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Study on challenges in
diagnosis of DR TB by
Gene X-pert in Nepal
2016

Challenges in the diagnosis of drug-resistant tuberculosis by GeneXpert MTB/Rif assay in Nepal



REPORT\654

National Tuberculosis Center
Bhaktapur, Nepal

Funded support by:
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Executive Summary

Introduction

In 2010, the World Health Organization approved and endorsed an automated, cartridge- based nucleic acid amplification test (NAAT) for TB based on the GeneXpert multi- disease platform – GeneXpert MTB/Rif assay (Cepheid, Sunnyvale, CA, USA). It can accurately detect both TB and resistance to Rifampicin in less than 2 hours. In Nepal, since the commencement of GeneXpert services in December 2011, there has been a steady uptake of this technology by the National TB Control Program. Nevertheless, a large number of drug-resistant TB cases remained undiagnosed. This study aims to examine the challenges in diagnosis of drug-resistant tuberculosis by the GeneXpert MTB/Rif assay in Nepal and explore the possible solutions.

Methods

This was a cross-sectional study consisting of two parts – a quantitative part assessing the individual demographic details and the relevant information and a qualitative part comprising of a focus group discussion on the challenges on the diagnosis of drug-resistant TB by GeneXpert MTB/Rif assay. Data were collected from the GeneXpert operators, clinicians and program managers from 16 centers at five locations across the country selected in such a way that they represented private, community or the public sectors as well as all the geographic terrains of the country. A sign in sheet collected information on the basic demographics and details on their work experience. Focus group discussions using a structured format captured the challenges faced in GeneXpert MTB/Rif assay utilization. Data were analyzed by IBM SPSS for Windows v23 (IBM Statistical Package for Social Sciences, 2015 IBM Corporation, New York, United States) and QDA Miner 4 Lite (QDA Miner 4 Lite, 2012 Provalis Research, Montreal, Canada). Descriptive statistics were used to summarize the socio demographic and other characteristics of the study participants using mean, standard deviation and proportions as appropriate.

Results

A total of 48 technical manpower contributed in the data collection. The mean age was 39.95 years (SD $\pm$ 11.5) and a majority of the participants (77.3%) were male. Most of the operators studied ran 2 to 3 test cycles per day and reported the results on the same or the next day. The CSF was the most frequently tested extra-pulmonary sample. Most of the program managers believed that the GeneXpert MTB/Rif assay was placed at the appropriate level of the health care delivery system. The major challenges were the inadequate training of the operators and clinicians, frequent power failure, difficulty in maintaining appropriate steady temperature, module failure which is often not replaced in time, issues with calibration and timely availability of cartridges as well as appropriate ways to store the new cartridges and safe disposal of the used cartridges.

Conclusion

The GeneXpert MTB/Rif assay has been of great help in the diagnosis of drug-resistant tuberculosis patients. Nevertheless, a number of challenges limit its optimal utilization. Solutions to the problems identified can potentially help in the diagnosis of drug-resistant TB patients and subsequent clinical decision making.

Discussion

The GeneXpert MTB/Rif was introduced in Nepal in 2011 through TB REACH initiative aimed at increasing TB case detection.²⁴ Since then, a number of GeneXpert machines have been rolled out at different locations across the country which accompanied the creation/dissemination of national algorithms and training for operators as well. It has been recommended that the GeneXpert machines can be placed anywhere, from a peripheral clinic to a reference laboratory, but the selection of a site depends on the workload, efficiency of referral networks, the infrastructure requirements, the human resources capacity and running costs. These considerations often result in GeneXpert machines being placed above the peripheral level, which requires the establishment of the reliable specimen or patient-referral networks.⁵ In a study from India, it has been shown that the installation and operations of the GeneXpert MTB/Rif assay in the lower levels of health systems were feasible and required minimal infrastructural modifications.²⁵ The Program Managers participated in the study felt that the GeneXpert MTB/Rif assays have been placed at the appropriate level of healthcare delivery system in Nepal which range from peripherally located Primary Health Care Centers to the National TB Reference Laboratories.

Available studies have demonstrated that the diagnosis of tuberculosis is cost effective in different settings from low income countries like Tanzania to high income low TB burden countries like the United States.^{26,27} A study assessing the cost of TB and drug resistant TB diagnosis by GeneXpert globally as well as in 36 high-burden countries reported that diagnosis of drug-resistant TB by GeneXpert would cost US\$70–90 million per year globally and be lower cost than conventional diagnostics.²⁸ No studies have examined the cost-effectiveness of the GeneXpert within the National TB Control Program of Nepal. However, the TB Program managers studied have been involved in the planning and budgeting activities of the programs and all except one respondent were of the opinion that the cost incurred by the GeneXpert MTB/Rif assays was justified for the benefits obtained.

Among the operators who participated in the study, 95.5% reported that they run 2 to 3 cycles of GeneXpert testing in a typical working day. While the number of test outputs in a day also depends on the number of modules available, given the less than 2 hour turn-around time, the World Health Organization estimates 16 tests in an 8-hour working day using 4 module GeneXpert machine that amounts to 4 cycles of tests per day.⁵ Increasing the number of test cycles per day, therefore, could potentially maximize the use of GeneXpert MTB/Rif assay. Considering the average number of

GeneXpert MTB/Rif assay requests made by clinicians studied in a typical working day as 4.85, the number of test cycles per day needs to be balanced against the number of requests of GeneXpert received for optimal outcome

Among the extra-pulmonary samples reportedly being requested and tested for GeneXpert MTB/Rif assay, CSF was the most common sample, followed by pus and gastric aspirate. The policy update on GeneXpert MTB/Rif assay by the WHO now

recommends considering CSF, pus, gastric aspirate for GeneXpert analysis with the pooled sensitivities of 79.5%, 84.9%, and 83.3% respectively.¹³ The WHO does not consider other body fluids, including the pleural fluids as an appropriate sample for the GeneXpert MTB/Rif assay. The pooled sensitivity of GeneXpert on detecting tuberculosis was 43.7% and data for other samples – pericardial fluid, ascitic fluid, urine, blood, and stool were limited. 7 operators and 9 clinicians participating in our study reported that they have been obtaining GeneXpert MTB/Rif assay samples for pleural fluids as well. While this may be justified for a limited number of reference labs or academic centers, routine testing of pleural fluids and other body fluids should be limited. Perhaps, updating the operators and clinicians with the latest national algorithms and evidence base can help achieve this goal.

Although the GeneXpert MTB/Rif assay procedure takes less than 2 hours, many clinicians report that they receive their results days later. Frequently cited problems include laboratory operations issues, including limited staff, practices of batching specimens, and other logistical barriers such as inefficient specimen referral and transport networks. In order to expedite the test results delivery to the clinicians, an SMS based tool called XpertSMS has been rolled out in TB REACH projects in a number of countries, including Pakistan and Bangladesh.²⁹ These services can potentially minimize the delays in treatment decision by the clinicians.

Human resources form an integral component of the efficient utilization of GeneXpert technology. The National TB Center conducts periodic basic training to the newly assigned operators and refresher training to update on the knowledge and skills of the existing operators. The clinicians requesting the GeneXpert tests and the program managers overseeing the operations are also trained on identifying the individuals to test for GeneXpert MTB/Rif assays, the test results interpretations and clinical decision- making based on the interpretations, in part through the drug-resistant TB basic training and PPM workshops. However, these activities seem to be inadequate in building up the confidence in operations and/or test results interpretations. While an escalation of training activities seem to be an obvious initial response, a further assessment of the specific needs and tailored approaches would ensure optimal utilization of the available resources. A short-term hands-on training at National Reference Labs by the trained colleagues/superiors can be an option for the selected operators. For clinicians, a wide dissemination of the most up-to-date diagnostic algorithms, including whom to test and what samples to send for the test is necessary and these algorithms need to be displayed in easy to access locations. Workshops for clinicians can be especially helpful in minimizing the use of GeneXpert MTB/Rif assay for treatment response monitoring, guiding the interpretations of discordant results and

clinical decision-making. An easy access to the technical advisory group can supplement in the clinical decision making process.

The GeneXpert machine requires a stable electric power supply. Interruptions in power may cause damage to the machine, results to be lost, cartridges to be wasted, and the need to obtain another specimen.⁵ Therefore, a power stabilizer and an uninterrupted power supply unit (UPS) are recommended for the GeneXpert instrument. In the testing

locations with frequent and longer power outages, an additional infrastructure to prevent the test cycle is needed. This will, in turn, prevent cartridges from being wasted and protect the equipment. Depending upon the frequency and duration of power outages, this may include an inverter with an external battery that can provide power for both the instrument and the computer for the average duration of the test – that is, 2 hours. An operational research in Uganda has demonstrated the feasibility of solar power at district/sub-district level with abundant sun-light.³⁰ A guideline from the Foundation for Innovative New Diagnostics is available that provides a clear guidance on GeneXpert power backup solutions to meet different power supply scenarios.³¹

The power supply irregularities also have implications in the GeneXpert machine operations as well as cartridge storage conditions. The manufacturer recommends that the ambient operating temperature be maintained between 15 °C and 30 °C for the GeneXpert machine operations and the cartridges and the specimen reagent be stored at 2–28 °C.⁵ The operating temperature recommended is not different from the operating temperatures recommended for a wide range of other laboratory equipment, household appliances, and computers. Therefore, an air condition is required in many locations, especially with a humid climate, in Nepal, the Southern Terai region of the country. Ensuring that appropriate temperature is of utmost importance because operating outside the recommended temperature range may increase the error rates since extreme temperatures interfere with thermo-cycling during the test. Though the manufacturer has stated that the cartridges are stable if kept at 2–45 °C for less than 6 weeks at 75% relative humidity, an uninterrupted power supply to the refrigerator storing the cartridges is important.

Equipment maintenance is an important problem in many resource-limited settings, including in Nepal as pointed out in this study as well. One of the important maintenance issues is the module failure. The repair and/or replacement requests of the failed modules have often been poorly addressed. An option of purchasing an extended warranty is now available, but with appropriate funding needs to be obtained. One possible solution outlined in this study is the provision of buffer modules and spare parts within the country, which can potentially minimize or prevent service interruptions. Resources from the manufacturer can be a useful resource to try tackling the maintenance problem locally.³² The GeneXpert modules require annual calibration. A remote calibration option is available that uses a kit containing special cartridges that are run on each module without specimens. The process lasts approximately 20 minutes and each kit can calibrate 4 modules. However, in some cases, remote calibration will not be sufficient. Therefore, provision of maintenance services and support for shipping and distribution of supplies, including calibration have been identified as key

demands.Recommendations

- Training support for operators - basic operations, software handling, testing strategies and interpretation of the test results, especially when there is discordance.
- Wide dissemination of the updated diagnostic algorithms among the clinicians as well as operators to minimize the confusions.
- Identify a focal point in the central TB reference lab in National TB Center, which can be a person or a group of persons who will respond to the requests for maintenance, cartridge supply, machine calibration etc. from the GeneXpert centers and proceed accordingly.
- Maintain a buffer stock of the modules (at least 25% of the total number of modules being operated) at the center so that they can be replaced soon to avoid or minimize lapses in services.

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Additional yield of active case finding through house hold survey in selected district of Kabul, Afghanistan, 2018

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Abstract

Every year, 10.4 million people get sick with Tuberculosis (TB) and 40 percent of them do not even receive care – they are “missed” by health systems. Missing TB cases is major challenge in fighting the disease, pose a serious threat to global health security. As per World Health organization estimation, in 2018, Afghanistan missed 27 percent TB case notification. Thus we conducted an active case finding through house hold survey from June – November, 2018 in Kabul city districts of Afghanistan.

This descriptive cross sectional study included all the residence of 21 sub-districts in Kabul City Afghanistan. After obtaining written consent, data was collected using a structured questionnaire from all presumptive TB cases. Later they went through standard laboratory

tests (Sputum for microscopy, Gene-Xpert and Chest X-ray) to confirm TB disease. These informations was entered into Epi-info version 7 and transferred to SPSS version 23 to analyze.

A total of 22,596 participants were included and 6740 were eligible. Among them 1614 (24.3%) individuals were screened positive to have presumptive TB and 105 (6.5%) had laboratory confirmed TB. Thus the case notification rate was 465 in 100,000 populations. Among the presumptive TB cases, mean age was 38.6 ± 18.5 years; 74% were female; 72% were housewives/unemployed; 2.6% were smokers.

The case notification rate and the point prevalence showed increase numbers then reports, which signifies Afghanistan National program was missing some cases. Hence active TB case finding might be an effective approach in terms of case notification in Afghanistan. We recommend to extend active community TB case finding in the TB surveillance of the country.

Key word:

Afghanistan, Tuberculosis, Prevalence, survey, household

Introduction

Worldwide, Tuberculosis (TB) is one of the top 10 causes of death and the leading cause from a single infectious agent. Millions of people continue to fall sick with TB each year. Globally, an estimated 10.0 million people fell ill with TB in 2018⁽¹⁾. There were an estimated 1.2 million (range, 1.1–1.3 million) TB deaths among HIV-negative people in 2018 (a 27% reduction from 1.7 million in 2000), and an additional 251 000 deaths among HIV positive people (a 60% reduction from 620 000 in 2000). TB affects people of both sexes in all age groups but the highest burden is in men (aged 15 years and over), who accounted for 57% of all TB cases in 2018. By comparison, women accounted for 32% and children (under 15 years of age) for 11%. Among all TB cases, 8.6% were people living with HIV (PLHIV). TB is an infectious disease caused by the bacterium *Mycobacterium tuberculosis*. Pulmonary TB is the most common form, caused by the inhalation of the bacterium ⁽²⁾. About 10 million new cases of TB occur globally each year, 70% of new cases are aged between 15 and 59 years and there are about 3 million deaths ⁽³⁾. TB is responsible for the second most number of deaths worldwide due to a single infectious agent, first being HIV/AIDS ⁽⁴⁾. The Low and middle

income countries bear 95% of the total burden of deaths. And in these countries TB is among the top three causes of deaths.

Tuberculosis is the leading cause of mortality of HIV infected patients; resulting in one fifth of all deaths ⁽⁴⁾. A TB patient can infect up to 10 to 15 other people in close contact within one year. Two thirds of people gotten ill with TB will die within a year if not properly treated ⁽⁴⁾. Though TB strikes in every part of the world, the largest number of new tuberculosis cases is from Asia; accounting for 60% of new cases globally ⁽⁴⁾.

Epidemiology of TB in Afghanistan

TB is a major health problem in Afghanistan, causing about 10,000 deaths per year (NTP annual report 2018 report)⁽⁵⁾. A number of factors, including ongoing conflict, make it difficult for health services to reach many parts of the country. Despite these challenges, the National Tuberculosis Program (NTP) has chosen to address the problem with interventions that are

proving successful. In 10 provinces, the NTP has started active case finding among targeted, previously underserved populations. because prompt case finding is an important pillar of global

tuberculosis (TB) control ⁽⁶⁾ In 2018, WHO estimated approximately 67,000 (CI: 40,000 - 88000) all types of TB cases with incidence rate of 189 per 100,000 population. Estimated mortality in 2018 was 10000 with the rate of 29 per 100000 populations. Also, cohort of 2017 shows treatment success rate for all TB cases of 90% and case notification rate was 154 per 100000 population in 2018. Total 48,800 TB cases were detected in 2018 (highest annual TB case notification so far in last decade). The progress is commendable because in 2001 only 9,581 cases were detected and from that point onwards, the trends shows increasing pattern except in 2008 and 2009 where a slight decline was seen in notified numbers as compared to previous year (2007). From 2010 onward, again the trends are upward ⁽⁷⁾.

In Afghanistan, TB case finding is mainly done by sputum examination of symptomatic patients who attend various types of health institutes (i.e. passive case detection). Delays in diagnosis through passive case detection have been associated with patient- and provider-related factors ^{(8), (9)}. Most studies on case finding have investigated risk factors associated with delay in diagnosis of TB patients found through passive case detection

(10), (11). Few studies have compared TB patients found through passive case detection with those identified through prevalence surveys or other active case finding efforts. Minimizing the time needed for case detection should be a key consideration for health planners in designing an effective TB control program. Understanding the barriers to care seeking is important in designing appropriate intervention to maximize the care seeking (12).

In many SAARC Countries including Afghanistan, there is a big difference between estimated number of TB cases and actual number of cases. There are many risk factors present in Afghanistan namely, ongoing conflict, poverty, overcrowding, smoking, drug addict and poor literacy etc. Even though these risk factors are abundant in Afghanistan case detection rates of tuberculosis remains low. We do not know whether TB presumptive cases are hidden in this area. According WHO estimation (2019) NTP Afghanistan will notify 70,000 (13) all TB cases yearly, however, NTP notified 48,800 (14) TB case during 2018 that shows Afghanistan NTP missed 21,200 cases during mentioned year that means 30% TB cases missed every years. Thus

we conducted a cross sectional study to estimate the point prevalence by active case finding through house hold survey in selected district of Kabul city, Afghanistan.

Methodology

This was a descriptive cross sectional study conducted in Kabul city of Afghanistan. Kabul province is the capital of Afghanistan with more than four million population suffering from pollution; overcrowding; poor sanitation; with poor TB indicators e.g., low case notification, high transfer rate, low treatment success rate, no proper system for presumptive case management, poor health infrastructure etc. Thus Kabul city was selected to carry out the study.

The study populations were ≥ 15 years of age and all residents located in 21 sub-districts in Kabul City. Pregnant women as well as all non-consented were excluded from the study. The population was selected as per cluster sampling method. After taking informed consent, data collections were done using a semi structured questionnaire. The presumptive TB (PTB) were defined as anybody with signs and symptoms for TB i.e. cough for more than two weeks, night sweat, cough and Sputum with blood, unintentional weight loss. Data was collected about the demographic and socioeconomic, previous and current treatment for TB, contact

with TB patients, BCG vaccination status. After primary screening all eligible presumptive TB patients went through laboratory tests. For that three sputum samples (on the spot, early morning and on the spot) were collected and Chest X- Ray (CXR) was done. For diagnosis and treatment of TB, the existing National Tuberculosis Control Program of Afghanistan algorithm was used ⁽¹⁵⁾.

Suggested diagnostic criteria were:

If symptoms positive, CXR negative, AFB negative do Gene X-pert
If symptoms negative, CXR positive, AFB negative do Gene X-pert
If symptoms positive, CXR positive, AFB negative do Gene X-pert
If symptoms positive, do Gene X-pert to identify RR/MDR-TB status

Operational definitions were clearly defined in the data collection tool. The strategy of screening for active TB and testing them for confirmation has been validated and recommended by the World Health Organization ⁽¹⁶⁾.

This information entered into Epi info version 7 and transferred to SPSS version 23 to analyze. Socio-demographic and other relevant characteristics of the study participants using mean,

standard deviation, median and percentages as appropriate. Data was statistically analyzed with the significance level setting as two-tailed and at p value <0.05. Descriptive statistics for continuous variables were described as means $\pm$ standard deviation, while categorical data were reported as frequency and percentage. Differences between the two groups (males and females) were compared using Fisher's exact test for categorical variables, or independent t-test for continuous variables.

Ethical approval

Ethical approval were taken from Institutional Review board of Islamic Republic of Afghanistan, Ministry of Public Health. All diagnosed TB patients were put into treatment with the government facility.

Result

During the study period, the study team approached 3,228 households with approximately 22,596 individuals in 21 districts of Kabul city of Afghanistan. Among them, 6,740 (29.82%)

individuals were eligible to be verbally screened for signs and symptoms of TB. The study team discovered 1,614(23.9%) individuals as presumptive to TB patients. The gender differences of the presumptive TB cases are plotted in figure 1. The age (mean $\pm$ standard deviation) of the presumptive TB cases were 31.75 + 16.38 year. The study team also registered all household members of presumptive TB patients as contact and they registered 4,875 individuals of the presumptive TB patients to keep them for further follow up.

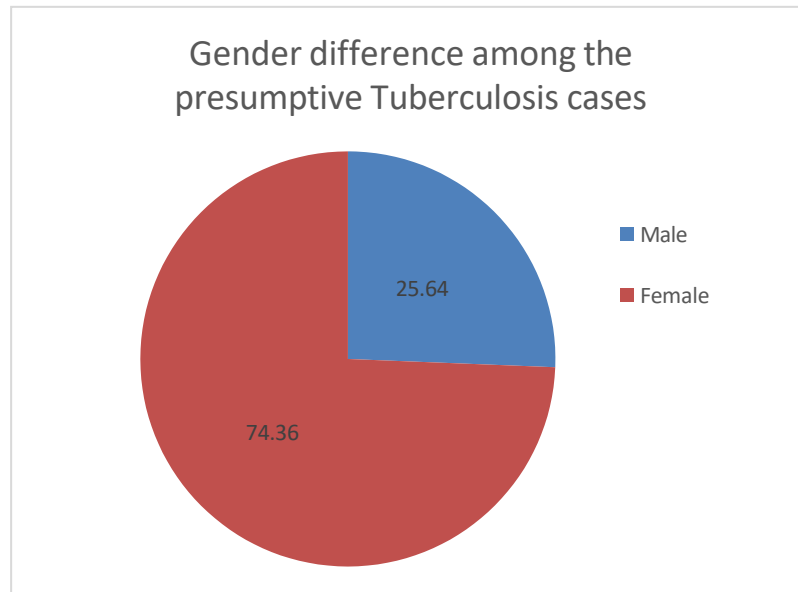


Figure 1: The gender differences of the presumptive TB cases, Kabul, Afghanistan, 2018

While analyzing the socio-demographic data, we found the mean family size was 8.4 ± 4.4 persons. The mean daily incomes of the households were Afghan Currency (AFN) 288.8/ day. Among the presumptive cases, 139 (8.6%) households reported to have at least one chronic smoker in their household members. Also, 42 (2.6%) of confirmed Pulmonary TB cases were smokers and average eleven cigarettes were consumed by them per day. The mean duration of cigarette smoking habit was 13.4 years. As the study was in Kabul districts, we found 1164 (72%) of the presumptive TB cases used natural gas and 435 (27%) used wood for regular household cooking.

We analyzed the level of education of presumptive TB patients and found that 223 (13.8%) could read and write, 121 (7.4%) had primary education, 82 (5%) had middle education, 98 (7%) were high school graduates and 28 (1.7%) were university graduates. The occupation were

diverse and the data is plotted in figure 2.

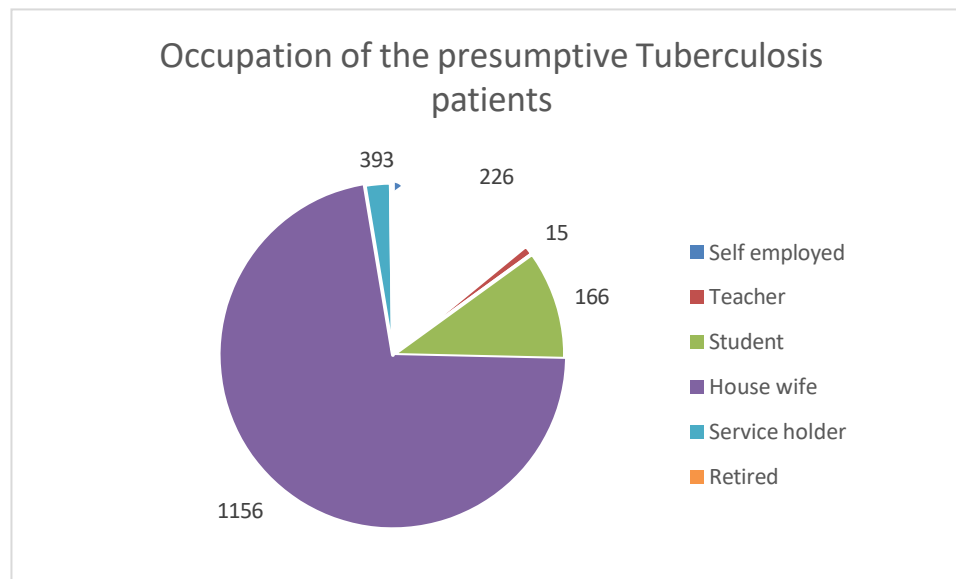


Figure 2: Occupational differences among the presumptive TB cases, Kabul, Afghanistan

While analyzing the symptoms, we found, 1145 (71%) of presumptive TB cases had cough, 696 (61%) had productive cough means both sputum and cough and the mean duration for having cough was 2.3 weeks. Contact history showed, 116 (7%) had history of TB with in their family members (exposure to TB patients) and 73 (4.5%) had reported to have TB before the survey time. It includes both those already completed their TB treatment and those still under treatment at the time of study. All 1614 presumptive TB cases were screened by digital X-ray. Among them 1171 (72.55%) showed negative study for having TB by the radiologists. Three sputum samples were collected from rest 443 Presumptive TB cases and 50 (11.28%) cases were confirmed by Gene-Xpert and 55 (12.41%) cases had definite clear lesion suggestive of TB suggested by radiologists. Thus a total of 105 cases were laboratory positive to have TB which comprises 6.5% of all presumptive TB cases and 23.70% of 2nd screening by chest X-ray. Thus the case notification rate was 465 in 100,000 populations.

Discussion:

The study found that the presumptive TB case identification was higher in Kabul population compared to this rate among general outpatient department (OPD) attendance in public health facilities. The study shows higher rate of 23.9%, however, this rate has been less than 3% out of OPD attendances over the past decade in Afghanistan⁽¹⁷⁾. Therefore, it suggests higher

prevalence of TB in Kabul city's population compared to the rests of the country and to that of WHO estimates for Afghanistan.

In addition, the findings are similar to the NTP and national health management information system (HMIS guideline, 2010) ⁽¹⁸⁾ of Afghanistan. There was higher rate of female attendances in the study which is same as the HMIS data and there is higher rate of female attendees to public OPD clinics. Also, the findings from NTP shows that there is higher rate of female attendees and presumptive TB patients identification in public facilities and that is almost 60% and this study finds this rate higher than it and it is 74%. This could be because the male members of the household attends jobs and other activities outside the home and female usually stays at home during the day and it is illustrated as majority of the study population had housewife job. Thus, it can be concluded that male members of the family are usually missed from screen and NTP needs to address their need through strategy to address it at their work environment.

The family size of the study population is higher than findings of Afghanistan health survey (AHS) 2018 ⁽¹⁹⁾. The household composition of the study population was 8.4%, although, this rate is 7.2% according to AHS 2018 ⁽¹⁹⁾.

In contrast to NTP surveillance data which shows the positivity rate of almost 7% out of all presumptive TB patients, but this study found that this rate is 11.28% among study population.

Interestingly, the findings shows the higher prevalence of TB disease among Kabul city's population compared to the WHO estimated rate in its global TB report 2019 ⁽²⁰⁾. The prevalence of TB in Kabul city found to be 465 in 100,000 populations which is higher than WHO estimates of 340 in 100,000 population.

Conclusion and Recommendations:

The findings of this study are interesting that shows higher rate of respiratory symptomatic patients in Kabul city. The presumptive TB patients identification rate was higher than the NTP data for over the past one decades. In addition, the positivity rate of new bacteriologically confirmed TB in study population is higher than the NTP data for the health facilities. That is 11.28% versus 7%, respectively. More interestingly, the prevalence of

pulmonary TB is higher

than the WHO estimated incidence and prevalence for Afghanistan. The yield of TB is 1.34% that is higher than WHO estimated prevalence of TB for Afghanistan. This is 2.4% higher than WHO estimated incidence of TB all forms among Afghan populations. Considering the findings of this study, we can conclude that TB is more prevalent in Kabul city compared to the rests of the country and the WHO estimates. In addition, this approach found to be an effective

mechanism to find missing TB cases in a densely populated city of Kabul. In order to have the exact and more accurate population estimate, we the study team recommend the conduction of prevalence survey in Afghanistan. Moreover, we recommend the NTP to sustaining this approach to find missed TB cases in sittings like Kabul.

The study found that there is higher rate of female participation and that male members of the family may have missed from screening as usually male members attend jobs during the day and therefore, NTP need to shape strategy to address the need of screening of male individuals, mostly at their workplaces.

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Conflict of interest

None

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Study on challenges in
diagnosis of DR TB by
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**Report
Study on challenges in
diagnosis of TB and MDR
TB by Gene-Xpert in
Bangladesh**

2016

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Abstract

Executive Summary

TB is a leading cause of morbidity and mortality worldwide. However, public health services globally reported only 66% of the estimated TB cases in 2014. Moreover, less than 5% of notified TB cases were tested for drug resistance which is often diagnosed after prolonged diagnostic delays. The main reasons for these gaps are inadequate diagnostic capacity and an over reliance on chest radiography and/or sputum smear microscopy as diagnostic tools. The „„classical““ diagnosis of HIV- associated and drug-resistant TB is complex, expensive, slow and technically demanding, relying on conventional culture and drug susceptibility testing (DST). Detecting more cases, detecting them early and rapidly identifying drug resistance are essential for improving individual patient health and avoiding transmission in the community. The Xpert MTB/RIF assay is an automated, real-time nucleic acid amplification technology represents a paradigm shift in the diagnosis of TB and drug-resistant TB by simultaneously detecting Mycobacterium tuberculosis and Rifampicin resistance-conferring mutations in a closed system suitable for use outside conventional laboratory settings in less than 2 hours, directly from sputum samples.

Objective: To find out the challenges in diagnosis of TB and MDR TB by Gene-Xpert in Bangladesh

Methodology;

Both quantitative and qualitative methods study designs were used. All the 43 Centres in Bangladesh where the Gene X pert test are carried out were included. In addition selective officials/staff from national and sub-national level were included as respondents for focus group discussion (FGD) and in-depth interview. Verbal informed consent was obtained from participants before starting interviews. Analysis of data was done using SPSS software program.

Results:

In about half (48.8%) of the sites machines are run twice a day, in 17 sites (39.5%) only once while in 5 sites (NGO supported sites) machines are run 3 times a day. In 19 (44.2%) sites 1-4 samples are tested per day and in 16(37.2%) sites 5-8 samples/day. Per day 9-20 samples are tested in 4(9.3%) centres while in other 4 centres 21-30 samples are tested daily. In all the centres a total of about 300 samples are tested in a single working day. In total, 38 sites informed about problems they faced in operating the machine. The most common problem was module failure (67.4%), followed by delay in maintenance support (46. 5%). Inadequate cartridge supply and load shading were faced by 16.3% and 11.6% respectively.

In 42 sites there were needs for support. The most common support they need is refresher training (93%), followed by maintenance training by 79.1%, and Software training by 18.6%. The responses from both FDG and in-depth interviews were as follows

Most common problem faced by the health workers were lack of timely maintenance of Machines, false result of Rif Resistance due to low bacterial load, module failure no proper sputum transport mechanism lack of appropriate centrifuge machine for processing of samples of EP cases and inadequate man power. Following suggestions given by the participants: A well maintained sputum transportation mechanism to be established, stable power supply is absolutely necessary. Discontinuation of electricity even for a fraction of second will cause erroneous test result, more machines need to be made available for easier access, machine operator needs refresher training including training related to day-to-day maintenance and software and ensure good quality sample in sufficient amount for Xpert testing to produce accurate results.

Conclusion:

Gene-Xpert machine is very useful in diagnosis of MDR and Rifampicin Resistance M. tuberculosis. Module failure is a common problem and their replacement takes longer time. Frequent errors are shown that might be due to poor quality of sample, unstable electricity supply or poor skill of machine operators. Proper training of operators and proper sputum transport system is urgently needed for efficient use of these machines.

Report

Study on challenges in diagnosis of TB and MDR TB by Gene-Xpert in Bangladesh-2016 Introduction:

Tuberculosis (TB) is a major global health problem. It causes ill-health among millions of people each year and ranks alongside the human immunodeficiency virus (HIV) as a leading cause of death worldwide. Bangladesh is among 30 countries with the high burden of TB and MDR-TB. The estimated incidence rate of all forms of tuberculosis was 225 per 100,000 population in 2015. In 2015 a total of 206,915 new and relapse cases were notified, among them 4% cases aged less than 15 years. The overall male female ratio was 1.5 in 2015. The treatment success rate among new and relapse cases (all forms) is above 90% since 2007. In 2014 cohort the overall treatment success rate was 93% and among bacteriologically confirmed cases it was 94%. (*ref Annual TB report 2016, NTP Bangladesh*)

NTP Bangladesh has conducted country's first nationwide drug resistance survey in 2010-2011. According to this survey report the proportion of new TB cases with MDR-TB is 1.4% and that of retreatment cases with MDR-TB is 28.5%. On this assumption the estimated total number of MDR-TB cases in 2015 in the country is 4662 (2198 among new PTB cases and 2464 among retreated PTB cases). (*ref Annual TB report 2016, NTP Bangladesh*)

The MDG target of halting and reversing TB incidence by 2015 was achieved globally, in all six WHO regions and in 16 of the 22 high TB burden countries (HBCs). The TB incidence rate has fallen at an average rate of 1.5% per year since the year 2000.

Globally, the TB mortality rate in 2015 was 47% lower than in 1990: the target of a 50% reduction was almost met. The target was achieved in four WHO Regions (the exceptions were the African and European regions), and in 11 HBCs. The TB prevalence rate in 2015 was 42% lower than in 1990. (1).The target of a 50% reduction was met in three WHO regions and in nine HBCs. Between

2000 & 2014, TB treatment alone saved 35 million lives among HIV-negative people; TB treatment and antiretroviral therapy saved an additional 8 million lives among HIV-positive people (1).

An estimated 3.3% (95% CI: 2.2–4.4%) of new cases and 20% (95%CI: 14–27%) of previously treated cases have MDR-TB; these levels have remained virtually unchanged in recent years. In 2014, there were an estimated 480 000 (range: 360 000–600 000) new cases of MDR-TB

worldwide, and approximately 190 000 (range: 120 000–260 000) deaths from MDR-TB.(1) Among patients with pulmonary TB who were notified in 2014, an estimated 300 000 (range: 220 000–370 000) had MDR-TB. More than half of these patients were in India, China and the Russian Federation. On average, an estimated 9.7% (95% CI: 7.4–12%) of people with MDR-TB have XDR-TB.

There was major progress in coverage of drug susceptibility testing (DST) between 2013 and 2014. Worldwide, 12% of new bacteriologically-confirmed TB cases and 58% of previously treated TB patients were tested for drug resistance in 2014, up from 8.5% and 17% respectively in 2013 (representing proportional increases of 43% and 223%, respectively)(1).

Moreover, globally in 2014, 123 000 patients with MDR -TB or rifampicin resistant tuberculosis (RR-TB) were notified, of whom about 75% lived in the European Region, India, South Africa or China. This was equivalent to 41% of the 300 000 notified TB patients who were estimated to have MDR-TB in 2014. The number of notified MDR/RR-TB cases in 2014 was almost the same as in 2013. People with MDR-TB or RR-TB are eligible for second-line treatment with MDR-TB regimens. A total of 111 000 people were started on MDR-TB treatment in 2014, an increase of 14% compared with 2013. Only 50% of patients on MDR-TB treatment were successfully treated, largely due to high rates of mortality and loss to follow-up (2–4)

TB Epidemiology in the SAARC Region:

The SAARC region, with an estimated annual incidence of 3.1 million TB cases, carries 32% of the global burden of TB incidence. Four of the eight Member Countries in the Region are among the 22 high burden countries, with India accounting for 23 % of the world's TB cases. Among 3.1 million incident TB cases, 2.1 million are notified new and relapse cases.

In 2014, there were an estimated 3.1 million incident cases of TB, equivalent to 185 cases per 100 000 population. This carries 31% of the global burden of TB incidence. The absolute number of

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incident cases is falling slowly, from 2000 to 2014. The incidence rate was relatively stable from 1990 up until around 2000, and then started to fall, achieving the MDG target far ahead of the 2015 deadline. (2)

There were an estimated 0.37 million TB deaths in 2014. TB ranks alongside HIV as a leading cause of death from an infectious disease. India accounted for about one third of global TB deaths (both including and excluding those among HIV-positive people.

In the SAARC region, the mortality rate (excluding deaths among HIV positive people) had achieved the target of 50% reduction from 1990 levels

MDR-TB situation in the SAARC Region:

The MDR TB cases in the region range from less than one to four percent (1-4%) among new TB cases and it ranges from less than one to almost 35 percent among the retreatment TB cases. In 2014 Pakistan has 4.3% of new tuberculosis cases with MDR-TB, which is highest in the SAARC region. However, in India there were 24,000 new MDR-TB cases among notified pulmonary TB cases. In case of retreatment Bhutan has 35% of new tuberculosis cases with MDR-TB, which is highest in the SAARC region. However, in India there were 47,000 MDR-TB cases among retreatment TB cases.

MDR-TB situation in Bangladesh (3)

The results of the first national DRS completed in 2012 confirmed a low proportion of new TB cases that have MDR-TB (1.4%, confidence intervals 0.7–2.5), but the proportion among retreated cases was revised upwards (28.5%, confidence intervals 24–34). The total number

of estimated MDR-TB cases among notified cases in 2015 was 4662. Coverage of routine surveillance of drug resistance is still low. (3)

Issues related to conventional method of diagnosis TB:

With 9.6 million incident cases of tuberculosis (TB) and 1.5 million deaths estimated in 2014 TB is a leading cause of morbidity and mortality worldwide. However, public health services globally reported only 66% of the estimated TB cases in 2014. Moreover, less than 5% of notified TB cases were tested for drug resistance which is often diagnosed after prolonged diagnostic delays (4-6)

The main reasons for these gaps are inadequate diagnostic capacity and an over reliance on chest radiography and/or sputum smear microscopy as diagnostic tools. Patients with HIV-associated TB, those with sputum smear-negative and/or extra pulmonary disease, and drug-resistant TB patients are particularly affected by the failure of microscopy as a primary diagnostic tool. The „classical“ diagnosis of HIV-associated and drug-resistant TB is complex, expensive, slow and technically demanding, relying on conventional culture and drug susceptibility testing (DST). The long delay (up to several weeks) required to obtain results has devastating consequences for patients who go undiagnosed (and therefore untreated or inappropriately treated), or are diagnosed too late (7). Detecting more cases, detecting them early and rapidly identifying drug resistance are essential for improving individual patient health and avoiding transmission in the community. This requires universal access and early detection using contemporary tools and innovative strategies (8, 9, 10) The past decade has seen unprecedented growth in the TB diagnostic pipeline and accelerated efforts to establish the necessary laboratory infrastructure (10). Nevertheless, although recommended by WHO, the latest generation liquid culture diagnostics and molecular line probe assays for rapid detection of MDR-TB have not yet solved the diagnostic dilemma in most resource-limited settings, largely due to the need for expensive laboratory infrastructure, extensive bio-safety precautions and specialized staff (10)

A new rapid test that overcomes many of the current operational difficulties was recommended for use by WHO in December 2010: the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) is an automated, real-time nucleic acid amplification technology run

on the multi-disease platform GeneXpert (Cepheid). The Xpert MTB/RIF assay represents a paradigm shift in the diagnosis of TB and drug-resistant TB by simultaneously detecting *Mycobacterium tuberculosis* and Rifampicin resistance-conferring mutations in a closed system suitable for use outside conventional laboratory settings in less than 2 hours, directly from sputum samples (11,12)

Literature review on GeneXpert Technique:

Field demonstration studies

6648 individuals were prospectively enrolled in South Africa, Peru, India, Azerbaijan, the Philippines and Uganda, comparing Xpert MTB/RIF with microscopy in decentralized microscopy centres, and with culture and phenotypic DST in centralized laboratories. Xpert MTB/RIF detected 90.3% (933 out of 1033) of the culture-confirmed TB cases, compared with 67.1% (699 out of 1041) using microscopy. In sputum smear- negative, culture-positive TB patients Xpert MTB/RIF test sensitivity was 76.9% (296 out of 385) and specificity was 99.0% (2846 out of 2876).

Sensitivity for rifampicin resistance was 94.4% (236 out of 250) and specificity was 98.3% (796 out of 810) (13)

While HIV co-infection substantially decreased the sensitivity of microscopy (to 47%), Xpert MTB/RIF performance was not significantly affected. The median time to detection of TB was 0 days (inter-quartile range (IQR) 0–1) using Xpert MTB/RIF, compared to 1 day (IQR 0–1) for microscopy, 30 days (IQR 23–43) for solid culture and 16 days (IQR 13–21) for liquid culture. The median time to detection of rifampicin resistance was 20 days (IQR 10–26) for line-probe assay versus 106 days (IQR 30–124) for phenotypic DST.(13)

The Xpert MTB/RIF test reduced the median time to treatment for sputum smear-negative TB from 56 days (IQR 39–81) to 5 days (IQR 2–8). The indeterminate rate of Xpert MTB/RIF testing was 2.4% (126 out of 5321 samples) compared to 4.6% (441 out of 9690) for culture.(13)

Unpublished, single-centre evaluation studies Results from 12 studies with varying design and study populations reported Xpert MTB/RIF sensitivity in detecting TB ranging from

70% to 100% in culture-positive patients and 60% in those with smear-negative disease. Specificity ranged from 91% to 100%. Pooled average sensitivity for TB detection was 92.5% and pooled average specificity was 98%. Average rifampicin sensitivity and specificity were 98% and 99%, respectively.

Previous studies of the MTB/RIF assay have reported test sensitivities of 57 to 76.9% in cases of smear-negative, culture-positive pulmonary tuberculosis and 98 to 100% in cases of smear-positive, culture-positive pulmonary tuberculosis, while the test specificity remained at 99% to 100% (17, 18, 19, 20,)

In the previous studies, the sensitivity of the MTB/RIF test for detecting RIF resistance was 94.4 to 100% and the specificity was 98.3 to 100% (21).

Operational and logistical issues:

The available evidence confirmed the robustness of the Xpert MTB/RIF assay under varying temperature and humidity conditions, the need for minimal staff training, basic bio-safety requirements (as for sputum smear microscopy) and high levels of user satisfaction. Operational challenges included the requirement for an ambient temperature < 30°C (necessitating air conditioning in hot climates), and uninterrupted and stable electrical power supply (requiring generators in several sites). Storage space and conditions (28°C for cartridges, waste generated (considerably more than for microscopy), and the 12-month shelf-life of cartridges were listed as main operational challenges (14,15) Cost, affordability and cost-effectiveness analyses Using X₁₀ B/RIF for the diagnosis of smear-negative pulmonary TB was deemed cost-effective compared with existing diagnostic strategies in India, South Africa and Uganda, and within WHO acceptable incremental cost effectiveness ratios (14,15,16)

The Gene-Xpert MTB/RIF assay is a novel integrated diagnostic device that performs sample processing and heminested real-time PCR analysis in a single hands-free step for the diagnosis of tuberculosis and rapid detection of RIF resistance in clinical specimens. The assay can generally be completed in less than 2 h (10, 17).

Xpert MTB/RIF was first introduced in Bangladesh in March 2012 with the USAID supported TB CARE II project. Till December 2015, Xpert MTB/ RIF machines were functioning at 43 settings (including 5 sites supported by icddr,b) in the country. Since inception of this method, no evaluation the challenges in diagnosis of TB and drug resistant

TB in Bangladesh was done in a systematic way. Hence according to the decision of the 25th Governing Board Meeting of the STAC this study was conducted in Bangladesh from 06 January to 05 April 2017.

Objective: To find out the challenges in diagnosis of TB and MDR TB by Gene Xpert in Bangladesh

Methodology;

Study settings: All the institutions in Bangladesh where the Gene Xpert test (43 Centres in Bangladesh) are carried out were included in the study. In addition selective officials/staff from national and sub-national level were included as respondents for focus group discussion (FGD) and in-depth interview.

Study Design: This study used both qualitative and quantitative research methods and accordingly research instruments were developed to address the research objectives.

In quantitative part information related to Xpert Machines and Machine operator were included. In qualitative section Focus Group Discussion (FGD) and Key informant in-depth interviews were done.

Four FGDs were carried out with i) National level officials related to TB control and are involved in GeneXpert procurement, establishment, functioning and supervision/ monitoring ii) Microbiologists/Laboratory Technicians who are involved in Gene X- Pert Procedure and iii) Medical Officers/health workers who are currently treating/managing MDR TB patients. A set of questionnaire was used for conducting FDG.

In addition to FDG few Key Informant Interviews were carried out with the key personnel involved in Gene X Pert technique in national and sub-national level of the country.

Verbal informed consent was obtained from participants before starting interviews.

Analysis of data was done using SPSS software program. All the information from interview and FGDs were checked for ensuring quality of the data. Data coding/programming was done for entering the data. Then data were entered, and checked/ cleaned. Data analysis was done through frequency table and cross table on the basis of identified variables, and accordingly results/findings were highlighted in the draft report which was finalized after review and dissemination.

Findings

Xpert MTB/RIF was first introduced in Bangladesh in March 2012. Till December 2015, a total of 61 Xpert MTB/ RIF machines were functioning at 43 sites (including 5 sites supported by icddr,b) in the country. Data have been collected to achieve the objectives from all these 43 sites. During 2016 no machine was installed.

Seventeen (39.5%) out of total 43 GeneXpert sites are in Dhaka division and it includes five sites supported by ICDDR,B which working for TB control as a partner of NTP (Table 1). In 42 sites the GeneXpert machines are placed in a separate room and in one site it is placed along with other general pathological laboratory activities. During data collection it was found that in all sites necessary physical support like air-cooler, dust control (inbuilt with Xpert machine) , UPS as well as regular electricity and water supply were available. However in 2 sites air cooler was found non- functioning and in other 2 sites UPS was non-functioning (Table 2) which were under process of repair.

A total of 65 operators are operating the machines in 43 Gene Expert sites. In 23 centres only one operator, in 18 centres there are 2 operators and in 2 centres (Rajshahi CDH and Chittagong RTRL) 3 operators are working considering the workload (Table 3).

During data collection where 2 or 3 operators were involved, interview was taken only from the senior one. So in total interview were taken from 43 operators. Among those 35 (81.4%) were male and 8 (18.6%) were female. Their age ranged from 23 to 55 years with a mean age 32.28 ± 8 years. More than 50 % were less than 30 years, 14 were within the range of 30-44 years and only 7 were of more than 44 years, and these all 7 were males (Table 4).

Other key parameters/characteristics of the Machine operators:

Regarding **general education**, more than one- third of the operators were only SSC pass and less than one-fourth of them were HSC pass. About 87% (36/43) of them have **lab related** formal institutional educational background (e.g. Diploma in Medical Technology Lab) and seven has no such background or even no formal lab related training. **Professional designations** are not harmonized among government and non-government organizations. More than 50% (22/43) has

professional designation as MT Lab (under GOB) , while 14 (32.6%) are designated as Technical Asst. Lab (under NGO). Other designations are health worker (4), senior lab technician, research assistant and lab attendant (each one). All MT labs are qualified with Diploma in MT Lab and are Supported by Government of Bangladesh. On the other hand most of the operators supported by NGO (BRAC and ICDDR,B) are not diploma holder.

Length of Service: More than 60% (26) have been working for 30-40 months, 14% (6) for 50-59 months, 3 for 2 months and 8 (18.6%) for less than I year. All except two(2) have received 3 day training on GeneXpert machines (36 from form NTP and 5 from ICDDR,B).

The other 2 have working experience with trained colleagues and will also receive formal training. (Table 5). Length of service varies because of phage wise installation of machines and training also provided phage wise by NTP with the support of NTP and Challenge TB USAID.

Type and number of machines:

Out of 43 sites, 38 have 4-module and 5 sites have 16-module machine. In total 55 fourmodule machines (34 under NTP and 21 under ICDDR,B) and 6 sixteenmodule machines (all under NTP) have been established. Out of these only one 4modul machine was non-functioning (for five days due to software problem). Therefore total number of module is 316 (Table 6-9)

The table 10 shows that in 23 sites there were nonfunctioning modules and out of total 316 modules 57 (18.04%) were non-functioning. Higher proportion of nonfunctioning non-functioning modules might be due to lengthy process of module replacement by Cepheid, Netherland.

On an average in about half (48.8%) of the sites machines are run twice a day, in 17 sites (39.5%) only once while in 5 sites (NGO supported sites) machines are run 3 times a day (Table 11).

In 19 (44.2%) sites 1-4 samples are tested per day and in 16(37.2%) sites 5-8 samples/day. Per day 9-20 samples are tested in 4(9.3%) centres while in other 4 centres 21-30 samples are tested daily. In all the centres a total of about 300 samples are tested in a single working day (Table 12).

In total, 38 sites informed about problems they faced in operating the machine. The most common problem was module failure (67.4%), followed by delay in maintenance support (46.5%).

Inadequate cartridge supply and load shading were faced by 16.3% and 11.6% respectively (Table 13).

In 42 sites there were needs for support. The most common support they need is refresher training (93%), followed by maintenance training by 79.1%, and Software training by 18.6%. Only 3 (7.3%) centres asked for more Xpert machine. (Table 14).

In-depth interview was taken from 23 respondents form different institutions/organization; among them 11 were from Government organization and 12 from NGO; their distribution is shown below in Table 15 and 16:

For in-depth interview 3 questions were set as below:

- A) Problem faced in running the GeneXpert machine in diagnosis of TB and Gene Expert
- B) Any lesson Learned: though using the machines in diagnosing TB cases:
- C) Suggestions (if any) for further improvement.

We also arranged 4 Focus Group Discussions for officials of different levels to identify the challenges and probable suggestions for further improvement in optimum utilization of GeneXpert machines in diagnosis of TB and MDR TB.

The responses from both FDG and in-depth interviews were analyzed and were summarized in 3 broad areas as shown in Table 17.

Conclusion:

- Availability of GeneXpert Machine are inadequate in comparison to need.
- Though almost 100% of the available machines are functional, there is lack of adequate support for timely maintenance.
- Module failure is a common problem and their replacement takes longer time. (> 18% of total modules are found nonfunctional).
- Frequent errors are shown that might be due to poor quality of sample, unstable electricity supply or poor skill of machine operators. (only 3 days training received). Also might be due non existence of well established sputum transport mechanism.
- Non-existence of EQA
- Strong monitoring and supportive supervision is key to improve the situation. Recommendation:
 - Rapid expansion of GeneXpert facility is required to enhance detection of TB and MDR TB
 - Diagnostic algorithm needs to be revised to testing all presumptive TB and DR TB
 - System to be developed for timely maintenance of machines
 - Refresher training for Machine operator needs to be arranged
 - To ensure quality EQA system to be established.
 - Supervision/monitoring to be strengthened.

Table 1. Distribution of GeneXpert Sites by Division (N=43)

Sl No.	Division	Frequency	Percent	Remarks
1	Rajshahi	3	7.0	
2	Rangpur	4	9.3	
3	Khulna	5	11.6	
4	Barisal	3	7.0	
5	Dhaka	12	27.9	
6	Sylhet	3	7.0	
7	Chittagong	8	18.6	
8	ICDDR,B	5	11.6	All these 5 sites are in Dhaka
	Total	43	100.0	

Table 2 GeneXpert Room status and required facilities (N=43)

Status/facilities	Yes (%)	No (%)	Remarks
Placed in separate room	42 (97.7)	1(2.3)	Placed along with general Lab activities
Air cooler	43 (100)	00	2 were non functioning
Dust control	43 (100)	00	Inbuilt with Xpert machine
UPS	43 (100)	00	2 were non functioning
Regular Electricity	43 (100)	00	
Running Water supply	43 (100)	00	

Table 3. Number of operator per site (N=43)

Number of operator	Frequency (Number of Sites)	Percent	Remarks
1	23	53.5	
2	18	41.9	
3	2	4.7	Considering higher workload
Total	43	100.0	

Table 4. Age distribution of the machine operators (N=43)

Age group (yrs)	Frequency		Total (%)
	Male (%)	Female (%)	
23-29	17 (77.3)	5 (22.2)	22 (51.2)
30-44	11 (78.6)	3 (21.4)	14 (32.5)
45-55	7	0	7 (16.3)
Total	35 (81.4)	8 (18.6)	43 (100)

Table 5. Some other key parameters of the machine operators ((N=43)

Parameters	Frequency	%	Remarks
Education (general):			
SSC	29	67.4	
HSC	10	23.3	
Bachelor	3	7.0	
Master	1	2.3	
Total	43	100%	
Education (technical):			
None	7	16.3	
Laboratory related	36	83.7	
Total	43	100%	
Professional Designation			
MT Lab	22	51.2	
Technical Asst. Lab	14	32.6	
Senior Lab Technician	1	2.3	
Lab attendant	1	2.3	
Research Assistant	1	2.3	
Health worker	4	9.3	
Total	43	100%	
Working Duration			
< 1 year (2-23 months)	8	18.6	
1-2 year (24 months)	3	7.0	
30-48 months	26	60.4	
>4 years (50-59 months)	6	14.0	
Total	43	100%	
Training Received on GeneXpert			
Yes	41	95.3	
No	2	4.7	
Total	43	100%	
Training Received from			
NTP (NTRL)	36	83.7	All received 3 days training
ICDDR,B	5	11.6	

No formal Training	2	4.7	On the Job training form Trained operator who has left the job

Table 6. Type of machines based on number of modules (N=43)

Machine Type	Frequency	%	Remarks
16 Model machine	5	11.6	Under ICDDRB, there are four 4modules in 2 centres and five 4modules in 1 centre
4 module Machine	38	88.4	
Total	43	100%	

Table 7. Type and number of Machines per site (N=43)

Type	Number	Frequency	Percent	Remarks
4 modules	0	5	11.6	34 machines under NTP 21 machines under ICDDR,B
	1	34	79.1	
	4	2	4.7	
	5	1	2.3	
	8	1	2.3	
	Total	43	100.0	
16 module	0	38		All 6 machines are under NTP
	1	4		
	2	1		
	Total	43	100.0	

Table 8. Summary table for calculating number of machine and number of modules

Machine Type	#of sites where placed	Total machine	Number of modules
4- module	38	55	220
16- module	5	6	96
Total	43 sites	61 machines	316 modules

Table 9 Functional status of Machines

Machine Type	Functioning	nonfunctioning	total
4- module	54	1	55
16- module	6	0	6
Total	60	1	61

Table 10 Functional status of modules by sites

Number of non functioning modules (i)	frequency (sites) (ii)	%	Total non functioning modules (i)x(ii)	Remarks
0	20	46.5	00	Total nonfunctioning modules 57/316 (18.04%)
1	9	20.9	9	
2	8	18.6	16	
4	4	9.3	16	
7	1	2.3	7	
9	1	2.3	9	
Total	43	100.00	57	

Table 11. number of times machines run per day

Number of times machines run per day	frequency	%
1	17	39.5
2	21	48.8
3	5	11.6
Total	43	100.00

Table 12. Average number of samples test per day

Average number of samples test per day	frequency	%
1-4	19	44.2
5-8	16	37.2
9-20	4	9.3
21-30	4	9.3
Total	43	100.00

Table 13. Type of problems faced in operating machine (multiple responses)

Type of problem faced	frequency	%
No problem	5	11.6
Load shading	5	11.6
Voltage up-down	7	16.3
Inadequate modules compared to workload	4	9.3
Module failure	29	67.4
In-adequate cartridge supply	7	16.3
Delay in maintenance	20	46.5
Laptop problem	2	4.7

Table 14. Type of support needed

Type of support	frequency	%
No support is needed	1	2.3
More Xpert machine	3	7.3
Refresher training	40	93.0
Software training	8	18.6
Maintenance training	34	79.1

Table 15: Distribution of Respondents (for in-depth interview) by institution

Institution/organization	Number of Respondent
Chest Disease Hospital (CDH)	4
NTP Bangladesh	4
Damien Foundation Bangladesh	1
BRAC	2
Challenge TB (CTB) Bangladesh	2
TB Hospital	4
ICDDR,B	6
Total	23

Table 16: Distribution of Respondents (for in-depth interview) by their principal responsibility

principal responsibility	Number of Respondent
Managerial	11
Technical	7
Supervisory	5
Total	23



Additional yield of activities
case finding through house
survey in selected district of
Afghanistan with lower
case notification rate
in Afghanistan
2018

Table 17

Response from the respondents on these 3 areas (multiple challenges)

Problem /challenges faced	Frequency
Sputum transport mechanism is not well established	2
Lack of timely maintenance of Machines	8
Module failure	3
Frequent error	9
False result of Rif Resistance due to low bacterial load	3
Lack of appropriate centrifuge machine for processing of samples of EP cases	3
Inadequate manpower	1
Lesson Learned	
Availability of GeneXpert is very less in comparison to presumptive cases	4
Diagnostic algorithm has limited indication for diagnosis of TB and MDRTB	2
GeneXpert is a very useful tool for quick and accurate diagnosis of TB and MDR TB	1 5
Selection criteria for referring to GeneXpert has limited scope	3
Incremental yield for Bacteriological cases	1
Ambient temp and regular cleanliness (at least once in a month) of filter to keep the machine well functioning.	3
Well oriented staff can operate the machine nicely	2
Error rate is higher in samples of EP TB due to low bacterial load and appropriate centrifuge machine is required for processing	3
As only one sample is tested, good quality sample is required	2
Regular maintenance and calibration should be in place for smooth implementation of GeneXpert	4
“Dedicated well trained staff should be there to operate the system for ensuring quality lab services”	1
Stable power supply is absolutely necessary. Discontinuation of electricity even for a fraction of second will cause erroneous test result	3
“The module replacement process kills lot of time as we have to acquire the replacement modules from Cepheid, Netherland”	1
Suggestions (if any) for further improvement	
A well maintained sputum transportation mechanism to be established	2
Diagnostic algorithm needs to be revised to testing all presumptive TB and DR TB	2
More machines need to be made available for easier access	5
Ensure regular and timely maintenance	5
Machine operator needs refresher training including training related to day- to-day maintenance and software.	5
Ensure good quality sample in sufficient amount for Xpert testing to produce accurate results	2
Diagnostic criteria should be more broadened so that more presumptive TB and DR TB can be tested.	2

Additional yield of active case finding through house hold survey in selected district of Kabul, Afghanistan, 2018

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Abstract

Every year, 10.4 million people get sick with Tuberculosis (TB) and 40 percent of them do not even receive care – they are “missed” by health systems. Missing TB cases is major challenge in fighting the disease, pose a serious threat to global health security. As per World Health organization estimation, in 2018, Afghanistan missed 27 percent TB case notification. Thus we conducted an active case finding through house hold survey from June – November, 2018 in Kabul city districts of Afghanistan.

This descriptive cross sectional study included all the residence of 21 sub-districts in Kabul City Afghanistan. After obtaining written consent, data was collected using a structured questionnaire from all presumptive TB cases. Later they went through standard laboratory tests (Sputum for microscopy, Gene-Xpart and Chest X-ray) to confirm TB disease. These informations was entered into Epi-info version 7 and transferred to SPSS version 23 to

analyze.

A total of 22,596 participants were included and 6740 were eligible. Among them 1614 (24.3%) individuals were screened positive to have presumptive TB and 105 (6.5%) had laboratory confirmed TB. Thus the case notification rate was 465 in 100,000 populations. Among the presumptive TB cases, mean age was 38.6 ± 18.5 years; 74% were female; 72% were housewives/unemployed; 2.6% were smokers.

The case notification rate and the point prevalence showed increase numbers then reports, which signifies Afghanistan National program was missing some cases. Hence active TB case finding might be an effective approach in terms of case notification in Afghanistan. We recommend to extend active community TB case finding in the TB surveillance of the country.

Key word:

Afghanistan, Tuberculosis, Prevalence, survey, household

Introduction

Worldwide, Tuberculosis (TB) is one of the top 10 causes of death and the leading cause from a single infectious agent. Millions of people continue to fall sick with TB each year. Globally, an estimated 10.0 million people fell ill with TB in 2018⁽¹⁾. There were an estimated 1.2 million (range, 1.1–1.3 million) TB deaths among HIV-negative people in 2018 (a 27% reduction from 1.7 million²⁵ in 2000), and an additional 251 000 deaths among HIV positive people (a 60% reduction from 620 000 in 2000). TB affects people of both sexes in all age groups but the highest burden is in men (aged 15 years and over), who accounted for 57% of all TB cases in 2018. By comparison, women accounted for 32% and children (under 15 years of age) for 11%. Among all TB cases, 8.6% were people living with HIV (PLHIV). TB is an infectious disease caused by the bacterium *Mycobacterium tuberculosis*. Pulmonary TB is the most common form, caused by the inhalation of the bacterium⁽²⁾. About 10 million new cases of TB occur globally each year, 70% of new cases are aged between 15 and 59 years and there are about 3 million deaths⁽³⁾. TB is responsible for the second most number of deaths worldwide due to a single infectious agent, first being HIV/AIDS⁽⁴⁾. The Low and middle income countries bear 95% of the total burden of deaths. And in these countries TB is among the top three causes of deaths. Tuberculosis is the leading cause of mortality of HIV infected patients; resulting in one fifth of all deaths⁽⁴⁾. A TB patient can infect up to 10 to 15 other people in close contact

within one year. Two thirds of people gotten ill with TB will die within a year if not properly treated ⁽⁴⁾. Though TB strikes in every part of the world, the largest number of new tuberculosis cases is from Asia; accounting for 60% of new cases globally ⁽⁴⁾.

Epidemiology of TB in Afghanistan

TB is a major health problem in Afghanistan, causing about 10,000 deaths per year (NTP annual report 2018 report)⁽⁵⁾. A number of factors, including ongoing conflict, make it difficult for health services to reach many parts of the country. Despite these challenges, the National Tuberculosis Program (NTP) has chosen to address the problem with interventions that are proving successful. In 10 provinces, the NTP has started active case finding among targeted, previously underserved populations. because prompt case finding is an important pillar of global tuberculosis (TB) control ⁽⁶⁾ In 2018, WHO estimated approximately 67,000 (CI: 40,000 - 26 0) all types of TB cases with incidence rate of 189 per 100,000 population. Estimated mortality in 2018 was 10000 with the rate of 29 per 100000 populations. Also, cohort of 2017 shows treatment success rate for all TB cases of 90% and case notification rate was 154 per 100000 population in 2018. Total 48,800 TB cases were detected in 2018 (highest annual TB case notification so far in last decade). The progress is commendable because in 2001 only 9,581 cases were detected and from that point onwards, the trends shows increasing pattern except in 2008 and 2009 where a slight decline was seen in notified numbers as compared to previous year (2007). From 2010 onward, again the trends are upward ⁽⁷⁾.

In Afghanistan, TB case finding is mainly done by sputum examination of symptomatic patients who attend various types of health institutes (i.e. passive case detection). Delays in diagnosis through passive case detection have been associated with patient- and provider-related factors ^{(8), (9)}. Most studies on case finding have investigated risk factors associated with delay in diagnosis of TB patients found through passive case detection ^{(10), (11)}. Few studies have compared TB patients found through passive case detection with those identified through prevalence surveys or other active case finding efforts. Minimizing the time needed for case detection should be a key consideration for health planners in designing an effective TB control program. Understanding the barriers to care seeking is important in designing appropriate intervention to maximize the care seeking ⁽¹²⁾.

In many SAARC Countries including Afghanistan, there is a big difference between estimated number of TB cases and actual number of cases. There are many risk factors present in Afghanistan namely, ongoing conflict, poverty, overcrowding, smoking, drug addict and poor literacy etc. Even though these risk factors are abundant in Afghanistan case detection rates of tuberculosis remains low. We do not know whether

TB presumptive cases are hidden in this area. According WHO estimation (2019) NTP Afghanistan will notify 70,000 ⁽¹³⁾ all TB cases yearly, however, NTP notified 48,800 ⁽¹⁴⁾ TB case during 2018 that shows Afghanistan NTP missed 21,200 cases during mentioned year that means 30% TB cases missed every years. Thus we conducted a cross sectional study to estimate the point prevalence by active case finding through house hold survey in selected district of Kabul city, Afghanistan.

Methodology

This was a descriptive cross sectional study conducted in Kabul city of Afghanistan. Kabul province is the capital of Afghanistan with more than four million population suffering from pollution; overcrowding; poor sanitation; with poor TB indicators e.g., low case notification, high transfer rate, low treatment success rate, no proper system for presumptive case management, poor health infrastructure etc. Thus Kabul city was selected to carry out the study.

The study populations were ≥ 15 years of age and all residents located in 21 sub-districts in Kabul City. Pregnant women as well as all non-consented were excluded from the study. The population was selected as per cluster sampling method. After taking informed consent, data collections were done using a semi structured questionnaire. The presumptive TB (PTB) were defined as anybody with signs and symptoms for TB i.e. cough for more than two weeks, night sweat, cough and Sputum with blood, unintentional weight loss. Data was collected about the demographic and socioeconomic, previous and current treatment for TB, contact with TB patients, BCG vaccination status. After primary screening all eligible presumptive TB patients went through laboratory tests. For that three sputum samples (on the spot, early morning and on the spot) were collected and Chest X-Ray (CXR) was done. For diagnosis and treatment of TB, the existing National Tuberculosis Control Program of Afghanistan algorithm was used ⁽¹⁵⁾.

Suggested diagnostic criteria were:

If symptoms positive, CXR negative, AFB negative do Gene X-pert
If symptoms negative, CXR positive, AFB negative do Gene X-pert
If symptoms positive, CXR positive, AFB negative do Gene X-pert
If symptoms positive, do Gene X-pert to identify RR/MDR-TB status

Operational definitions were clearly defined in the data collection tool. The strategy of screening for active TB and testing them for confirmation has been validated and recommended by the World Health Organization ⁽¹⁶⁾.

This information entered into Epi info version 7 and transferred to SPSS version 23 to analyze. Socio-demographic and other relevant characteristics of the study participants using mean,

standard deviation, median and percentages as appropriate. Data was statistically analyzed with the significance level setting as two-tailed and at p value <0.05 . Descriptive statistics

for continuous variables were described as means $\pm$ standard deviation, while categorical data were reported as frequency and percentage. Differences between the two groups (males and females) were compared using Fisher's exact test for categorical variables, or independent t-test for continuous variables.

Ethical approval

Ethical approval were taken from Institutional Review board of Islamic Republic of Afghanistan, Ministry of Public Health. All diagnosed TB patients were put into treatment with the government facility.

Result

During the study period, the study team approached 3,228 households with approximately 22,596 individuals in 21 districts of Kabul city of Afghanistan. Among them, 6,740 (29.82%) individuals were eligible to be verbally screened for signs and symptoms of TB. The study team discovered 1,614(23.9%) individuals as presumptive to TB patients. The gender differences of the presumptive TB cases are plotted in figure 1. The age (mean $\pm$ standard deviation) of the presumptive TB cases were 31.75 + 16.38 year. The study team also registered all household members of presumptive TB patients as contact and they registered 4,875 individuals of the presumptive TB patients to keep them for further follow up.

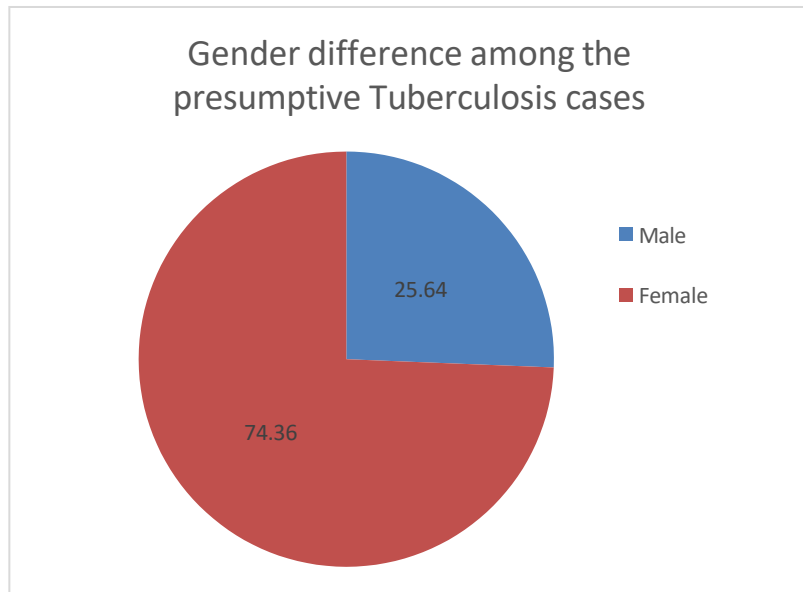


Figure 1: The gender differences of the presumptive TB cases, Kabul, Afghanistan, 2018

While analyzing the socio-demographic data, we found the mean family size was 8.4 ± 4.4 persons. The mean daily incomes of the households were Afghan Currency (AFN) 288.8/day. Among the presumptive cases, 139 (8.6%) households reported to have at least one chronic smoker in their household members. Also, 42 (2.6%) of confirmed Pulmonary TB cases were smokers and average eleven cigarettes were consumed by them per day. The mean duration of cigarette smoking habit was 13.4 years. As the study was in Kabul districts, we found 1164 (72%) of the presumptive TB cases used natural gas and 435 (27%) used wood for regular household cooking.

We analyzed the level of education of presumptive TB patients and found that 223 (13.8%) could read and write, 121 (7.4%) had primary education, 82 (5%) had middle education, 98 (7%) were high school graduates and 28 (1.7%) were university graduates. The occupation were diverse and the data is plotted in figure 2.

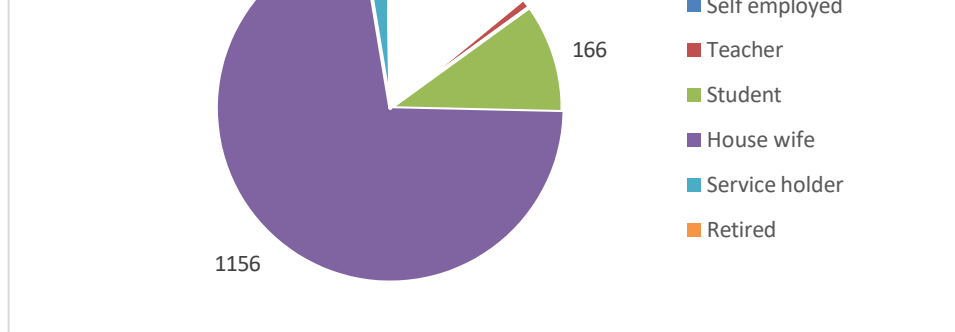


Figure 2: Occupational differences among the presumptive TB cases, Kabul, Afghanistan

While analyzing the symptoms, we found, 1145 (71%) of presumptive TB cases had cough, 696 (61%) had productive cough means both sputum and cough and the mean duration for having cough was 2.3 weeks. Contact history showed, 116 (7%) had history of TB with in their family members (exposure to TB patients) and 73 (4.5%) had reported to have TB before the survey time. It includes both those already completed their TB treatment and those still under treatment at the time of study. All 1614 presumptive TB cases were screened by digital X-ray. Among them 1171 (72.55%) showed negative study for having TB by the radiologists. Three sputum samples were collected from rest 443 Presumptive TB cases and 50 (11.28%) cases were confirmed by Gene-Xpert and 55 (12.41%) cases had definite clear lesion suggestive of TB suggested by radiologists. Thus a total of 105 cases were laboratory positive to have TB which comprises 6.5% of all presumptive TB cases and 23.70% of 2nd screening by chest X-ray. Thus the case notification rate was 465 in 100,000 populations.

Discussion:

The study found that the presumptive TB case identification was higher in Kabul population compared to this rate among general outpatient department (OPD) attendance in public health facilities. The study shows higher rate of 23.9%, however, this rate has been less than 3% out of OPD attendances over the past decade in Afghanistan ⁽¹⁷⁾. Therefore, it suggests higher

prevalence of TB in Kabul city's population compared to the rests of the country and to that of WHO estimates for Afghanistan.

In addition, the findings are similar to the NTP and national health management information system (HMIS guideline, 2010) ⁽¹⁸⁾, of Afghanistan. There was higher rate of female attendances in the study which is same as the HMIS data and there is higher rate of female attendees to public OPD clinics. Also, the findings from NTP shows that there is higher rate of female attendees and presumptive TB patients identification in public facilities and that is almost 60% and this study finds this rate higher than it and it is 74%.

This could be because the male members of the household attends jobs and other activities outside the home and female usually stays at home during the day and it is illustrated as majority of the study population had housewife job. Thus, it can be concluded that male members of the family are usually missed from screen and NTP needs to address their need through strategy to address it at their work environment.

The family size of the study population is higher than findings of Afghanistan health survey (AHS) 2018 ⁽¹⁹⁾. The household composition of the study population was 8.4%, although, this rate is 7.2% according to AHS 2018 ⁽¹⁹⁾.

In contrast to NTP surveillance data which shows the positivity rate of almost 7% out of all presumptive TB patients, but this study found that this rate is 11.28% among study population.

Interestingly, the findings shows the higher prevalence of TB disease among Kabul city's population compared to the WHO estimated rate in its global TB report 2019 ⁽²⁰⁾. The prevalence of TB in Kabul city found to be 465 in 100,000 populations which is higher than WHO estimates of 340 in 100,000 population.

Conclusion and Recommendations:

The findings of this study are interesting that shows higher rate of respiratory symptomatic patients in Kabul city. The presumptive TB patients identification rate was higher than the NTP data for over the past one decades. In addition, the positivity rate of new bacteriologically confirmed TB in study population is higher than the NTP data for the health facilities. That is 11.28% versus 7%, respectively. More interestingly, the prevalence of pulmonary TB is higher than the WHO estimated incidence and prevalence for Afghanistan. The yield of TB is 1.34% that is higher than WHO estimated prevalence of TB for Afghanistan. This is 2.4% higher than WHO estimated incidence of TB all forms among Afghan populations. Considering the findings of this study, we can conclude that TB is more prevalent in Kabul city compared to the rests of the country and the WHO estimates. In addition, this approach found to be an effective

mechanism to find missing TB cases in a densely populated city of Kabul. In order to have the exact and more accurate population estimate, we the study team recommend the conduction of prevalence survey in Afghanistan. Moreover, we recommend the NTP to sustaining this approach to find missed TB cases in sittings like Kabul.

The study found that there is higher rate of female participation and that male members of the family may have missed from screening as usually male members attend jobs during the day and therefore, NTP need to shape strategy to address the need of screening of male individuals, mostly at their workplaces.

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Conflict of interest

None

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Comparative Evaluation of
Treatment for MDR TB with
and without co-morbidity a
retrospective analysis
in Bangladesh
2018



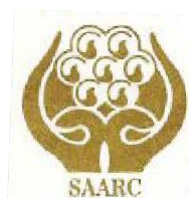
**Comparative Evaluation of Treatment Outcome
for Multidrug-resistant and Rifampicin-resistant
Tuberculosis with and without Comorbidity:
A Retrospective Analysis, Bangladesh, 2018**

Jointly Conducted by-



**National Tuberculosis Control Programme
Directorate General of Health Services
Ministry of Health and Family Welfare,
Bangladesh**

and



**SAARC Tuberculosis and HIV/AIDS Centre
(STAC) Kathmandu, Nepal**

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Abbreviations:

BDT	Bangladeshi Taka
BMI	Body Mass Index
CAT	Category
CDH	Chest Diseases Hospital
CI	Confidence Interval
CKD	Chronic Kidney Disease
COPD	Chronic Obstructive Pulmonary Disease
DGHS	Directorate General of Health Services
DM	Diabetes Mellitus
DOTS	Directly Observed Treatment Short course
DR-TB	Drug-resistant Tuberculosis
HIV	Human Immunodeficiency Virus
MDR-TB	Multidrug-resistant Tuberculosis
MDR/RR-TB	Multidrug-resistant or Rifampicin-resistant Tuberculosis
MTB	Mycobacterium Tuberculosis
NIDCH	National Institute of Diseases of the Chest and Hospital
NTP	National Tuberculosis Control Programme
PI	Principal Investigator
PMDT	Programmatic Management of Drug-resistant TB
RR-TB	Rifampicin-resistant Tuberculosis
RR	Risk Ratio
SAARC	South Asian Association for Regional Cooperation
SPSS	Statistical Package for the Social Sciences
STAC	SAARC Tuberculosis and HIV/AIDS Centre
TB	Tuberculosis
WHO	World Health Organization
XDR-TB	Extensive Drug-resistant Tuberculosis

Comparative Evaluation of Treatment Outcome for Multidrug-Resistant and Rifampicin-resistant Tuberculosis with and without Comorbidity: A Retrospective Analysis, Bangladesh, 2018

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1. Summary:

Multidrug-resistant and Rifampicin-resistant Tuberculosis (MDR/RR-TB) is a continuing threat to TB control especially in the developing countries. Compared to first line therapy, MDR-TB treatment is less effective with more adverse effect and cost. Thus MDR-TB has become a major barrier to achieve successful TB control. Treatment of MDR-TB is complex and outcome depends on many factors. Presence of comorbid conditions might be one of the factors. Despite being one of the high-burden MDR-TB country, Bangladesh has minimal data on treatment outcome of patients with MDR/RR-TB with other comorbid diseases. The overall objective of the study is to compare treatment response among patients with and those without comorbidities in a setting of MDR-TB care.

The study was a record based retrospective cohort study where MDR/RR-TB patients' medical records were retrospectively reviewed to evaluate the treatment outcome of the patients with and without comorbid disease. Data were collected from five DR-TB treatment initiation Hospitals who were enrolled into second line treatment from January to December 2015. Information of comorbid conditions were obtained by analyzing the baseline and follow-up laboratory investigations and from documents. The SPSS software (version 23) was used for data entry and analysis. Relative risk of unfavorable outcome was assessed between exposed and unexposed group. Comparisons of categorical variables was performed using the Pearson Chi square tests or Fisher's exact tests to compare different groups. P-value <0.05 was considered as statistically significant.

A total number of 657 MDR/RR-TB patients' data were retrieved. Among them, 428 (65.1%) were male and 229 (34.9%) were female. The mean age of the patients was 35.24±14.82 years and majority of the patients (n=472, 71.9%) were between 15-44 years of

age. 35.3% of the patients BMI below 18.5 kg/m². Risk of unfavorable treatment outcome increased for the patients above 55 years of age and with lower BMI. 539 patients had known HIV status and none of them were HIV positive. One hundred forty-nine (22.7%) patients had one or more comorbidity. Among them, 103(15.7%) patients had diabetes, 32(4.9%) COPD, 17(2.6%) hypothyroidism, 12(1.8%) chronic kidney disease and only 2(0.003%) patients had chronic liver disease. Among all the patients, 508(77.3%) were successfully treated and 149(22.7%) had unfavorable treatment outcomes: 75(11.4%) died, 11(1.7%) treatment failed, 54(8.2%) were lost to follow up and 9(1.4%) patients' outcome were not evaluated. Among who had been successfully treated (n=508), patients with comorbidity had success rate of 67.8% where it was 80.1% without comorbidity which was statistically significant (p=0.002). Among unfavorable treatment outcome, death was 19.5% and 9.1%, failure was 3.4% and 1.2%, lost to follow up was 8.7% and 8.1% for the patients with and without comorbidity respectively. Lower treatment success rate was observed among the patients with comorbidities, treatment success rate was 68.9%(p=0.027) with diabetes, 65.6%(p=0.105) with COPD, 41.7%(p=0.003) with chronic kidney disease and 52.9%(p=0.033) with hypothyroidism. Risk ratio of unfavorable outcome with comorbidity was found 1.6(95%CI 1.2-2.1) where RR for diabetes was 1.5(95%CI 1.1-2.5), COPD 1.6(95%CI 0.9-2.6), chronic kidney disease 2.7(95%CI 1.6-4.4) and for hypothyroidism RR was 2.1(95%CI 1.3-3.6). Mortality rate was found highest (33.3%) among the patients with chronic kidney disease.

Our cohort study revealed that comorbidities had serious impact on treatment outcomes and were associated with significant lower successful treatment outcomes and higher death rate. Special care and measures should be taken to improve quality of care of the drug resistant TB patients with comorbidities.

2. Background:

Multidrug-resistant and Rifampicin-resistant Tuberculosis (MDR/RR-TB) is a continuing threat to TB control especially in the developing countries [1]. MDR-TB refers to *Mycobacterium tuberculosis* (MTB) strains with in vitro resistance to the most effective two anti-tuberculosis drugs, e.g., isoniazid (H) and rifampin (R) where RR-TB refers to MTB strains with resistance to rifampicin [2]. Resistant to Isoniazid, rifampicin that forms the back bone of the short course chemotherapy would require use of drugs that are more toxic, costly and that administered for long period [3]. It is a growing public health concern, with an estimation of 3.6% (95% CI: 2.6–4.8) of new TB cases and 17% (95% CI: 5.6–33) of those previously treated for TB turning to MDR/RR-TB. The WHO estimated the incidence of MDR/RR-TB to be 558,000 (95% CI: 483,000–639,000) with 230,000 deaths in 2017 where most of the deaths occurred in Asia [4]. Bangladesh is one of the 30 high MDR-TB burden countries with estimated annual incidence of 5,800 (95% CI: 3,800–7,800) among notified pulmonary TB cases in 2017. The proportion of MDR/RR-TB among notified new and previously treated pulmonary TB cases are 1.6% (95% CI: 0.59–2.6) and 29% ((5% CI: 22– 36) respectively [4]. Compared with standard first line therapy, treatment of MDR-TB is less effective along with more adverse effect and cost, thus MDR-TB has become a major barrier to achieving successful control of TB. In recent years, treatment success rate of MDR-TB has increased slightly. Overall, the proportion of MDR/RR-TB patients in the 2015 cohort who successfully completed treatment globally (i.e. cured or treatment completed) was 54% [4]. Bangladesh maintained better treatment success over the years than the global average. In 2015 cohort 77% of the MDR/RR-TB patients were treated successfully under WHO endorsed 20-month regimen where the success rate of 9-month regimen under operational research was 84% in the same cohort [5].

Treatment of MDR-TB is complex and chemotherapy course's outcome depends on many factors where comorbidities may complicate TB disease and effect treatment response [6]. But there is limited data available to compare the treatment success rate of MDR/RR-TB with other comorbid diseases like diabetes mellitus (DM), liver diseases, renal diseases, malnutrition etc. Although studies showed that the prevalence of TB-DM is higher in low- and middle-income countries where TB and DM are most prevalent [7]. Among the ten countries with the highest number of DM patients worldwide, six are classified as “high burden” for TB by the World Health Organization, meaning they contribute to 80% of the TB cases worldwide [7]. According to previous studies [8-11] and our clinical experience, the co- morbidities including diabetes, COPD, malnutrition, chronic hepatitis, hepatic dysfunction and renal dysfunctions could be risk factors associated with poor treatment outcome among patients with MDR/RR-TB.

Literature review:

One cause of unsuccessful treatment outcomes may be comorbid conditions. A cohort study in Georgia revealed that comorbidities had serious impact on treatment outcomes and were associated with significant lower successful treatment outcomes and about twice higher death rate [6]. Another study in China showed that, low BMI, hypoalbuminemia, cavitory disease and previous anti-TB treatment are independent prognostic factors for poor outcome in patients with MDR-TB [12]. The impact of comorbidities on drug sensitive TB treatment is well-described, with conditions such as human immunodeficiency virus (HIV) infection, diabetes mellitus, chronic kidney disease (CKD) and alcohol misuse all associated with worse treatment outcomes [2, 13-15]. MDR-TB treatment programs often report high proportions of these comorbidities, with the prevalence of HIV, DM and alcohol misuse exceeding 10–20% in several large MDR-TB cohort studies [16-18]. Unfortunately, the relationship between comorbid conditions and MDR-TB treatment outcomes remains poorly described. One individual patient [19] and four aggregate meta-analyses [2, 20-22] have studied the outcomes of different treatment regimens in MDR /XDR-TB. Most cohorts included in these meta-analyses were from high-burden MDR-TB countries, but the Bangladeshi cohort has never been included. Moreover, the relative role of different exposure variables (e.g. cavitory disease, DM, CKD, liver dis 40 malnutrition etc.), other than treatment, has been poorly explored in the literature.

3. Rationale of the study:

Although Bangladesh is a high burden MDR-TB country, there is little information available on treatment outcome of patients with MDR/RR-TB with other comorbid diseases. Therefore, to explore this concern, a study is required to determine the characteristics, treatment outcomes and risk factors associated with poor treatment outcome among patients with MDR/RR-TB with or without comorbid diseases. We performed a retrospective record review of the five major DR-TB treatment initiation hospitals in Bangladesh to assess the association between specific comorbidities and MDR/RR-TB treatment outcomes and also compared treatment outcomes between different groups of patients with and without comorbidities.

Objectives:

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3.1. General objective:

The general objective of the study is comparison of treatment response among patients with and those without comorbidities in a setting of MDR-TB care.

3.2. Specific objectives:

- To observe socio-demographic distribution of treatment outcomes of MDR/RR-TB patients.
- To determine the treatment outcomes of MDR/RR-TB patients with different comorbidities.
- To compare treatment outcomes of MDR/RR-TB patients with and without comorbidity
- To identify risk factors for unfavorable treatment outcome of MDR/RR-TB patients.

4. Methods:

4.1. Study design:

The study was a record based retrospective cohort study. MDR/RR-TB patients' medical records were retrospectively reviewed to evaluate the treatment outcome of the patients who were exposed and those who were unexposed. The exposed group was defined as the MDR/RR-TB patients who had one or more comorbidities and the unexposed group was defined as patients who did not have any comorbidity. Ratio of favorable and unfavorable outcome between exposed and unexposed group was be assessed.

4.2. Study population:

All patients with rifampicin resistance diagnosed by GeneXpert and all culture proven MDR- TB registered from January to December 2015 who were enrolled under WHO recommended 20-month regimen.

4.3. Study site:

Study sites were all 5 (five) Drug-resistant (DR-TB) treatment initiation hospitals in Bangladesh where patients were enrolled under WHO recommended 20-month regimen. The hospitals for this study were National Institute of Diseases of the Chest and Hospital

(NIDCH) in Dhaka and 4 (four) Chest Diseases Hospitals (CDHs) in Chattogram, Khulna, Sylhet and Pabna.

4.4. Study period:

Study duration was six months starting from April 2018 to September 2018. Data was retrospectively collected from hospital records of January 2015 to December 2015.

4.5. Sample size:

All the MDR/RR-TB patients who were enrolled under WHO recommended 20-month regimen from January to December 2015 at mentioned 5 (five) DR-TB treatment initiation hospitals except the patients who meet the exclusion criteria were taken as sample. However, to assess the strength of the study, the required sample size was calculated using OpenEpi, version 3, an open source web-compatible calculator program for cohort study. (<http://www.openepi.com>)

Clear

Calculate

Sample Size: X-Sectional, Cohort, & Randomized Clinical Trials		
Two-sided confidence level(%)	95	(1-alpha) usually 95%
Power (1-beta or % chance of detecting)	80	Usually 80%
Ratio of Unexposed to Exposed in sample	4	For equal samples, use 1.0
Percent of Unexposed with Outcome	20	Between 0.0 and 99.9
Please fill in 1 of the following. The others will be calculated.		
Odds ratio		
Percent of Exposed with Outcome		Between 0.0 and 99.9
Risk/Prevalence Ratio	2	
Risk/Prevalence difference		Between -99.99 and 99.99

Two-sided significance level(1-alpha):	95
Power of the study (1-beta, % chance of detecting):	80
Ratio of sample size, Unexposed/Exposed: (According to program experience)	4
Percent of Unexposed with Outcome: (Unfavorable outcome was taken into account according to program experience)	20
Percent of Exposed with Outcome: (Auto generated)	40
Odds Ratio: (Auto generated)	2.7
Risk/Prevalence Ratio: (As it is unknown, default value of 2 was selected)	2
Risk/Prevalence difference: (Auto generated)	20

Cohort group	Sample Size
Exposed (MDR/RR-TB patients with comorbidity)	45
Unexposed (MDR/RR-TB patients without comorbidity)	179
Total sample size:	224

OpenEpi calculated the sample size using Kelsey's formula [23]. The sample size formula for the method described in Kelsey et. al.is:

$$N_{Kelsey} = \frac{(z_{\alpha/2} + z_{\beta})^2 p(1-p)(r+1)}{r(p_0 - p_1)^2}$$

And

$$N_1 = rN$$

Where

N_{Kelsey} = required sample size for the unexposed group using Kelsey formula

N_1 = number of unexposed

$Z_{\alpha/2}$ = standard normal deviate for two-tailed test based on alpha level (relates to the confidence interval level)

Z_{β} = standard normal deviate for one-tailed test based on beta level (relates to the power level)

r = ratio of unexposed to exposed

p_0 = proportion of exposed with disease

p_1 = proportion of unexposed with disease

Exclusion criteria:

The MDR/RR-TB patients having the following criteria will be excluded from study-

- Patients who had any serious illness requiring admission to Intensive Care Unit (ICU)
- Any patient who required surgical intervention
- Extra-pulmonary TB
- MDR/RR-TB patients enrolled under shorter regimen
- MDR/RR-TB patients who received new drugs i.e. Bedaquiline and Delamanid

4.6. Data collection method:

A standardized anonymous pretested data collection tool was used to collect patients' socio- demographic and clinical information. Information of comorbid conditions were obtained by analyzing the baseline and follow-up laboratory investigations and any known comorbidity which is recorded. Data quality was assured by trained supervisors and the principal investigator who regularly checked for data completeness. Six data collectors collected information retrospectively from five DR-TB treatment initiation centers. Data collectors were deployed according to MDR/RR-TB patient burden in the respective hospitals. Data collectors worked until all the patients' information were collected. Two data collectors worked in NIDCH for two months as patient burden is high where one data collector each worked in rest of the CDHs ranging from two weeks to six weeks duration. The filled-up data collection tool was sent to PI weekly either by courier service or by hand. Proper measures were taken to maintain the confidentiality of data.

4.7. Data collection tool:

A semi-structured data collection tool was used and the variables included are- age, sex, occupation, previous history of TB, previous history of TB treatment, contact history with TB patients, smoking history, weight, body mass index (BMI), cavitation in X-ray chest, diabetes, kidney diseases, liver diseases, COPD, HIV status, other comorbid diseases present, treatment regimen used, smear microscopy results, month of culture conversion, duration of hospitalization, and treatment outcome.

4.8. Operational definitions:

Treatment outcomes was recorded according to criteria defined in the WHO guidelines for the management of MDR-TB [24]. Cure and treatment completed were grouped under

favorable outcomes, whereas died, treatment failed and lost to follow-up were categorized as unfavorable outcomes. The measured outcomes were according to the definitions of National PMDT guidelines of NTP Bangladesh [25].

Cured: A DR-TB patient who has completed treatment according to NTP protocol without evidence of failure and has at least three consecutive negative cultures from samples collected at least 30 days apart in the final 12 months of treatment. If only one positive culture is reported during that time, and there is no concomitant clinical evidence of deterioration, a patient may still be considered cured, provided that this positive culture is followed by a minimum of three consecutive negative cultures taken at least 30 days apart.

Treatment completed: A DR-TB patient who has completed treatment according to programme protocol but does not meet the definition for cure because of lack of bacteriological results (i.e. fewer than three cultures were performed in the final 12 months of treatment).

Died: A DR-TB patient who dies for any reason during the course of DR-TB treatment.

Failed: Treatment terminated or need for permanent regimen change of at least two anti-TB drugs because lack of conversion in the continuation phase or bacteriological reversion in the continuation phase after conversion to negative or evidence of additional acquired resistance to fluoroquinolones and 2nd line injectable drug or adverse drug reaction.

Lost to follow up: A DR-TB patient whose treatment was interrupted for two or more consecutive months for any reason.

4.9. Pretesting of the data collection tool:

The data collection tool was pretested by an independent expert operationalization in the field.

4.10. Monitoring:

The funding agency of the study, SAARC Tuberculosis and HIV/AIDS Centre (STAC) monitored the overall progress. Beside this the PI and co-PIs visited all the sites at least

once during data collection period. Locally the director, PMDT coordinator, superintendent or consultant of the respective hospitals monitored the data collection activities and checked the validity and completeness of data.

Statistical Analysis:

Data abstraction was performed by two reviewers. When there was any disagreement, differences were resolved by consensus of PI and Co-PIs. SPSS software (version 23) was used for data entry and analysis. The primary end of the study was the favorable or unfavorable outcomes of MDR-TB treatment. Possible predicting factors for the favorable or unfavorable outcome of therapy were assessed against this end. Relative risk of unfavorable outcome was assessed between exposed and unexposed group. Comparisons of categorical variables was performed using the Pearson Chi square tests or Fisher's exact tests to compare different groups. Comparisons of continuous variables was performed using the T tests to compare different groups. P-value <0.05 was considered as statistically significant.

4.11. Ethical considerations:

Approval for the study was taken from National TB Research Committee, DGHS. As data was extracted from hospital records, approval from the head of institutions was also taken to access data. Confidentiality and anonymity were maintained by coding data with ID number and was stored safe. The study did not involve any invasive procedure and there was no physical risk. Any drugs, placebo, organs, body fluids, the fetus or the abortus was not used in this study. There was no direct involvement of human subjects.

5. Results:

A total number of 680 MDR/RR-TB patients were put into second line treatment in the selected five DR-TB treatment initiation centers. Among them 657 patients met the inclusion criteria and their data were retrieved from the treatment card, hospital records and relevant reports. All the patients were treated with standard MDR-TB regimen according to National PMDT guidelines [27]. Patients were treated with at least 8 months of intensive phase and at least 12 months of continuation phase. The standard regimen was as follows-

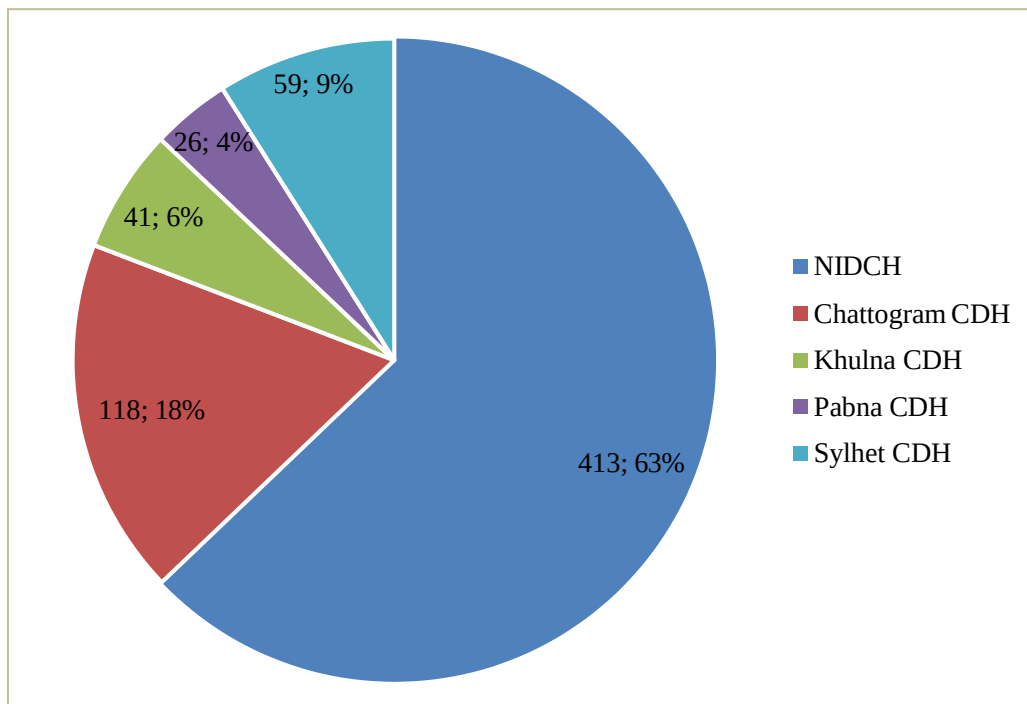
8(Km-Lfx-Eto-Cs-Z)- 12(Lfx-Eto- Cs-Z)

Km= Kanamycin, Lfx= Levofloxacin, Eto= Ethionamide, Cs= Cycloserine, Z= Pyrazinamide

Majority of the patients, 413(63%) were enrolled at NIDCH in Dhaka followed by 118 (18%) in Chattogram CDH, 59(9%) in Sylhet CDH, 41(6%) in Khulna CDH and the rest of the 26(4%) in Pabna CDH.

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Figure-1: Distribution of MDR/RR-TB patients by DR-TB treatment initiation centers



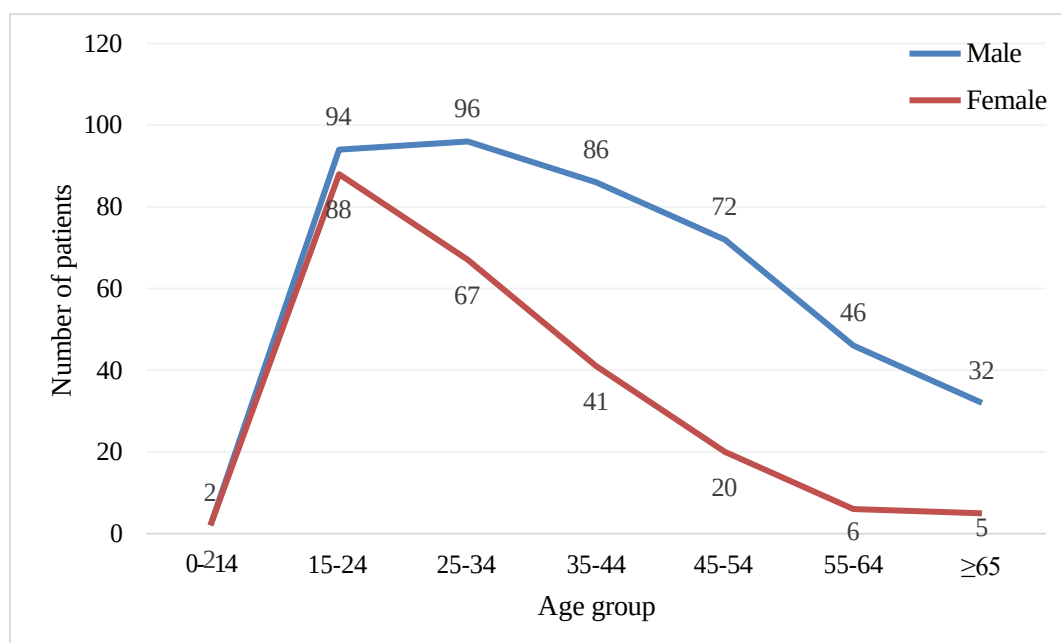
The mean age of the patients was 35.24 ± 14.82 years and majority of the patients (n=472, 71.9%) were between 15-44 years of age. The lowest number of patients (n=4, 0.6%) were observed among children under 15 years of age followed by the elderly people of 65 years and above (n=37, 5.6%).

Table-1: Distribution of MDR/RR-TB patients by age group (n=657)

Age group	Frequency	Percentage (%)
0-14	4	0.6
15-24	182	27.7
25-34	163	24.8
35-44	127	19.3
45-54	92	14.0
55-64	52	7.9
65 and above	37	5.6

Among 657 eligible patients, males were 428(65.1%) and females were 229(34.9%) with female ratio of 0.53. It was observed that, with the increase of age, especially patients aged 25 and above, the number of female patients decreases significantly ($p < 0.001$).

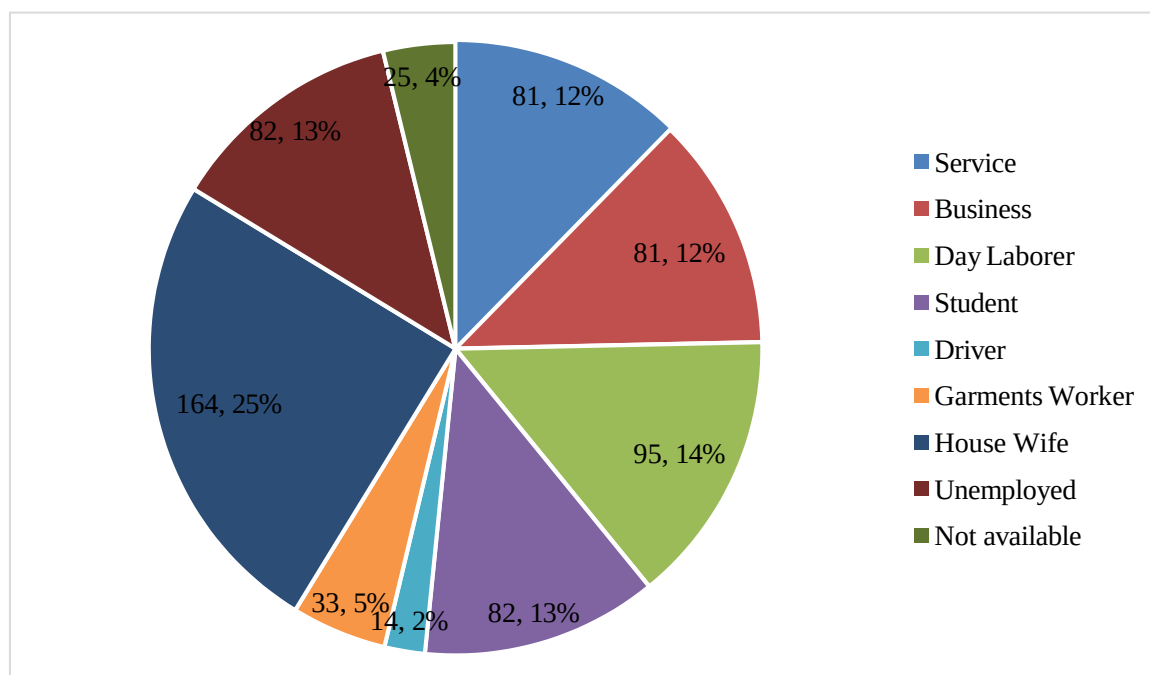
48

Figure-2: Age-sex distribution of MDR/RR-TB patients

Apart from housewife 164(25%) which was found most common, the highest number 95(14.5%) of patients were found to be day laborer followed by 82(12.5%) patients who were unemployed and another 82(12.5%) patients were students. Both service holders and businessmen were found 81(12.3%) each, 33(5.0%) were garments worker and

14(2.1%) were drivers. Occupational data was not available for 25 patients.

Figure-3: Distribution of MDR/RR-TB patients by their occupation (n=657)



Most of the patients' (n=611, 93.0%) monthly family income was below 25,000 BDT and 138(21.0%) had monthly family income below 5,000 BDT where 201(30.6%) had monthly family income between 5,000 to 10,000 BDT and 272(41.4%) patients were found between 10,000 to 25,000 BDT. Only 7(1.1%) patients had monthly family income above 40,000 BDT and 14(2.1%) had between 25,000 to 40,000 BDT.

Table-2: Distribution of MDR/RR-TB patients by their monthly family income (n=657)

Monthly family income	Frequency	Percentage (%)
<5,000 BDT	138	21.0
5,000 to 10,000 BDT	201	30.6
10,000 to 25,000 BDT	272	41.4
25,000 to 40,000 BDT	14	2.1
>40,000 BDT	7	1.1
Not available	25	3.8

Only 548 patients' BMI could be calculated as height of 109 patients was not available. Among them, 232(35.3%) patients' BMI was found below 18.5 kg/m², 265(40.3%) patients had BMI within normal range, 23(3.5%) patients had BMI between 25.0 to 29.9 and were marked as overweight where 28(4.3%) patients had BMI over 30.0 kg/m² and were marked as obese.

Table-3: Distribution of MDR/RR-TB patients by their BMI (n=548)

BMI (Kg/m²)	Frequency	Percentage (%)
<18.5	232	35.3
18.5–24.9	265	40.3
25.0–29.9	23	3.5
>30.0	28	4.3

Among all the patients 67(10.2%) were new and 590(89.8%) were previously treated. Relapse after CAT-I treatment 216(32.9%) was found as the highest among previously treated patients followed by non-converter of CAT-I to be 175(26.6%) patients. Failure of CAT-I and CAT-II were 89(13.5%) and 21(3.2%) respectively.

Table-4: Distribution of MDR/RR-TB patients by TB treatment history (n=657)

TB treatment history		Frequency	Percentage (%)
New	New	67	10.2
Previously treated	CAT-I Relapse	216	32.9
	CAT-II Relapse	46	7.0
	CAT-I Failure	89	13.5
	CAT-II Failure	21	3.2
	CAT-I Non-converter	175	26.6
	CAT-II Non-converter	20	3.0
Treatment after lost to follow up	CAT-I	17	2.6
Treatment after lost to follow up	CAT-II	6	0.9

Among all MDR/RR-TB patients, 508(77.3%) were successfully treated and 149(22.7%) had unfavorable treatment outcomes: 75(11.4%) died, 11(1.7%) treatment failed, 54(8.2%) were lost to follow up and 9(1.4%) patients' outcome were not evaluated.

Beside the comorbidities, host characteristics and socio-demographic factors were also found to be associated with treatment outcomes. Risk of unfavorable treatment outcome increased with the age which was statistically found highly significant ($p<0.001$). Up to

34 years of age treatment success rate was more than 87% which declined to around 70% in the age group 25 to 54 years. Treatment success further declined to 57.7% in the age group of 55 to 64 years and was lowest (40.5%) among the patients above 65 years. Females were found to have better treatment success (81.7%) than the males (75.0%) but were not statistically significant. The risk ratio of unfavorable treatment outcome of male gender was 1.4 (95%CI 1.0-1.9). Treatment outcome of the patients were found significantly different among different occupations ($p < 0.001$). Unfavorable outcome was found highest (37.8%) among the unemployed followed by day laborer (30.5%) whereas, treatment success rate was highest among the students (92.7%) followed by businessman (84.0%). Monthly family income did not show any pattern to have any effect on treatment outcome.

Table-5: Sociodemographic predictors of treatment outcome among MDR/RR-TB patients

Characteristics	Favorable outcome	Unfavorable outcome	p-value	Risk ratio (95% CI)
Age (n=657)				
0-34	306 (87.7%)	43 (12.3%)	<0.001	
35-44	157 (71.7%)	62 (28.3%)		
55-64	30 (57.7%)	22 (42.3%)		
65 and above	15 (40.5%)	22 (59.5%)		
Gender (n=657)				
Male	321 (75.0%)	107 (25.0%)	0.052	1.4 (1.0-1.9)
Female	187 (81.7%)	42 (18.3%)		
Occupation (n=632)				
Service	63 (77.8%)	18 (22.2%)	<0.001	
Business	68 (84.0%)	13 (16.0%)		
Day laborer	66 (69.5%)	29 (30.5%)		
Student	76 (92.7%)	6 (7.3%)		
Driver	11 (78.6%)	3 (21.4%)		
Garments Worker	27 (81.8%)	6 (18.2%)		
House Wife	130 (79.3%)	34 (20.7%)		
Unemployed	51 (62.2%)	31 (37.8%)		
Monthly family income (n=632)				
<5,000 BDT	107 (77.5%)	31 (22.5%)	0.037	
5,000 to 10,000 BDT	163 (81.1%)	38 (18.9%)		
10,000 to 25,000 BDT	207 (76.1%)	65 (23.9%)		
25,000 BDT and above	18 (85.7%)	3 (14.3%)		

Life styles, TB treatment history and treatment adherence are also considered as a determinant of treatment outcomes. Of all the patients 321(48.9%) patients' smoking history could be retrieved where 130(40.5%) were smoker. Significant difference was seen in treatment outcome between smokers and non-smokers ($p=0.003$). Unfavorable outcome was 30.0% among the smokers whereas it was limited to 16.2% among the non-smokers. The relative risk of unfavorable outcome for smoking was 1.8 (95% CI 1.2-2.8). BMI also showed

significant difference in treatment outcome of MDR/RR-TB patients ($p=0.017$). BMI below

18.5 kg/m² had 25.4% unfavorable treatment outcome which reduced to 17.1% with increase of BMI to 18.5 kg/m² and above. Relative risk of an unfavorable outcome with low BMI was found 1.5 (95%CI 1.1-2.1). There was no significant difference observed in treatment outcomes between new and previously treated MDR/RR-TB patients ($p=0.952$). Highly significant ($p<0.001$) difference in treatment outcome was observed with treatment adherence. The MDR/RR-TB patients with no treatment interruption had treatment success of 82.9% and when the patients had treatment interruption of any duration, success sharply reduced to 28.4%. The relative risk of unfavorable treatment outcome among the patients with bad adherence was found 4.2 (95%CI 3.3-5.3).

Table-6: Effect of smoking, BMI, TB treatment history and treatment adherence to treatment outcome of MDR/RR-TB patients

Characteristics	Favorable outcome	Unfavorable outcome	p-value	Risk ratio (95% CI)
Smoking history (n=321)				
Smoker	91 (70.0%)	39 (30.0%)	0.003	1.8
Non-smoker	160 (83.8%)	31 (16.2%)		
Body Mass Index (n=548)				
<18.5 kg/m ²	173 (74.6%)	59 (25.4%)	0.017	1
≥18.5 kg/m ²	262 (82.9%)	54 (17.1%)		1.5
TB treatment history (n=657)				
New	52 (77.6%)	15 (22.4%)	0.952	(1.1-2.1)
Previously treated	456 (77.3%)	134 (22.7%)		
Treatment adherence (n=657)				
Interruption	19 (28.4%)	48 (71.6%)	<0.001	4.2 (3.3-5.3)
No interruption	489 (82.9%)	101 (17.1%)		

Treatment success rate was found to be lower (67.8%) with the presence of comorbidity where higher treatment success (80.1%) was observed among the patients without any comorbidities which was found statistically significant ($p=0.002$). It was also found that death, failure and lost to follow-up all increased among the patients who had one or more comorbidities. Among unfavorable treatment outcome, death was 19.5% and 9.1%, failure

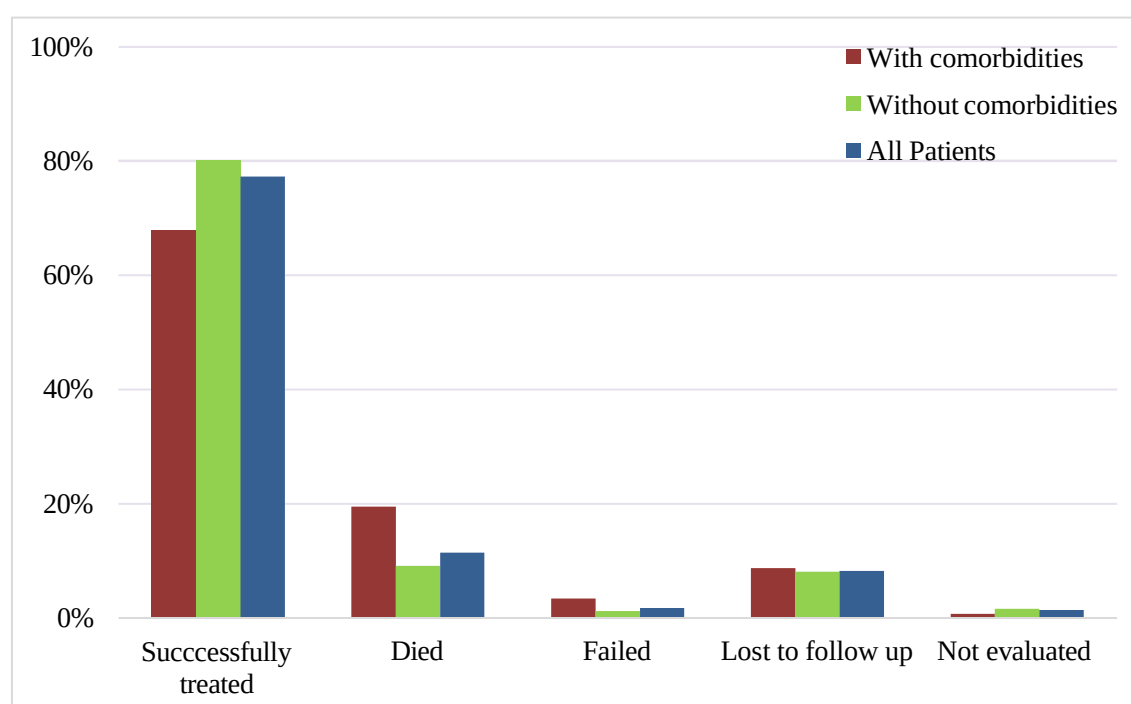
was 3.4% and 1.2%, lost to follow up was 8.7% and 8.1% for the patients with and without comorbidity respectively.

One hundred forty-nine (22.7%) patients had one or more comorbidity. Among them, 103(15.7%) patients had diabetes, 32(4.9%) COPD, 17(2.6%) hypothyroidism, 12(1.8%) chronic kidney disease and only 2 (0.003%) patients had chronic liver disease. Of all the patients 539(82.0%) patients had known HIV status and none of them were found HIV positive.

Table-7: Treatment outcome of MDR/RR-TB patients with and without comorbidities

Category of patients	Successfully treated	Unfavorable treatment outcome				p-value
		Died	Failed	Lost to follow up	Not evaluated	
With comorbidities	101 (67.8%)	29 (19.5%)	5 (3.4%)	13 (8.7%)	1 (0.7%)	0.002
Without comorbidities	407 (80.1%)	46 (9.1%)	6 (1.2%)	41 (8.1%)	8 (1.6%)	
Total	508 (77.3%)	75 (11.4%)	11 (1.7%)	54 (8.2%)	9 (1.4%)	

Figure-4: Treatment outcome of MDR/RR-TB patients with and without comorbidities



Lower treatment success rate was found among the patients with comorbidities, treatment success rate was 68.9% (p=0.027) among the MDR/RR-TB patients with diabetes, 65.6% (p=0.105) with COPD, 41.7% (p=0.003) with chronic kidney disease and 52.9% (p=0.033) for hypothyroidism. Risk ratio of unfavorable outcome with comorbidities was found 1.6 (95%CI 1.2-2.1). Except COPD other comorbidities showed significantly poor treatment outcome (p<0.05). Risk ratio of unfavorable treatment outcome among the patients with diabetes was found 1.5 (95%CI 1.1-2.5), COPD 1.6 (95%CI 0.9-2.6), chronic kidney disease 2.7 (95%CI 1.6-4.4) and for hypothyroidism 2.1 (95%CI 1.3-3.6)

Table-8: Treatment outcome of MDR/RR-TB patients with different comorbidities and risk ratio of unfavorable outcome (n=657)

Comorbidity	Status of comorbidity	Favorable outcome	Unfavorable outcome	p-value	Risk ratio (95% CI)
Diabetes	Yes	71 (68.9%)	32 (31.1%)	0.027 ^a	1.5 (1.1-2.1)
	No	437 (78.9%)	117 (21.1%)		
COPD	Yes	21 (65.6%)	11 (34.4%)	0.105 ^a	1.6 (0.9-2.6)
	No	487 (77.9%)	138 (22.1%)		
Chronic kidney disease	Yes	5 (41.7%)	7 (58.3%)	0.003 ^b	2.7 (1.6-4.4)
	No	503 (78.0%)	142 (22.0%)		
Hypothyroidism	Yes	9 (52.9%)	8 (47.1%)	0.033 ^b	2.1 (1.3-3.6)
	No	499 (78.0%)	141 (22.0%)		

^a = Pearson Chi-Square, ^b = Fisher's Exact Test

Of all the patients who had unfavorable outcomes, 11.4% of them died and death was evident among the patients with comorbidities. Highest proportion of death (33.3%) occurred among the patients who had chronic kidney disease followed by hypothyroidism (23.5%), COPD (21.9%) and diabetes (18.4%). Treatment failure occurred in 1.7% of the total patients but higher proportions were observed among different comorbid conditions. Patients with chronic kidney disease had highest (25.0%) treatment failure rate. There were no visible

differences in lost to follow-up among the patients with and without comorbidities, it was found in a higher side (17.6%) among the patients with hypothyroidism.

Table-9: Breakdown of unfavorable treatment outcomes of MDR/RR-TB patients with different comorbidities (n=657)

Comorbidity	Died	Failed	Lost to follow up	Not evaluated
Diabetes (n=103) (n=32)	19 (18.4%)	4 (3.9%)	8 (7.8%)	1 (1.0%)
COPD (n=12)	7 (21.9%)	2 (6.3%)	2 (6.3%)	0 (0.0%)
Chronic kidney disease (n=17)	4 (33.3%)	3 (25.0%)	0 (0.0%)	0 (0.0%)
Hypothyroidism	4 (23.5%)	1 (5.9%)	3 (17.6%)	0 (0.0%)

However, as there were only 2 (0.003%) patients who had chronic liver disease and both of them died and were not further analyzed due to small sample size. Beside this, no significant association was found between unfavorable treatment outcomes and abnormal chest X-ray

i.e. cavitory lesion, military TB or bilateral lung involvement.

6. Discussions:

In this retrospective study, complete information was extracted from MDR-TB registration documents and hospital records for a total of 657 registered MDR/RR-TB patients of five DR-TB treatment centers in Bangladesh in 2015. We found 508 (77.3%) patients were treated successfully which is a bit higher than those recommended (75%) by the World Health Organization Global Plan to Stop TB [26]. Better treatment success rate was achieved mainly due to a decrease of lost to follow-up (8.2%) than the previous cohorts [5]. This could be attributed to the free treatment, monthly nutritional support and transport allowance provided to patients and their incentives to their DOT providers and the establishment of links between patients, the PMDT unit and the nearest health centre. The consistent and reliable supply of drugs and patient tracing by field staff with strict DOT could be other reasons for the comparatively low lost to follow-up rate.

A higher mortality rate (11.4%) was observed in our study than reported elsewhere [2,21,27-28]. As in Peru, high mortality (53.2%) was observed among lost to follow-up cases of MDR-TB treatment [29]. A possible reason for the lower mortality rates in the aforementioned studies [2,21,27-28] might be the masking of deaths by high lost to follow-up rates.

MDR-TB associated morbidity occurs mainly in the economically productive age group [30]. In this study, 71.9% of the registered MDR/RR-TB patients fall in the age range between 15-

44 years, the most productive age group. This may pose challenges to the social and economic development of the community in the area and the nation at large. In line with our findings, older age has previously been reported to have of poor treatment outcomes in MDR/RR-TB patients [31]. Treatment success rate was as low as 40.5% among the patients above 65 years of age in the current study. In Turkey, Babalik et al. found that the treatment success rate was lower in patients 65 years of age and older [32]. Several studies have demonstrated an association between advance age and unsuccessful treatment outcomes. It is assumed to be related to increased comorbidity, overall physiological deterioration, and difficulty in accessing several healthcare opportunities along with increasing age [12,32].

Most of the patients (65.1%) in this study cohort were male, this could be due to underutilization of the DOTS service by females or higher proportion of males being exposed to the infection in the area. A study conducted in Bangladesh on access to TB diagnosis and treatment also documented that women have poorer access to public outpatient clinics than men [33]. In our study, being male was associated with a higher risk of an unfavorable outcome. Similar result was also observed in other studies like one in South Africa [34-35].

Unemployment was identified was found as one of the factors that leads to unfavorable treatment outcomes in this study. Day laborer also had poor treatment outcomes whereas, treatment success rate was highest among the students followed by businessman. The effect of occupation on treatment outcome may be linked with educational status. As it was a retrospective study, educational status of the patients could not be retrieved. Several studies showed that various social and economic characteristics (lower education level, low income level, unemployment and family size) have been found to be associated with negative treatment results [12,36-37]. However,

in the current study monthly family income did not show any influence on treatment outcomes. This finding may be attributed to the free of cost treatment and successful implementation of the DOT for each socioeconomic group.

As smoking history is not included in the MDR-TB treatment card, the information is not routinely documented. However, of all the patients smoking history of 321(48.9%) patients' data could be retrieved from treatment file where 130(40.5%) were smoker. In this study smoking was significantly associated with poor treatment outcome with 1.8 times higher risk of poor treatment outcome. Similar result was observed in other studies in different settings [12,35,45].

In the current study, as elsewhere [2,12] a significantly poor treatment outcomes in patients with low baseline body mass index (BMI) was observed. BMI alone is an objective measure of underweight; however, as height was not measured at the study site for all the cohort of patients, we calculated BMI of 548 (83%) patients. The association between poor outcome with BMI is not unexpected. Body mass index, as a symptom of severe disease and low socioeconomic status requires aggressive intervention [2]. To our knowledge, there are no comparative studies examining the optimal nutritional intervention in an MDR-TB population. Moreover, there are no evidence-based guidelines on nutritional supplementation in adults or children [38].

It is well known that previous TB is the strongest risk factor for being ill with MDR-TB [39]. Therefore, special attention has to be paid to improving the treatment adherence of re- treatment cases. Previous studies confirm that previous anti-TB treatment significantly increases the risk of poor treatment outcome in MDR-TB [40]. But this study did not find any difference in treatment outcomes between new and previously treated cases. This might be due to strong community-based DOT and programme monitoring for all the DR-TB patients. However, treatment adherence was found to be a significantly associated with treatment outcomes. The MDR/RR-TB patients who had treatment interruption of any duration showed 4.2 times higher risk of poor treatment outcome and similar result was observed in a study in South Africa [34].

The presence of one or more comorbidities lead to significantly poor treatment outcomes ($p=0.002$) in the current study cohort. Diabetes mellitus, the most prevalent comorbidity (15.6%) in our study, has previously been reported as a comorbidity with poor treatment outcomes in MDR/RR-TB patients [12,41]. This can be due to a number of reasons, including immunological dysfunction and possible non-adherence to anti-tuberculosis treatment due to the complex medication schedule [42]. A recent meta-analysis on the impact of diabetes on TB treatment outcome reported that diabetic patients have a risk ratio (RR) for the poor treatment outcome of 1.69 (95% CI, 1.36 to 2.12) among the 23 unadjusted studies which is almost similar to our current study, and 4.95 (95% CI, 2.69 to 9.10) among the 4 studies that adjusted for age and other potential confounding factors [39-40,43-44]. A recent meta-analysis also showed the similar findings [45]. In the current study, information of HbA1C was not available as it is not indicated as baseline and monitoring investigation in the National PMDT guidelines [25]. Few more patients were identified in this cohort with high random blood glucose but could not be concluded as diabetes due to further evidence and eventually excluded from diabetes group.

A multi centre study in China showed poor treatment outcome among MDR-TB patients with COPD [12]. Similar findings observed in our study with 1.6 times higher risk of unfavorable outcomes and high proportion of deaths.

Chronic Kidney Disease (CKD) was found to be associated with significantly poor treatment outcomes with 2.7 folds higher risk in our study. A recent meta-analysis described CKD to be associated with worse outcomes but also mentioned that analysis of primary and secondary outcomes was not possible due to insufficient data [45]. We also suffered to find sufficient study to come to conclusion. The data in the current study might be affected with small sample size and further study is recommended for proper assessment.

Hypothyroidism is well documented as an adverse event of MDR-TB treatment especially with para amino salicylic acid (PAS) and ethionamide [25]. But effect of hypothyroidism in MDR-TB treatment outcomes was not found after extensive literature review. However, concomitant baseline hypothyroidism with MDR/RR-TB was found to be associated with poor treatment outcomes in this study though the data might have chance to be affected by small sample size.

This study also found higher proportion of deaths in this group. Future largescale studies might help to establish possible association and effect of hypothyroidism on MDR-TB treatment outcomes.

Due to insufficient data MDR/RR-TB patients with chronic liver disease could not be analyzed in this study but some studies identified poor treatment success among the MDR/RR-TB patients with liver disease [6].

7. Limitations of the study:

The strength of the present study is its capacity to provide representative country-wide data: all diagnosed MDR/RR-TB patients who started second line treatment were included. This pertains also to laboratory data. However, there were several limitations. Owing to the retrospective nature of the study, we were only able to analyze the available data within treatment card and hospital records where under reporting as well reporting bias is possible.

Some important socio-demographic information and data on alcohol consumption, drug abuse and psychiatric condition were missing, thus these variables were excluded from the analyses. As there is no option to record smoking history in the DR-TB treatment card, this data was not routinely documented but 321(48.9%) patients' smoking history could be retrieved from treatment file. Another significant limitation is the lack of HIV positive patients. In total 539 of 657 patients (82%) patients had known HIV status but none of them was positive and could not be analyzed. As during the study period, the outcome of shorter MDR-TB regimen was not available it could not be assessed.

8. Conclusion:

Our cohort study revealed that comorbidities had serious impact on treatment outcomes and were associated with significant lower successful treatment outcomes and higher death rate. Older age, male gender, unemployment, smoking, low BMI during treatment initiation and treatment interruption was found as predicting factors for poor treatment outcomes. Comorbid conditions like Diabetes, COPD, CKD and hypothyroidism were found to lead to poor treatment outcomes. Special care and measures should be taken to improve quality of care of the drug resistant TB patients with risk factors and comorbidities.

The current results indicate that special attention should be paid and measures should be taken to improve quality of care of the drug resistant TB patients with risk factors and comorbidities in order to improve adherence and treatment results. The addition of more comprehensive treatment strategies may help improve outcomes. But, an evidence-based approach, in addition to comprehensive, individualized therapy, is necessary to contain MDR-TB. Without high quality evidence to support treatment decisions, including data from high-risk populations, high mortality, lost to follow-up, and failure rates will persist. Interventions are required to improve adherence and reduce the high number of unfavorable DR-TB treatment outcomes. Interventions such as social and financial support and nutritional supplementation should be optimized as well as shorter, more effective treatment should be considered.

Further studies should be conducted for better understanding of MDR-TB treatment outcomes with different comorbidities using individualized regimen as well as shorter MDR- TB regimen.

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13. Annexures:

Data Collection Tool

Code
No:

Date: ____/____/____

Title of the study: Comparative evaluation of treatment outcome for MDR/RR-TB with and without comorbidity: A retrospective analysis in Bangladesh

1. Name of the initial treatment centre:

☐ NIDCH	=1
☐ Chattogram CDH	=2
☐ Khulna CDH	=3
☐ Pabna CDH	=4
☐ Sylhet CDH	=5

2. DR-TB registration number: _____

3. eTB manager number: _____

4. Date of DR-TB registration: ____/____/____

5. Date of DR-TB treatment started: ____/____/____

6. Age of the patient (in Year)

7. Gender of the patient:

☐ Male	=1
☐ Female	=2

8. Residing district of the patient: _____

9. Current occupation of the patient:

☐ Service	= 1
☐ Business	= 2
☐ Day Laborer	= 3
☐ Student	= 4
☐ Driver	= 5
☐ Garments Worker	= 6
☐ House Wife	= 7
☐ Unemployed	= 8
☐ Others	= 9[(Specify)_____]
☐ Not available	= 99

10. Patient's monthly family income:

☐ <5,000 BDT	= 1
☐ 5,000 to 10,000 BDT	= 2
☐ 10,000 to 25,000 BDT	= 3
☐ 25,000 to 40,000 BDT	= 4

- ☐ >40,000 BDT = 5
☐ Not available = 9

11. Registration group of the patient:

- ☐ CAT-I Non-converter = 1
☐ CAT-I Failure = 2
☐ Treatment after lost to follow up CAT-I = 3
☐ CAT-I Relapse = 4
☐ CAT-II Non-converter = 5
☐ CAT-II Failure = 6
☐ Treatment after lost to follow up CAT-II = 7
☐ CAT-II Relapse = 8
☐ Close contact of DR-TB = 9
☐ Pulmonary (New) = 10
☐ Unknown = 99

12. Previous tuberculosis treatment history:

- ☐ Treated once for DS-TB = 1
☐ Treated twice for DS-TB = 2
☐ Treated more than twice for DS-TB = 3
☐ Treated for DR-TB = 4
☐ Unknown = 9

13. Initial weight of the patient (in kg):

14. Initial height of the patient (in cm):

15. Drugs used in the DR-TB regimen: (Tick multiple)

Z	Km	Lfx	Eto	Cs	Cm	PAS	Cfz	Amx/Clv	Trd	Lzn	Mfx
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

16. Month of first Negative culture:

- ☐ Baseline = 1
☐ Month-1 = 2
☐ Month-2 = 3
☐ Month-3 = 4
☐ Month-4 = 5
☐ After month-4 = 6
☐ Not available = 9

17. Was there any Bacteriological Reversion (Negative to Positive)?

- ☐ Yes = 1
☐ No = 2

18. Duration of hospital stay (in days):

19. Duration of intensive phase (in months):

20. Duration of continuation phase (in months):

21. Interruption of DR-TB treatment:

- ☐ None = 1
☐ <7 days = 2
☐ 7 to 15 days = 3
☐ 15 to 30 days = 4
☐ >30 days = 5

22. HIV status of the patient:

- ☐ HIV positive = 1
☐ HIV negative = 2
☐ Unknown = 9

23. Smoking history of the patient:

- ☐ Smoker = 1
☐ Non-smoker = 2
☐ Not available = 9

24. Any known (documented) comorbid conditions: (Tick multiple, if applicable)

- ☐ Diabetes
☐ COPD
☐ Bronchial Asthma
☐ Chronic Liver Disease
☐ Chronic Kidney Disease
☐ Heart Disease
☐ Hypothyroidism
☐ Other [(Specify) _____]

25. Findings of the Baseline Chest X-ray: (Tick multiple, if applicable; keep blank if not available)

- ☐ Normal Chest X-ray
☐ Cavitary Lesion
☐ Consolidation
☐ Miliary TB

- ☐ Pleural Effusion
- ☐ Other [(Specify)_____]

26. Results of Baseline investigations:

Hb (g/dl)	
ESR	
Blood Glucose (mmol/L)	
HbA1C	
Serum Bilirubin (mg/dl)	
SGPT	
Alkaline Phosphatase	
Serum Creatinine (mg/dl)	
TSH	

27. Final outcome of the patient:

- ☐ Cured = 1
- ☐ Treatment Completed = 2
- ☐ Died = 3
- ☐ Failed = 4
- ☐ Lost to Follow up = 5
- ☐ Transferred out = 6

For any query and clarification:

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Government of the People's Republic of Bangladesh Office
of the Director MBDC and Line Director TBL&ASP
Directorate General of Health Services
Mohakhali, Dhaka-1212

Memo no. 5-66/TB-Lep/NTP-Research/2017-2018/I1'64

Date: 05-07-2018

Dr. Nazis Arefin Saki

Medical Officer and

Focal person for Drug-resistant TB, TB/HIV and TB-IC National
Tuberculosis Control Programme

DGHS, Mohakhali, Dhaka-1212

Subject: National TB Research Committee Approval.

With reference to your application as Principal Investigator on the above mentioned subject, this is to inform you that your Research Proposal entitled "**Comparative evaluation of treatment outcome for MDR/RR-TB with and without comorbidity: A retrospective analysis in Bangladesh**" has been reviewed and approved by the National TB Research Committee in its meeting held on 02 July 2018.

You are requested to submit the final report to the National TB Research Committee after completion of the research.



Prof. (Dr.) Md.-Shamiul Islam

Prof. (Dr.) Md.-Shamiul Islam

Director MBDC and Line Director TBL&ASP and
Chairperson, National TB Research Committee
DGHS, Mohakhali, Dhaka-1212

Timeline:

Sl no.	Task	April 2018				May 2018				June 2018				July 2018				August 2018				September 2018			
1	Literature review																								
2	Draft proposal																								
3	Meeting with National TB research committee (extended)																								
4	Feedback and revised proposal																								
5	Development of data collection tool																								
6	Pretest and finalize data collection tool																								
7	Approval from TB research committee																								
8	Recruit/ hire survey staff and training																								
9	Draft and test electronic template																								
10	Data collection																								
11	Data input and cleaning																								
12	Data analysis																								
13	Report writing																								
14	Dissemination																								



Identification of Risk factors of Extra-pulmonary Tuberculosis in Bhutan **2018**

Identification of Risk Factors of Extra pulmonary Tuberculosis in Bhutan, 2018

A Case-Control Study

Jointly conducted by the NTCP, DoPH, Ministry of Health, Bhutan & STAC with



The funding support of the SAARC Tuberculosis & HIV/AIDS Centre (STAC) Kathmandu, Nepal.



Afghanistan



Bangladesh



Bhutan



India



Maldives



Nepal



Pakistan



Sri Lanka



SAARC Tuberculosis and HIV/AIDS Centre (STAC)

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1. Research project title

Identification of Risk Factors of Extrapulmonary Tuberculosis in Bhutan, 2018.

2. Summary

Tuberculosis (TB) is primarily a diseases of lungs caused by bacteria called the *Mycobacterium tuberculosis* in humans. But it can also cause TB of other body sites besides lungs such as the pleural, lymphatic, genito-urinary, bone-joints, meningeal, peritoneal, gastrointestinal, cutaneous, miliary, larynx and others like epididymis, ocular etc. These variety of TB is called as “Extrapulmonary tuberculosis” or EPTB. Tuberculosis is preventable and treatable disease. The four forms of TB known to mankind are drug sensitive Pulmonary TB (PTB) and EPTB, the deadly Multidrug Resistant TB (MDR-TB) and the emerging XDR-TB. Extrapulmonary tuberculosis although less discussed and seemingly given less priority in the public health care sphere is however diagnosed at a higher proportion compared to the PTB and MDR-TB. Hence, this has triggered huge economic burden in terms of treatment expenditures and caused an alarm among the public health policy makes in the country and beyond.

TB is spread from person to person through droplet born infection through the medium of air. When patient with pulmonary TB coughs, sneezes or spit, they expel the TB germs into the air and hence spread from person to person. It is estimated that people infected with TB bacteria have 5-15% lifetime risk of falling ill with TB. However, persons with compromised immune systems, such as people living with HIV/AIDS, malnutrition, diabetes, on steroids or people who use tobacco and alcohol have a much higher risk of falling ill with TB.

In Bhutan, over the last one and a half decade, there has been rapid increase in proportion of EPTB being diagnosed and treated at respective health centres. Although one study suggests that from 2001 to 2010 the proportion of all TB cases registered as EPTB over a period of 10 years varied from 30% to 40%, there has been no identified risk factor or proper epidemiology described for EPTB in Bhutan. Therefore, the current research proposal was devoted to

identify the risk factors of EPTB and also describe its epidemiology in Bhutan by sampling patients diagnosed as EPTB as cases and PTB as control (Case-control study) from 1st April 2018 till the sample size was achieved during data collection period (September 2018).

3. Problems the project addresses

The project titled Identification of Risk Factor of Extrapulmonary Tuberculosis (EPTB) in Bhutan, 2018 was aimed to firstly identify risk factors associated with EPTB diagnosed patients in Bhutan for the first time ever, and to present the descriptive epidemiology of PTB and EPTB including its burden. It is also expected that through this research activity, we can estimate the expenditure incurred on treatment of EPTB cases for drugs alone or in combination of other associated costs. As the diagnostic modalities varies from diagnosing TB based on bacteriologically confirmed, on clinical grounds, using X-ray or ultrasound, examination of body fluids (e.g., pleural or cerebrospinal fluid), histopathology when available and circumstantial evidence, this research was aimed to standardise diagnostic procedure and classification of EPTB in Bhutan. The outcomes of this study is also expected to produce teaching materials on EPTB and help the National Tuberculosis Control Program (NTCP) in achieving its goals and targets for detection, management and successful treatment outcomes of tuberculosis in general. The spatio-temporal distribution and burden estimation will help us allocate resources across the country while the identification of risk factors will guide us at targeted tuberculosis intervention programs.

4. Background and justification

According to the World Health Organization (WHO), tuberculosis falls in one of the top 10 causes of global death. About 10.4 million people has fallen sick with TB, and 1.7 million have died from TB (including 0.4 million among people with HIV) in 2016 with over 95% of TB deaths occurring in low and middle income counters (WHO, 2017b). The End TB Strategy was endorsed by WHO's

194 Member State during the 2014 World Health Assembly, that is for the period from 2016- 2035. The Social Developments Goals (SDGs) were adopted by the UN Member States in September 2015, that is for the period 2016-2030. Both the SDGs and End TB Strategy share a common goal: to end the global TB epidemic. The End TB Strategy targets to reduce 90% in TB deaths and aims to reduce 80% in TB incidence by 2030, compared to 2015 (WHO, 2017a). Tuberculosis which is an infectious disease caused by the bacillus *Mycobacterium tuberculosis* typically affects lungs (Pulmonary TB) and it can also affect other body sites (Extrapulmonary TB). The disease is spread through respiratory droplet infection when the in ⁷³ patients coughs, sneezes or spits. It is estimated that a small proportion (5-15%) of the estimated 1.7 billion people infected with *M. tuberculosis* could develop TB during their lifetime. In general, the probability of developing TB is higher among people affected with HIV/AIDS, under-nutrition, diabetes, smoking and alcohol consumption (WHO, 2017a). Diagnosis is achieved by rapid molecular tests, sputum smear microscopy and culture-based methods as confirmatory tests, besides the clinical history and presentation.

The National Tuberculosis Control Programme (NTCP) in the Ministry of Health in Bhutan was established in 1986 and the WHO universally recommended DOTS TB treatment was introduced in 1997. In 2001 the proportion of EPTB of all the TB patients was 26%. In 2010 the proportion of EPTB has increased to 39 % and an alarming increase was seen by the end of 2016, wherein almost half (49%, *Figure 1*) of all TB cases registered were classified as EPTB (Jamtsho et al., 2013; NTCP, 2016).

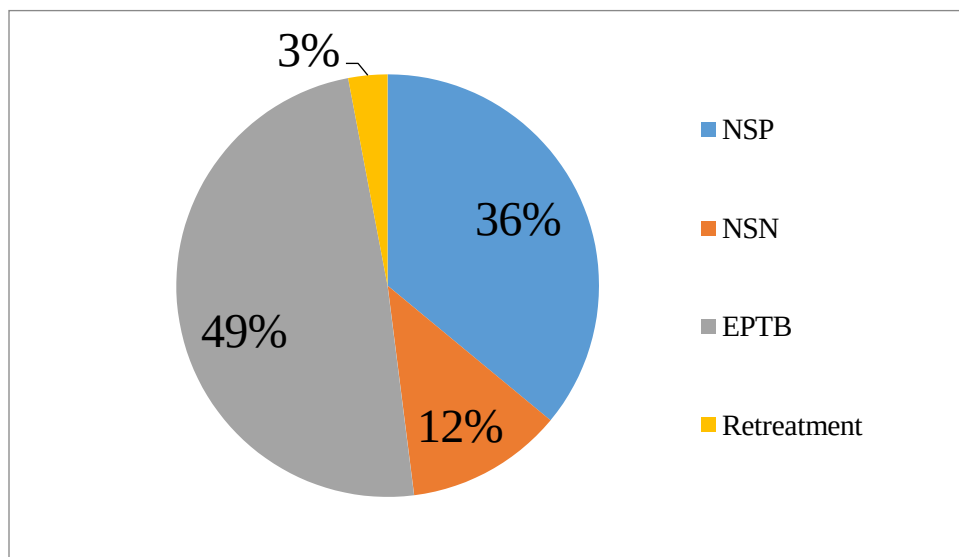


Figure 1. Notified TB cases by classification, 2016 (Source:(NTCP, 2016))

Although TB incidence and prevalence is on the decline compared to 1990s (*Figure 2*), it's found that the highest affected are in the most productive age group. All forms of TB and MDR-TB have been found most commonly affecting the age range of 15 to 34 years in Bhutan. The number of suspected EPTB tested, bacteriologically confirmed EPTB cases and proportion of MDR-TB among the EPTB presented in (*Table 1*) shows that a good percentage of EPTB (15% in 2014 and 8% in 2015) have given ⁷⁴ to MDR-TB.

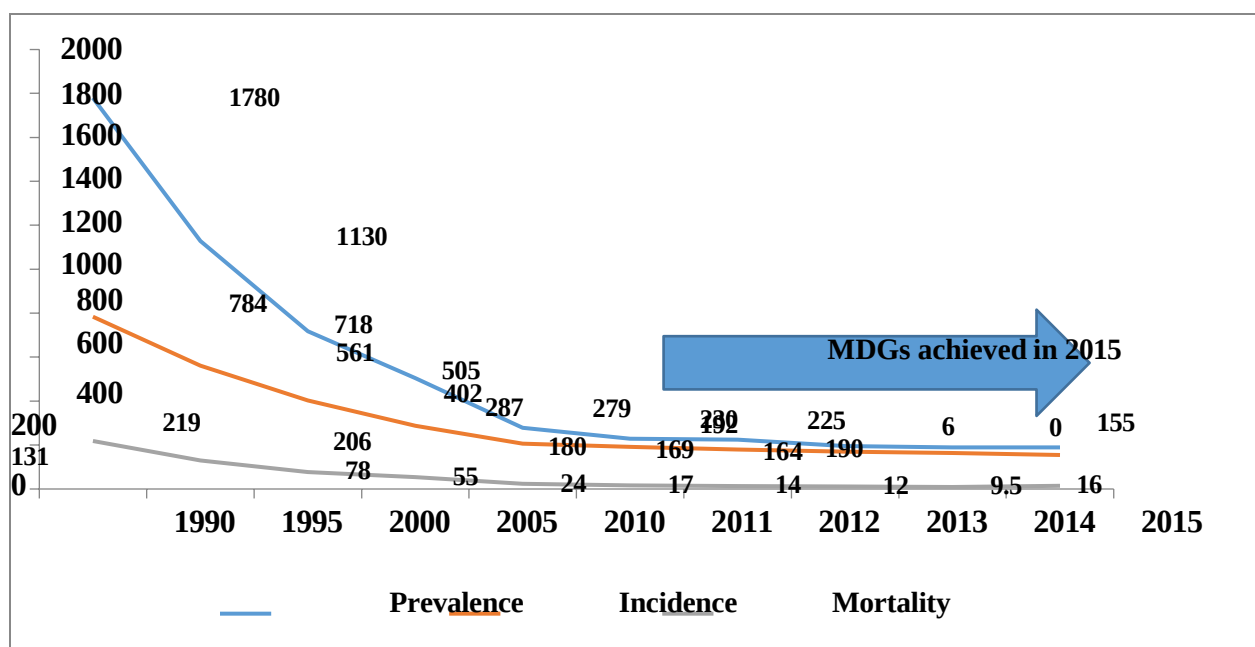


Figure 2. Estimate of all forms of TB burden from 1990 to 2015 (NTCP, 2016).

Table 1: Laboratory confirmation of EPTB cases (NTCP, 2016).

Activity	2014	2015	2016
No. of EPTB suspected cases tested	411	690	598
No. of EPTB bacteriologically confirmed	59 (14%)	97 (14%)	66 (11%)
No. of MDR-TB in EPTB confirmed	9 (15%)	8 (8%)	8 (9%)

Table 2: National level TB cases compilation for 2015-2017 (NTCP, 2016).

Classification	2015	2016	2017
Pulmonary positive (PBC)	453 (46.46%)	448 (39.12%)	449 (50.96%)
Pulmonary negative (PCD)	55 (05.64%)	137 (11.97)	73 (8.29%)
EPTB (BC & CD)	467 (47.90%)	560 (48.91%)	359 (40.75%)
Total	975 (100%)	1145 (100%)	881 (100%)

It is evident from (Table 2) that EPTB cases are on the rise and constitute the highest proportion amongst all the cases, which call for further research to know the risk factors and describe disease

epidemiology. However, by the end of 2017, the percentage of EPTB diagnosed has dropped to about 41%, compared to preceding two years, showing a decreasing trend.

Researchers around the globe has studied risk factors of extrapulmonary TB and they have documented various parameters attributed to EPTB. The risk factors of EPTB has been related to age, sex, occupation, comorbidity conditions like diabetes, cirrhosis and neoplasia, use of immunosuppressive agents, past history of pulmonary TB, smoking habits, previous alcohol use, history of contact with PTB patients, Bacillus Calmette Guerin (BCG) vaccination status, HIV status, injection drug use etc.

In a study by García-Rodríguez et al. (2011), female sex (OR=2.05) and younger age (OR=1.02) has been found as the risk factors of EPTB patients when they were compared with the PTB patients. In the US, women (OR, 1.98; 95% CI, 1.25–3.13), non-Hispanic blacks (OR, 2.38; 95% CI, 1.42–3.97), and HIV-positive persons (OR, 4.93; 95% CI, 1.95–12.46) had significantly higher risk for extrapulmonary tuberculosis than men, non-Hispanic whites, and HIV-negative persons (Yang et al., 2004). In Nepal, younger age group (<25 years) and female gender were identified as independent risk factors of EPTB. The authors have also suggested that smoking, use of immunosuppressive drug or steroids, diabetes and past history of TB were more likely to be associated with PTB (Sreeramareddy, Panduru, Verma, Joshi, & Bates, 2008). In Thailand, a multicentre observational cohort study was performed by Kingkaew et al. (2009) and in their proportional hazards model, patients with extrapulmonary TB had an increased risk of death if they had meningitis, and a CD4+ T-lymphocyte count <200 cells/ml.

Extrapulmonary TB can occur alone or along with a primary focus in the lungs. The HIV/AIDS pandemic has resulted in changing epidemiology and has once again brought EPTB into limelight. In the majority of the cases, lymph nodes are the most common site of involvement followed by pleural effusion but it can virtually affect every site of the body. As the clinical presentation of EPTB is not typical like the PTB, the conventional diagnostic methods have

faced with difficulties in confirmation and oftentimes diagnosis have been delayed. The availability of computerised tomographic scan (CT-scan), magnetic resonance imaging (MRI), laparoscopy, endoscopy and aspiration biopsy have tremendously helped in anatomical localisation and sampling of EPTB sites in diagnostic confirmation (Sharma & Mohan, 2004).

As it's evident from *Figure 1* and *Table 1*, EPTB occupying about half of the tuberculosis infection proportion in Bhutan is a cause of concern and at the same time definitely a huge economic burden for a small developing country like ours. Hence, this draws us to strain our nerves a bit more as it certainly merits further research as proposed herewith, to identify the risk factors and then further improve and standardise diagnostic modalities and to make recommendations for good patient care and successful treatment outcome as well.

In Bhutan we have a total 32 TB treatment centres with at least one each for a district and some bigger districts like Chukha, Samtse and Trashigang have two to three TB treatment centres (NTCP, 2016).

5. Project Objectives

5.1. Broad

This proposed study was aimed to identify the risk factors of EPTB in Bhutan and describe its epidemiology to further direct the NTCP to embark on strategic prevention and control programmes whilst the specific objectives set herewith in conjunction with this project is attained thereof.

5.2. Specific

- i. To identify the risk factor of EPTB by recruiting cases diagnosed as EPTB/PTB from 1st April 2018 till the sample size was achieved during data collection period (September 2018) in Bhutan.
- i. To describe the epidemiology EPTB (cases) and PTB (controls) diagnosed from 1st April to September 2018 in Bhutan.
- ii. To describe the diagnostic modalities used to classify study EPTB cases and report any inconsistency in classification.

6. Intended Outcomes

- i. The risk factors for EPTB with target patients across all the 20 districts of Bhutan will be determined.

- ii. Will be able to provide more accurate diagnostic modalities for EPTB as per the availability of diagnostic facilities.
- iii. Make recommendations for practical, cost-effective and targeted prevention and control measures to reduce risk of EPTB in people in Bhutan.
- iv. Estimate the overall expenditure of an individual patient treatment and national estimate as per the disease burden.

7. Materials and Methods

7.1. Study description

We conducted a case-control study to achieve the objectives of this research project.

7.1.1.Type of study Case-control study:

We conducted a case-control study by recruiting EPTB as cases and PTB as controls among the cases diagnosed as EPTB and PTB from 1st April 2018 till the sample size was achieved during data collection period (September 2018).

7.1.2. Epidemiological unit of interest

The epidemiological unit for case-control study were the individual patient diagnosed as EPTB and PTB cases and being treated or receiving treatment at the district TB treatment centres. Both cases and controls were selected amongst the TB treatment centres as per the case and control definitions given below.

As per the National Guidelines for Management of Tuberculosis 2016, EPTB and PTB is defined as follows;

PTB: Any bacteriologically confirmed or clinically diagnosed case of TB that involves the lung parenchyma or the trachea-bronchial tree with or without the involvement of any other organs in the body. Miliary TB is classified as PTB because there are lesions in the lungs.

EPTB: Any bacteriologically confirmed or clinically diagnosed cases of TB involving organs other than the lungs, e.g. pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, meninges, tuberculous intrathoracic lymphadenopathy (mediastinal and/or hilar) or tuberculosis pleural effusion, without radiographic abnormalities in the lung parenchyma constitutes a case of EPTB.

However, in case of pleural involvement and/or lymph node involvement, involvement of the lungs should be ruled out. As already stated, in case of lung involvement, even in the absence of symptoms, the case is classified as a pulmonary TB Case.

A patient with both pulmonary and EPTB should be classified as a case of PTB (MoH, 2016).

Case and control definition of our study:

- **Case definition:** A case of EPTB was defined as a patient of any age diagnosed as having extrapulmonary tuberculosis at the hospital/TB treatment centre in Bhutan based on **bacteriological confirmation** by sputum smear, culture, GeneXpert or PLA.
- **Control definition:** A control was defined as a case of pulmonary tuberculosis (PTB) diagnosed in Bhutan based on **bacteriological confirmation** by sputum smear, culture, GeneXpert or PLA, besides the typical clinical features of PTB and X-rays.

All EPTB cases and PTB controls, **bacteriological confirmed** were recruited from among those under treatment from 1st April to October 2018 or until the required sample size was achieved. The case and control definitions and their selections in our proposed study was placed based upon the previous similar study conducted by Yang et al. (2004) to identify the risk factor of EPTB in the USA.

7.2. Study sites

The case-control study to identify the risk factor of EPTB was conducted across 31 (out of 32) TB Treatment Centres of the 20 districts in Bhutan. The sample size calculation is detailed in the following specific section.

7.3. Study period

The case-control study project was conducted from April to September 2018. Data collection period was scheduled in the months September 2018.

7.4. Sampling strategy

7.4.1. Target population

The whole of the Bhutanese people is the target population for this study project. As the study was conducted nationwide with samples across all the 20 districts and all 32 TB Treatment Centres, the findings can be extrapolated to the whole of the Bhutanese population.

7.4.2. Source population

All the EPTB and PTB cases registered and under treatment from 1st April to October 2018 at the 32 TB Treatment Centres formed the source population for this case-control study.

7.4.3. Study population

7.4.3.1. TB Treatment Centres

<i>Selection method:</i>	For this case-control study, we selected all the TB centres in 20 districts.
<i>Inclusion criteria:</i>	All the TB centres that have both the EPTB cases and PTB controls under treatment and easily accessible for interview across Bhutan.
<i>Exclusion criteria:</i>	Gasa district located in the Greater Himalayas has not reported any form of TB. We excluded Gasa TB Treatment Centre.
<i>Number to sample:</i>	31 TB Treatment Centres as ultimate study site sample.

7.4.3.2. Individuals

Selection method:	Cases and controls meeting the case and control definitions will be selected as per the inclusion and exclusion criteria.
Inclusion criteria:	All diagnosed cases and controls (EPTB and PTB) from 1 st April to September 2018 within the data collection time frame will be recruited for the study.
Exclusion criteria:	Cases and controls diagnosed outside Bhutan, MDR-TB or XDR-TB cases were excluded. Clinically diagnosed EPTB cases and PTB controls.
Type of samples:	Individual EPTB cases and PTB controls were sampled.
Number to sample:	By using an estimated exposure risk factor prevalence of 20% in the exposed (target) population and $n = 80$ to detect an odds ratio (OR) of at least 2.5 at 95% confidence interval and 80% power of detection, a sample size of 69 cases and 138 controls were required for an unmatched case-control study, cases to control ratio of 1:2). We have used EpiInfo 7 STATCALC for case-control sample size estimation. To make up for non-response, drop out cases or missing information, we have rounded up the sample size to <u>80 cases and 160 controls</u> for the study (20% increase). The screenshot of sample size estimation is attached herewith for your reference (<i>Annexure 01</i>). For the sample size estimation we have taken the average odds ratio (approximately 2.5) of the significant risk factors (related to female sex, age, race and HIV) observed in the similar studies by García-Rodríguez et al. (2011); (Sreeramareddy et al., 2008; Yang et al., 2004).

8. Data collection

8.1.1. Survey or study questionnaire

8.1.1.1. Individual EPTB and PTB patients.

What data will be collected (Survey form or questionnaire enclosed separately)

Demographical, clinical and epidemiological data, diagnostic history, exposure history to assess environmental and other risk factors data were collected from individual case and control patients. Questionnaire form is enclosed separately as EPTB_Case-Control_Questionnaire_Version_04.

Who will design the survey form or questionnaire and how will this be tested

The research team led by the Principal Investigator have designed the questionnaire, and will be reviewed by the approving authority as appropriate. The questionnaire was pre-tested at JDWNRH, Gidakom, Paro, Wangdue, CRRH Gelephu, Phuentsholing hospitals and their

feedback have been incorporated before the actual data collection in the field, once approved the REBH.

How will the data be collected (include any consents required)

Data was collected by interviewing the individual cases and controls after they have consented voluntarily to participate in the study. Both the cases and controls were interviewed by the selected and trained TB In-charges/Nurses/other competent health workers and even by the investigators wherever feasible. The cases and controls were interviewed when they came to the centres for their monthly refill, but given the short duration of data collection, interviewers travelled to where the selected cases and control resided and questionnaire administered after they have agreed and consented to participate in the study.

Who will collect and record the data (include qualification and skills required)

The TB In-charges, Health Assistants and Nurses have collected and recorded the data in hard copy at the field level. Later, the data was transferred to data-base designed in EpiInfo version 7 managed by the Bhutan EPTB study team.

Training, mentoring and supervision

Training on interviewing of cases and controls were provided to the TB In-charges/Data collector. The investigators and supervisors visited the data collection places for data completeness and verification and collection of final data from the field.

List possible sources of risk and how these will be mitigated

As there were no biological sample collection involved in this study, our proposed study was a non-invasive procedural research project and a questionnaire based research study. The possible risk of contracting tuberculosis infection by the interviews were mitigated by using universal precautions like wearing surgical or N-95 masks by both the patients and the interviewers, keeping a safe distance of one meter from the interviewers, interviewing in a well ventilated room and maintain privacy of the interviewee at all times by interviewing them in a TB Treatment Centre Room or in a VCT room in the hospitals after their consent.

9. Data management and analysis

9.1.1. Database / data recording

Data collected from the field was entered, stored, processed and managed in EpiInfo 7 (CDC, Atlanta). Data was entered by trained data punchers from the Ministry of Health. Double cross- checking method was used to check for errors both at the field and after entering the data into the data management system. Data base was built in EpiInfo 7 by the Principal Investigator and other expert in this field.

9.1.2. Data analysis and interpretation

A descriptive statistics was used to analyse the variables related to clinical profile of cases and variables related to demography and risk factors of cases and controls. A univariable association between an outcome and a risk factor was analysed using chi-square test. A multivariable logistic regression model was used to estimate the adjusted effect of an individual risk factors. A liberal p-value of ≤ 0.2 was used for selection of a variable into the multivariable logistic regression model. A 5% ($p=0.05$) significant level was chosen to identify significant risk factors for chi-square test and to retain the variable in the final logistic regression model. For all analyses STATA version 14 (Stata Corp, USA) was used.

10. Results

10.1. Descriptive statistics

10.1.1. Socio-demographic Characteristics

The study participants included 73 (66.36%) females and 37 (33.64%) males in EPTB

(Extrapulmonary Tuberculosis) case group and 128 (54.47%) females and 107 (45.53%) males in PTB (Pulmonary Tuberculosis) control group. The median age of cases and controls were 25 years and 29 years, which ranged 9-79 years and 13-84 years respectively. The mean (SD) BMI at the time of diagnosis and start of TB treatment were 21.612 (3.620) and 19.946 (3.671) kg/m^2 for cases and controls respectively. Students dominated in both the groups with 37 (33.64%) and 54

(22.98%) respectively. Among cases, 78 (70.91%) resided in the town and 32 (29.09%) resided in the rural areas (Table 1).

Table 1. Socio-demographic characteristics of EPTB and PTB study participants in Bhutan, 2018.

Variables	Case (N=110)		Control (N=235)	
	Frequency	Percentage	Frequency	Percentage
Age (years)				
Mean $\pm$ SD	30.773 $\pm$ 14.171		35.179 $\pm$ 17.397	
Median (IQR)	25 (36 - 21)		29 (44 - 23)	
Mini - Max	9 - 79		13 - 84	
BMI (Start)				
Mean $\pm$ SD	21.612 $\pm$ 3.620		19.946 $\pm$ 3.671	
Median (IQR)	21.170 (23.244 - 19.591)		19.477 (21.929 - 17.543)	
Mini - Max	10.939 - 34.240		12.840 - 41.666	
BMI (Current)				
Mean $\pm$ SD	22.193 $\pm$ 3.621		21.050 $\pm$ 3.767	
Median (IQR)	21 (23.922 - 19.918)		20.832 (23.183 - 18.420)	
Mini - Max	12.366 - 34.240		11.944 - 36.805	
Gender				
Female	73	66.36	128	54.47
Male	37	33.64	107	45.53
Household size				
4-6 people	63	57.27	124	52.77
7-10 people	12	10.91	29	12.34
> 10 people	4	3.64	9	3.83
<=3 people	31	28.18	73	31.06
Family income				
<=Nu.5000	23	20.91	40	17.02
Nu.5001-Nu.15000	30	27.27	76	32.34
Nu.15001-/Nu.25000	27	24.55	61	25.96
Nu.25001-Nu.35000	13	11.82	24	10.21
>Nu.35000	17	15.45	34	14.47
Individual income				
Dependent	45	40.91	88	37.45

<Nu.5000	14	12.73	38	16.17
Nu.5001-10000	12	10.91	30	12.77
Nu.10001-15000	13	11.82	37	15.74
Nu.150001-20000	9	8.18	24	10.21
>Nu.20000	17	15.45	18	7.66
Occupation				
Farmer	22	20	48	20.43
Housewife/Househusband	13	11.82	37	15.74
Corporate worker	7	6.36	13	5.53
Armed forces	2	1.82	4	1.7
Students	37	33.64	54	22.98
Monk/Nun/Gomchen	1	0.91	1	0.43
Construction worker	1	0.91	7	2.98
Private business	20	18.18	48	20.43
Civil servant	7	6.36	23	9.79
Education				
No schooling	24	21.82	59	25.11
NFE	3	2.73	8	3.4
Primary school(<=6)	6	5.45	21	8.94
Lower secondary school	11	10	24	10.21
Higher secondary school	44	40	83	35.32
Monastic	0	0	6	2.55
Degree	22	20	34	14.47
Ethnicity				
Sharchop	61	55.45	113	48.09
Lhotshampa	23	20.91	44	18.72
Ngalop	26	23.64	78	33.19
Marital status				
Unmarried	50	45.45	81	34.47
Separated	5	4.55	18	7.66
Married	55	50	136	57.87
Area of residence				
Town	78	70.91	126	53.62
Rural	32	29.09	109	46.38

10.1.2. Distribution of EPTB by body site involvement

Of the 110 EPTB cases, lymphatic TB was the most predominant (n=78 (70.91%)) followed by genitourinary TB (n=4 (3.64%)). Lymphatic TB were mostly present in females than males (67.90% vs 32.10%), while genitourinary TB were present equally between the two (two cases each). Three cases (3.64%) had multiple extra-pulmonary sites involving tuberculous intrathoracic lymphadenopathy (Mediastinal/hilar) and the lymphatic system. In 12% of the cases, EPTB sites were not mentioned.

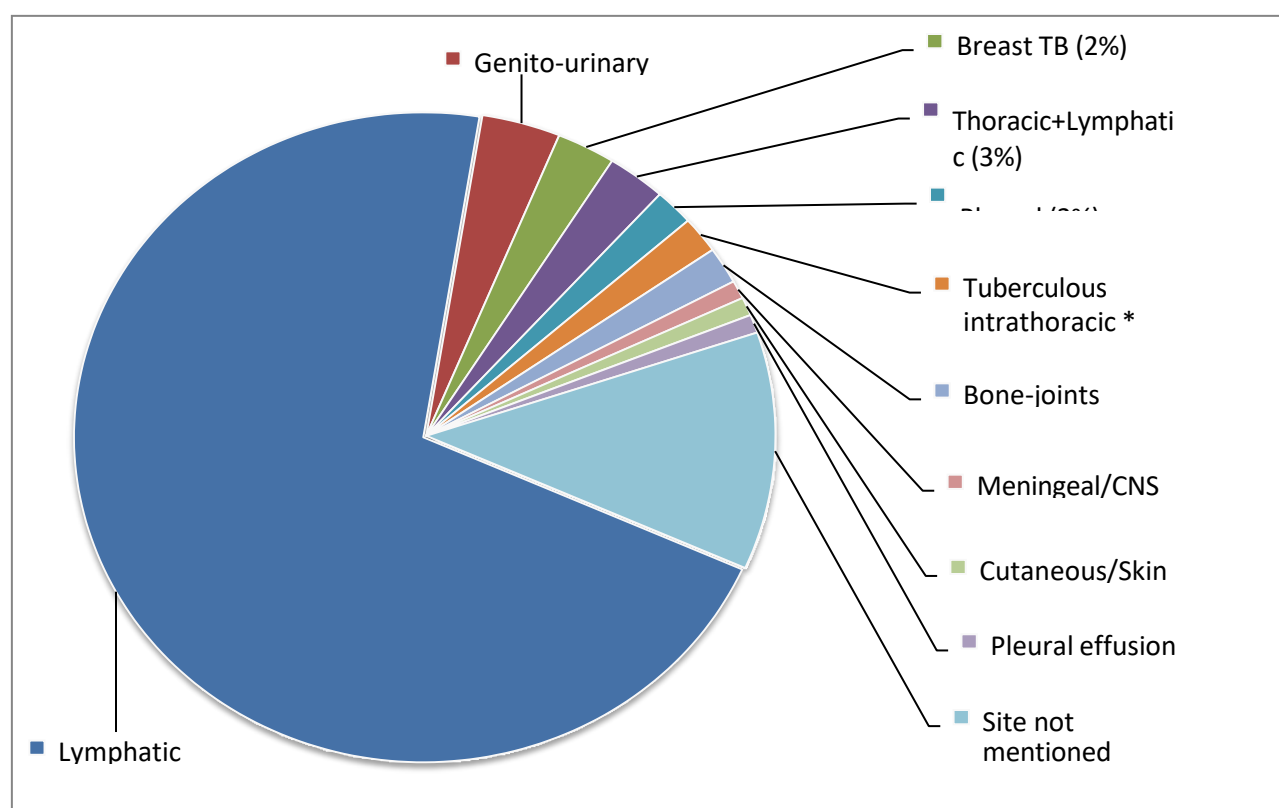


Figure 3. Distribution of EPTB sites by body site involvement in Bhutan, 2018.

**Tuberculous intrathoracic lymphadenopathy (Mediastinal/hilar)*

10.1.3. Clinical manifestations and Diagnosis

Most of the EPTB cases had lymphadenopathy (71.82%) followed by fever (32.73%) and weight loss (27.27%). Cough (87.23%), weight loss (57.87%) and chest pain (49.36%) were the most common clinical features among PTB control groups (Figure 3).

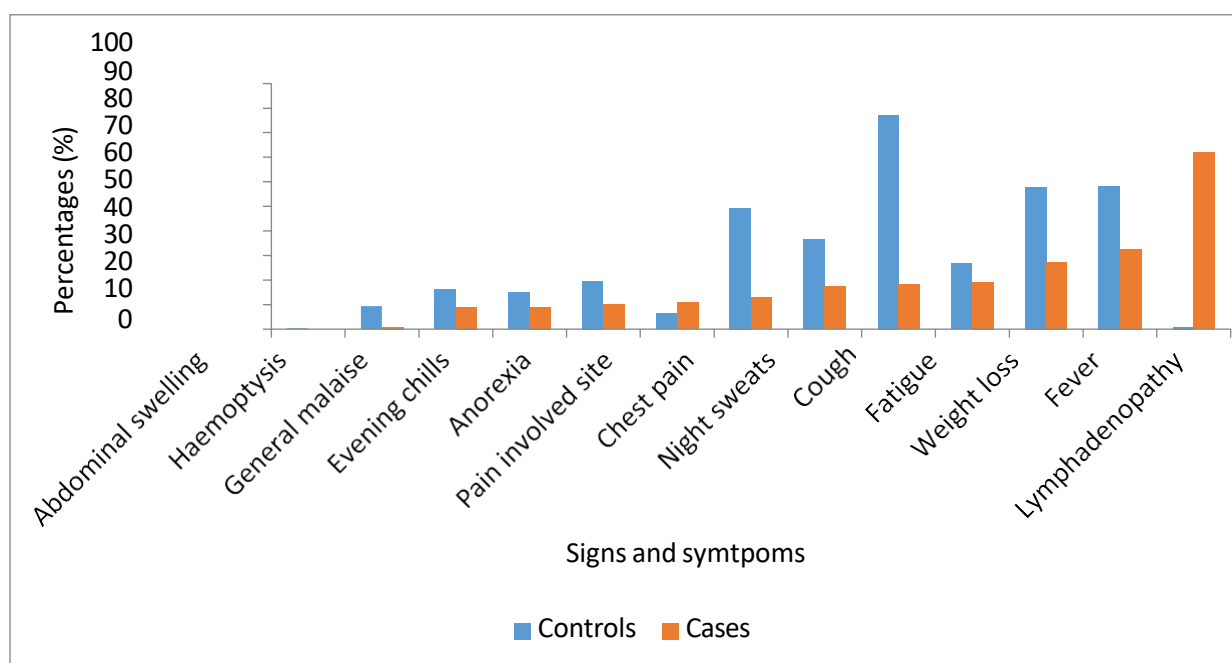


Figure 4. Signs and symptoms of EPTB and PTB participants in Bhutan, 2018.

10.1.4. History of medication and hospitalization

The highest duration of illness for both EPTB and PTB patients ranged from one to two weeks before being diagnosed as TB. Among EPTB cases, illness duration of more than two months was 27.27%. About 80% of the PTB patients were hospitalized, while only 16% of the EPTB patients were hospitalized. Traditional healers were visited by 13.64% of the EPTB patients, while 24.26% of PTB patients have also visited the traditional healers before seeking medication in allopathic medication (Table 2).

Table 2. History of medication and hospitalization before the diagnosis of EPTB and PTB study participants in Bhutan, 2018.

Variables		Case (EPTB)		Control (PTB)	
			<u>Percentage</u>	<u>Frequency</u>	<u>Percentage</u>
Illness duration					
3-4 weeks	16		14.55	42	17.87
1-2 months	17		15.45	51	21.7
>2 months	30		27.27	33	14.04
1-2 weeks	47		42.73	109	46.38
			87		

Visited other health centre				
Yes	64	58.18	124	52.77
No	46	41.82	111	47.23
Aware of receiving antibiotic before ATT?				
Yes	57	51.82	72	30.64
No	53	48.18	163	69.36
Hospitalized after diagnosed as TB				
No	93	84.55	48	20.43
Yes	17	15.45	187	79.57
Admission duration				
1-14 days	16	94.12	89	47.34
15-30 days	0	0	66	35.11
>30 days	0	0	33	17.55
Visited traditional healer				
Yes	15	13.64	57	24.26
No	95	86.36	178	75.74
TB History				
Yes	15	13.64	34	14.47
No	95	86.36	201	85.53
Surgery in last 1-2 years				
Yes	12	10.91	30	12.77
No	98	89.09	205	87.23
Psychiatric illness				
Yes	0	0	4	1.7
No	110	100	231	98.3

10.1.5. Co-morbidities

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Gastritis was the predominant co-morbidities present in both cases and controls (17.27% vs 14.47%) followed by hypertension (6.36% vs 8.51%) and diabetes mellitus (2.73% vs 5.11%)

(Table 3).

Table 3. Co-morbidities of EPTB and PTB participants in Bhutan, 2018.

Co-morbidities	Case (EPTB)		Control (PTB)	
	Frequency	Percentage	Frequency	Percentage
Gastritis	19	17.27	34	14.47
Hypertension	7	6.36	20	8.51
Diabetes mellitus	3	2.73	12	5.11
Malignancy/Ca	2	1.82	0	0
Migraine headache	2	1.82	10	4.26
Cardiovascular disease	1	0.91	1	0.43
Chronic kidney disease	1	0.91	2	0.85
Chronic hepatitis	1	0.91	2	0.85
COPD	1	0.91	3	1.28
Haematological disease	1	0.91	0	0
Auto-immune disorder	1	0.91	0	0
Silicosis	0	0	1	0.43
Asbestosis	0	0	0	0
Congenital anomaly	0	0	0	0
Convulsion disorder	0	0	1	0.43
Transplantation	0	0	1	0.43

10.1.6. X-ray chest and sputum examination

Diagnosis of EPTB was confirmed by the detection of *Mycobacterium Tuberculosis* (MTB) by sputum microscopy (58.18%) and/or by culture (5.45%) and/or by GeneXpert (40%) and/or by LPA (1.82%). Among the cases, 78 (70.91%) and 55 (50%) had done their X-ray chest and sputum examination at the time of diagnosis (Table 4).

Table 4. History of X-ray chest, sputum examination and findings of EPTB and PTB participants in Bhutan, 2018.

Variables		Case (EPTB)	Control (PTB)		
		Frequency	Percentage	Frequency	Percentage
Bacteriologically confirmed					
Smear positive		64	58.18	216	91.91
Culture positive	6		5.45	73	31.06
GeneXpert		44	40	162	68.94
LPA	2		1.82	29	12.34
Clinically diagnosed					
X-ray chest		31	28.18	161	68.51
MRI	5		4.55	8	3.4
CT scan	8		7.27	12	5.11
USG	7		6.36	12	5.11
Histopathology					
FNAC	93		84.55	11	4.68
Biopsy	54		49.09	11	4.7
89					
X-ray chest at diagnosis					
Yes	78		70.91	206	87.66
No	32		29.09	29	12.34
Sputum exam at diagnosis					
Yes	55		50	229	97.45
No	55		50	6	2.55

10.1.7. Behavioural and lifestyle related characteristics

Participants in both EPTB and PTB groups had the highest proportion of non-smokers (73.64% vs 64.26%) followed by those who quit at-least six months ago (17.27% vs 27.66%). Substance use was nearly proportionate in case and controls with 8 (7.27%) and 17 (7.23%) respectively. The most common substance use in both the groups was in tablet form (62.5% vs 47.06%) followed by smoking (37.5% vs 41.18%). From a total of 39 participants who had a history of EPTB, 12 (10.91%) of the cases had the family history of EPTB. Exercise was prevalent in 64 (58.18%) and 157 (66.81%) of the cases and controls respectively (Table 5).

Table 5. Behavioural and lifestyle related characteristics of EPTB and PTB participants in Bhutan, 2018.

Variables	Case (EPTB)		Control (PTB)	
	Frequency	Percentage	Frequency	Percentage
Smoking				
Never smoked	81	73.64	151	64.26
Currently smoking	5	4.55	6	2.55
Quite at least 6 months ago	19	17.27	65	27.66
Passive smoking	5	4.55	13	5.53
Alcohol				
Never drank	63	57.27	107	45.53
Occasional drinker	26	23.64	39	16.6
Quite 6 months ago	21	19.09	89	37.87
Substance use				
Yes	8	7.27	17	7.23
No	102	92.73	218	92.77
Substance name				
Tablet	5	4.55	8	3.36
Sniffing	0	0	2	0.84
Smoking form	3	2.73	7	2.97
BCG vaccination				
Vaccinated	90	81.82	181	77.02
Not vaccinated	20	18.18	54	22.98
Steroid use				
Yes	2	1.82	8	3.4
No	108	98.18	227	96.6
Family history of EPTB				
Yes	12	10.91	27	11.49
No	98	89.09	208	88.51
Family history of PTB				
Yes	25	22.73	59	25.11
No	85	77.27	176	74.89

Family history of MDR-TB					
Yes	3		2.73	5	2.13
No	107		97.27	230	97.87
History EPTB contact					
No contact	92		83.64	205	87.23
Contact in last 1-3 years	9		8.18	21	8.94
Contact in last >=4 years	9		8.18	9	3.83
History of PTB contact					
No contact	82		74.55	161	68.51
Contact in last 1-3 ye	11		10	46	19.57
Contact in last >=4 ye	17		15.45	28	11.91
History of MDR-TB contact					
Yes	5		4	7	2.98
No	105		95.45	228	97.02
HIV status					
HIV-Positive	1		0.91	4	1.7
HIV-Negative	108	91	98.18	227	96.6
HIV-Status unknown	1		0.91	4	1.7
Exercise					
Yes	64		58.18	157	66.81
No	46		41.82	78	33.19

10.1.8. Environmental exposures

Majority of the case and control participants resided in modern concrete houses (59.09% vs 52.34%) with three rooms (32.73% vs 31.06%) respectively. Domestic animals were present mostly in controls than cases (55.74% vs 38.18%). Most of the cases and controls were non- vegetarian (63.64% vs 65.53%). Participants among EPTB worked mostly for 4-5 hours, while those in PTB worked mostly for 8-9 hours (Table 6).

Table 6. Environmental and exposure associated characteristics of EPTB and PTB in Bhutan, 2018.

Variables	Case (EPTB)		Control (PTB)	
	Frequency	Percentage	Frequency	Percentage
Residency type				
Traditional Bhutanese	38	34.55	88	37.45
Modern concrete house	65	59.09	123	52.34
Bamboo thatched/makeshift	7	6.36	24	10.21
Number of rooms				
One room	16	14.55	29	12.34
Two rooms	26	23.64	46	19.57
Three rooms	36	32.73	73	31.06
Four rooms	20	18.18	48	20.43
Five rooms	4	3.64	19	8.09
>5 rooms	8	7.27	20	8.51
Type of fuel				
Firewood		92		
Yes	19		56	23.83
No	91	82.73	179	76.17
LPG				
Yes	93	84.55	202	85.96
No	17	15.45	33	14.04
Electrical				
Yes		108	230	97.87
No	2	1.82	5	2.13
Kerosene heater				
Yes	2	1.82	0	0
No	108	98.18	235	100
Domestic animals				
Yes	42	38.18	131	55.74
No	68	61.82	104	44.26
Dogs				
>= Two dogs	6	5.45	16	6.81
1 dog	10	9.09	50	21.28
No dog	94	85.45	169	71.91

Cats					
>= Two cats	4		3.64	14	5.96
One cat	12		10.91	44	18.72
No cat	94		85.45	177	75.32
Cattles					
>5 cattle	7		6.36	21	8.94
<=5 cattle	10		9.09	39	16.6
No cattle	93		84.55	175	74.47
Pigs					
Yes	109		99.09	8	3.40
No pig	1		0.91	227	96.60
Horse					
Yes	5		4.55	5	2.13
No	105		95.45	230	97.87
Goat					
Yes	2		1.82	15	6.38
No	108		98.15	220	93.62
Poultry					
> five birds	2		1.82	13	5.53
=< five birds	4	93	3.64	16	6.81
No birds	104		94.55	206	87.66
Working hour					
=>10 hours	11		10	36	15.32
8-9 hours	30		27.27	80	34.04
6-7 hours	34		30.91	46	19.57
4-5 hours					
	35		31.82	73	31.06
Sleeping hour					
<7 hours	20		18.18	35	14.89
7-8 hours	63		57.27	133	56.6
>8 hours	27		24.55	67	28.51
Diet					
Vegetarian	10		9.09	26	11.06
Non-vegetarian	70		63.64	154	65.53
Ovo-vegetarian	6		5.45	7	2.98
Both	24		21.82	48	20.43

Un-boiled milk				
Yes	21	19.09	52	22.13
No	89	80.91	183	77.87
Butter milk				
Yes	87	79.09	184	78.3
No	23	20.91	51	21.7
Curd				
Yes	82	74.55	174	74.04
No	28	25.45	61	25.96
Work/study abroad				
Yes	16	14.55	29	12.34
No	94	85.45	206	87.66
Travel abroad				
Yes	21	19.09	42	17.87
No	89	80.91	193	82.13

10.1.9. Pregnancy and contraception related

The study included additional tools for assessing pregnancy and contraception practices among women participants. For assessing term delivery, students, nuns and pre-school children were omitted from the analysis. From a total of 50 EPTB cases, 19 (38.00%) of them did not have history of delivery, while most of the PTB cases had 1-2 term delivery (43.88%). 94 PA/OCP was the most commonly used contraceptive methods by both cases and controls (66.67% vs 58.97%). More than half of the EPTB participants completed eight ANC visits (59.38%), while the same for PTB was 44.29%. History of EPTB and PTB during and after pregnancy was less than 4% in both EPTB and PTB cases (Table 7).

Table 7. Pregnancy-related and contraception practices among women participants between EPTB and PTB in Bhutan, 2018.

Variables	Case (EPTB)		Control (PTB)	
	Frequency	Percentage	Frequency	Percentage
Term deliveries (n=148)				
>= 3 term	14	28	27	27.55
1-2 term	17	34	43	43.88
None	19	38	28	28.57

Contraception (n=210)				
Yes	18		24.66 39	30.47
No	55		75.34 89	69.53
Contraception method (n=57)				
DMPA/OCP	12		66.67 23	58.97
Copper-T/Tubectomy	2		11.11 6	15.38
Condom	4		22.22 10	25.64
ANC visits (n=102)				
None	5		15.63 11	15.71
< Eight times	8	25	28	40
Eight times	19		59.38 31	44.29
PNC visits (n=101)				
None	7		21.88 18	26.09
< Four visits	9		28.13 25	36.23
Four visits	16	50	26	37.68
EPTB/PTB during pregnancy (n=166)				
Yes	1		1.85 4	3.57
No	53		98.15	108 96.43
EPTB/PTB during postpartum period (n=167)				
Yes	2		3.64 3	2.68
No	53		96.36	109 97.32

10.2. Univariable analysis

10.2.1. Socio-demographic characteristics

From the socio-demographic characteristics, younger age groups were significantly associated with development of EPTB as compared to elderly age group ($p=0.023$). As compared to males, females were 1.65 times at risk of developing EPTB ($p=0.038$). Increase in Body Mass Index (BMI) had significant association with EPTB ($p<0.001$). Participants residing in urban areas were 2.11 times at risk of developing EPTB as compared to those residing in rural areas ($p=0.003$). Other socio-demographic factors were not significantly associated in developing EPTB (Table 8).

Table 8. Univariable logistic regression analysis of socio-demographic characteristics of EPTB in Bhutan, 2018.

Variables	Frequency	COR*	95% CI	P-value
Age(Years)	345	0.982	0.968-0.998	0.023
BMI	345	1.128	1.058-1.203	<0.001
Gender				
Female	201	1.650	1.029-2.643	0.038
Male	144	1.000		
Household size				
4-6 people	187	1.196	0.712-2.001	0.498
7-10 people	41	0.974	0.441-2.154	0.949
> 10 people	13	1.047	0.299-3.654	0.943
<=3 people	104	1.000		
Family income				
<=Nu.5000	63	1.15	0.529-2.498	0.724
Nu.5001-Nu.15000	106	0.7895	0.384-1.620	0.520
Nu.15001-/Nu.25000	88	0.8852	0.423-1.851	0.746
Nu.25001-Nu.35000	37	1.0833	0.444-2.641	0.860
>Nu.35000	51	1		

Individual income					
	Dependent	133	0.541	0.254 - 1.151	0.111
	<Nu.5000	52	0.390	0.158 - 0.961	0.041
	Nu.5001-10000	42	0.424	0.165 - 1.087	0.074
	Nu.10001-15000	50	0.372	0.148 - 0.130	0.034
	Nu.150001-20000	33	0.397	0.144 - 1.093	0.074
	>Nu.20000	35	1.000		
Occupation		96			
	Farmer	70	1.506	0.562 - 4.033	0.415
	Housewife/Househusband	50	1.154	0.401 - 3.319	0.790
	Corporate worker	20	1.769	0.507 - 6.170	0.371
	Armed forces	6	1.643	0.246 - 10.946	0.608
	Students	91	2.251	0.876 - 5.785	0.092
	Monk/Nun/Gomchen	2	3.286	0.181 - 59.599	0.421
	Construction worker	8	0.469	0.048 - 4.497	0.512
	Private business	68	1.369	0.507 - 3.699	0.536
	Civil servant	30	1.000		
Education					
	No schooling	83	0.629	0.307 - 1.286	0.204
	NFE	11	0.580	0.138 - 2.424	0.455
	Primary school(<=6)	27	0.442	0.153 - 1.267	0.128
	Lower secondary school	35	0.708	0.290 - 1.729	0.449
	Higher secondary school	127	0.819	0.428 - 1.567	0.547
	Monastic	6	-	-	-
	Degree	56	1.000		
Ethnicity					
	Sharchop	174	1.619	0.942 - 2.786	0.081
	Lhotshampa	67	1.568	0.801 - 3.070	0.189
	Ngalop	104	1.000		
Marital status					
	Unmarried	131	1.526	0.953 - 2.446	0.079
	Separated	23	0.687	0.243 - 1.942	0.479
	Married	191			
Residence					
	Urban	204	2.109	1.299 - 3.424	0.003
	Rural	141	1.000		

COR*=Crude Odds Ratio

10.2.2. Duration of illness, medication and hospitalization

EPTB cases were likely to be diagnosed after more than two months of illness by 2.11 folds as compared to PTB controls ($p=0.015$). EPTB cases were aware of receiving antibiotics by 2.43 times compared to PTB controls ($p<0.001$). EPTB cases were less likely to be admitted in the hospital by 21.31 times compared to PTB controls ($p<0.001$) (Table 9).

Table 9. Univariable logistic regression analysis of history of illness, medication and hospitalization before diagnosed as EPTB in Bhutan, 2018.

Variables		Frequency	COR	94% CI	P-value
Illness duration					
	3-4 weeks	58	0.883	0.452 - 1.726	0.717
	1-2 months	68	0.773	0.404 - 1.476	0.435
	>2 months	63	2.108	1.155 - 3.846	0.015
	1-2 weeks	156	1.000		
Visited other health centre					
	Yes	188	1.245	0.788 - 1.968	0.347
	No	157	1.000		
Aware of receiving antibiotic before ATT?					
	Yes	129	2.435	1.528 - 3.879	<0.001
	No	216	1.000		
Hospitalized after diagnosed as TB					
	No	204	21.312	11.621 - 39.087	<0.001
	Yes	141	1.000		
Visited traditional healer					
	Yes	72	0.493	0.265 - 0.917	0.026
	No	273	1.000		
TB History					
	Yes	49	0.933	0.485 - 1.796	0.837
	No	296	1.000		
Surgery in last 1-2 years					
	Yes	42	0.837	0.411 - 1.704	0.623
	No	303	1.000		

10.2.3. X-ray chest and sputum examination:

X-ray chest and sputum examination at the time of diagnosis was less likely to be done for the EPTB cases as compared to the PTB controls with significance level of ($p < 0.001$) each (Table 10).

Table 10. Univariable logistic regression analysis X-ray and sputum examination at the time of diagnosis of EPTB study participants in Bhutan, 2018.

Variables	Frequency	COR	95% CI	p-value
X-ray chest at diagnosis				
Yes	284	0.343	0.194 - 0.604	<0.001
No	61	1.000		
Sputum exam at diagnosis				
Yes	284	0.026	0.011 - 0.064	<0.001
No	61	1.000		

10.2.4. Behavioural and lifestyle characteristics

EPTB development was less likely among the participants who quit smoking and alcohol six months ago with significant level of ($p=0.040$) and ($p=0.002$) respectively. Family history of TB was not significantly associated with EPTB development. Participants with history of contact with TB in the last 1-3 years was less likely associated in EPTB development ($p=0.037$) (Table 11).

Table 11. Univariable logistic regression analysis of lifestyle, behavioural, exposure history related characteristics of EPTB study participants in Bhutan, 2018.

Variables	Frequency	COR	95% CI	p-value
Smoking				
Currently smoking	11	1.553	0.460 - 5.247	0.478
Quit 6 months ago	84	0.545	0.306 - 0.971	0.040
Passive smoking	18	0.717	0.247 - 2.082	0.541
Never smoked	232	1.000		
Alcohol				
Occasional drinker	65	1.132	0.630 - 2.034	0.678
Quit 6 months ago	110	0.401	0.227 - 0.707	0.002
Never drank	170	1.000		

Substance used					
	Yes	25	1.006	0.420 - 2.407	0.99
	No	320	1.000		
BCG vaccination					
	Not vaccinated	74	0.745	0.420 - 1.319	0.313
	Vaccinated	271	1.000		
Steroid medication					
	Yes	10	0.525	0.109 - 2.516	0.421
	No	335	1.000		
Family history of EPTB					
	Yes	39	0.943	0.458 - 1.940	0.874
	No	306	1.000		
Family history of PTB					
	Yes	84	0.877	0.514 - 1.498	0.631
	No	261	1.000		
Family history of MDR-TB					
	Yes	8	1.290	0.303 - 5.496	0.731
	No	337	1.000		
History EPTB contact					
Contact in last 1-3 years		30	0.955	0.421 - 2.165	0.912
Contact in last >=4 years		18	2.228	0.856 - 5.797	0.100
	No contact	297	1.000		
History of PTB contact					
Contact in last 1-3 years		57	0.470	0.230 - 0.954	0.037
Contact in last >=4 years		45	1.192	0.616 - 2.303	0.601
	No contact	243	1.000		
History of MDR-TB contact					
	Yes	12	1.551	0.481 - 5.001	0.462
	No	333	1.000		

HIV status	HIV-Positive	5	0.525	0.058 - 4.757	0.567
	HIV-Status unknown	5	0.525	0.058 - 4.757	0.567
	HIV-Negative	335	1.000		
Exercise	Yes	221	0.691	0.433 - 1.102	0.12
	No	124	1.000		

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10.2.5. **Environmental exposures** EPTB cases were less likely to possess domestic animals such as dogs, cats, cattle, pig, horse, goat and poultry as compared to PTB controls ($p=0.003$). ETPB cases were not significantly associated with resident type, number of rooms, working hour, sleeping hour, diet and exposure to dairy products ($p>0.05$) (Table 12).

Table 12. Univariable logistic regression analysis for environmental exposure risk factors of EPTB study participants in Bhutan, 2018.

Variables	Frequency		COR	95% CI	p-value
Residency type					
	Traditional Bhutanese	126	0.817	0.503 - 1.327	0.414
	Bamboo thatched / makeshift	31	0.552	0.225 - 1.349	0.193
	Modern concrete house	188	1.000		
Number of rooms					
	One room	45	1.379	0.496 - 3.833	0.537
	Two rooms	72	1.413	0.546 - 3.656	0.476
	Three rooms	109	1.233	0.495 - 3.069	0.653
	Four rooms	68	1.042	0.394 - 2.752	0.934
	Five rooms	23	0.526	0.135 - 2.039	0.353
	>5 rooms	28	1.000		
Type of fuel					
Firewood	Yes	75	0.667	0.374 - 1.189	0.171
	No	270	1.000		
LPG	Yes	295	0.894	0.473 - 1.686	0.729
	No	50	1.000		
Electrical	Yes	338	1.174	0.224 - 6.147	0.849
	No	7	1.000		

Domestic animals					
	Yes	173	0.490	0.309 - 0.779	0.003
	No	172	1.000		
Dogs					
	>= Two dogs	22	0.674	0.255 - 1.781	0.426
	1 dog	60	0.360	0.174 - 0.741	0.006
	No dog	263	1.000		
Cats					
	>= Two cats	18	0.538	0.172 - 1.680	0.286
	One cat	56	0.514	0.259 - 1.019	0.057
	No cat	271	1.000		
Cattles					
	>5 cattle	101	0.627	0.257 - 1.525	0.305
	<=5 cattle		0.482	0.230 - 1.010	0.053
	No cattle	268	1.000		
Pigs					
	Yes	336	0.260	0.0321 - 2.107	0.207
	No pig	9	1.000		
Horse					
	Yes	10	0.260	0.032 - 2.108	0.207
	No	335	1.000		
Goat					
	Yes	17	0.272	0.061 - 1.209	0.087
	No	328	1.000		
Poultry					
	> five birds	15	0.305	0.067 - 1.375	0.122
	=< five birds	20	0.495	0.161 - 1.518	0.219
	No birds	310	1.000		
Working hour					
	=>10 hours	47	0.637	0.290 - 1.399	0.261
	8-9 hours	110	0.782	0.437 - 1.399	0.408
	6-7 hours	80	1.542	0.846 - 2.806	0.157
	4-5 hours	108	1.000		

Sleeping hour					
	<7 hours	55	1.206	0.645 - 2.256	0.557
	>8 hours	94	0.851	0.497 - 1.457	0.556
	7-8 hours	196	1.000		
Diet					
	Non-vegetarian	224	1.182	0.541 - 2.583	0.675
	Ovo-vegetarian	13	2.229	0.600 - 8.274	0.231
	Both	72	1.300	0.539 - 3.129	0.558
	Vegetarian	36	1.000		
Un-boiled milk					
	Yes	73	0.830	0.471 - 1.463	0.52
	No	272	1.000		
Butter milk					
	Yes	271	1.048	0.602 - 1.825	0.867
	No	74	1.000		
Curd		102			
	Yes	256	1.027	0.611 - 1.724	0.921
	No	89	1.000		
Work/study abroad					
	Yes	45	1.209	0.627 - 2.333	0.571
	No	300	1.000		
Travel abroad					
	Yes	63	1.084	0.607 - 1.938	0.785
	No	282	1.000		

10.3. Multivariable logistic regression

All variables which were significantly associated in the univariable logistic regression and those variables with threshold p-value of ≤ 0.2 were included in the multivariable logistic regression analysis. Each of these variables was tested for collinearity and possible interactions among those variables. The final model identified three significant risk factors for EPTB in Bhutan (Table 13):

Table 13. Multivariable logistic regression analysis of EPTB in Bhutan, 2018.

Variables	AOR**	95% CI	p-value
BMI	1.123	1.052 - 1.200	0.001
Alcohol use			
Quit 6 months ago	0.389	0.216 - 0.702	0.002
Occasional drinker	1.047	0.573 - 1.913	0.882
Never drank	1.000		
Residential area			
Urban	1.688	1.016 - 2.805	0.040
Rural	1.000		

AOR=Adjusted Odds Ratio**

- i. An increase in BMI was significantly associated with development of EPTB (OR 1.123, 95% CI 1.052-1.200, p=0.001) as compared to PTB.
- ii. The odds of getting EPTB was significantly reduced (OR 0.389, 95% CI 0.216-0.702, p=0.002) in participants who quit alcohol six months ago (past drinkers).
- iii. Participants residing in urban areas were at risk of developing EPTB (OR 1.688, 95% CI 1.016-2.805, p=0.040) than those residing in rural areas (Table 13).

11. Discussions

11.1. Multivariable analysis BMI and Urban residents

In the final multivariable logistic regression model, the significant risk factor of EPTB development identified in Bhutan were related to increase in BMI (OR 1.123, 95% CI 1.052-1.200, $p=0.001$) and urban residents (OR 1.688, 95% CI 1.016-2.805, $p=0.040$). Our study also found that the odds of getting EPTB was significantly reduced (OR 0.389, 95% CI 0.216-0.702, $p=0.002$) in participants who quit alcohol six months ago (past drinkers).

The fact that EPTB has urban affinity and among people with high BMI points out that the disease is more common among people with high income group with sedentary lifestyles. People living in the urban areas are likely to earn better than those residing in the rural areas and have more purchasing power on food and commodities. Urban dwellers are less likely to exercise, thereby leading to weight gain and increase in BMI ultimately.

Alternately, we can explain that people living in urban areas are more aware of EPTB and accessible to health care services compared to rural counterparts.

In our study, those who quit alcohol at least 6 months ago (past drinkers) were shown to be protective against EPTB development.

It is important to know the mode of spread of EPTB from the time of primary infection it occurs in the lungs. Spread of tubercle bacilli to other body parts occur by blood or lymphatic route where the bacilli sits there and remains dormant until triggered by immune deficit. EPTB is a non- contagious form of TB which is not usually accompanied with cough and high grade fever as with PTB. Hence, spread of EPTB is not related to immediate close contacts or overcrowding, as it exists the urban areas. As increase in MBI and urban residency has been significantly found to be associated in development of EPTB in our study, it indicates that reactivation of dormant tubercle bacilli may be related to gain in body weight or obesity, which needs to be further proven.

11.2. Univariable analysis

Age, Medication history & Diagnosis

In the univariate analysis, our study has shown that the risk of EPTB was significantly associated with younger age ($p=0.023$) with significant female predominance ($p=0.038$). In a study by García- Rodríguez et al. (2011), female sex ($OR=2.05$) and younger age ($OR=1.02$) has been found as the risk factors of EPTB patients when they were compared with the PTB patients. In Nepal, younger age group (<25 years) and female gender were identified as independent risk factors of EPTB (Sreeramareddy et al., 2008).

The diagnosis of EPTB cases were likely to take more than two months ($p=0.015$) as compared to PTB. As we know that presentation of EPTB is not similar or show typical features like PTB case presentation, diagnosis of EPTB cases are likely to be delayed over a period of two months once a person gets the disease. This is also supported by a finding in our study that EPTB cases were less likely to be hospitalized after diagnosis due to less severity of illness and potential of spread unlike the PTB cases ($p<0.001$).

Our results showed that EPTB cases were more likely to be aware of receiving antibiotic treatment before starting anti-TB treatment ($p<0.001$) and less

likely to have visited traditional healers ($p=0.026$). As EPTB cases are difficult to diagnose due to non-specific clinical presentation, the EPTB patients are likely to visit health centres more often and thereby remember antibiotic treatments provided. In a country where traditional beliefs still holds strong

even in times of severe illness, visiting traditional healers is still a practice. As the natural history of disease itself is non-specific and takes a longer duration (>2 months) to be recognized and diagnosed, their likelihood of visiting traditional healers is also low due to non-severe nature of illness. It also suggests that people living in the urban areas are more likely to be educated and prefer visiting hospitals than traditional practitioners.

X-ray and Sputum examination

Among the EPTB cases, 78 (70.91%) and 55 (50.00%) had done their X-ray chest and sputum examination at the time of diagnosis (Table 4). The proportion of EPTB who were NOT offered X-ray chest and sputum examination at the time of diagnosis was highly significant with ($p<0.001$) each in the univariate analysis. In contrast, all cases of EPTB should have undergone X-ray chest and sputum examination to rule out pulmonary involvement or miliary TB and for appropriate classification of TB. As significant number of EPTB cases were not subjected to X-ray and sputum examination at the time of diagnosis, probably misclassification of PTB cases as EPTB could have been possible which was beyond the scope of this study to assess.

Lifestyles, behavioural and TB contact

Those participants who quit smoking and alcohol at least 6 months ago were significantly protected from development of EPTB with ($p=0.040$) and ($p=0.002$) respectively.

We have also found that contact with PTB cases in the last 1-3 years had no risk or rather remained protected towards EPTB development ($p=0.037$) which further agrees that EPTB is not related to direct transmission of TB bacilli from PTB cases.

In a study by Sreeramareddy et al. (2008), the authors have also suggested that smoking, use of immunosuppressive drug or steroids, diabetes and past history of TB were more likely to be associated with PTB (Controls).

Environmental exposure

We assessed environmental exposure in terms of housing, fuel used, exposure to domestic animals, working and sleeping hours, history of travel abroad and dietary habit including exposure to dairy products such as milk, buttermilk and curd.

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Our study found that EPTB cases were less likely to possess domestic animals such as dogs, cats and cattle compared to PTB cases ($p=0.003$) in univariate analysis. As consumption of dairy products is quite common in our society, we have hypothesised that consumption of unpasteurised milk in the form of butter milk or curd could have led to transmission of bovine TB that cases abdominal TB in humans. However, neither possessing of domestic animals by the

cases nor exposure to milk, buttermilk and curd was associated risks of EPTB in our study. Classification of EPTB by body site involvement did not show any case of abdominal TB which may suggest that exposure to domestic animals and dairy products may not be a potential risk factor for EPTB development in Bhutan.

The fact that we have taken both cases and controls based on bacteriologically confirmation, our findings from this study is robust and the findings can be translated into recommendations for policy decisions making.

12. Recommendations:

- ☞ Health education and awareness program on Extrapulmonary TB should focus on urban dwellers with high BMI.
- ☞ Clinicians or health care workers should be made aware that EPTB cases are more predominant among the urban residents compared to the rural residents.
- ☞ As significant number of EPTB cases were not offered X-ray chest and Sputum microscopy at the time of diagnosis, clinicians should be made aware on subjecting EPTB cases to X- ray chest and sputum microscopy at the time of diagnosis to rule out pulmonary involvement and proper classification.
- ☞ We should also make our health care workers aware that EPTB cases are significantly associated with younger age with female predominance. 107
- ☞ Our clinicians should also be made aware that diagnosis of EPTB is likely to take more than two months (or > 2months of delayed diagnosis) and less likely to get admitted owing to non-specific and non-severe nature of presentation.
- ☞ In 12% of the cases, EPTB sites were not mentioned. Both the Clinicians and the TB In- charges should be reinforced on proper documentation in the patient treatment card.
- ☞ The NTCP is highly recommended to come up with an algorithm for diagnoses of EPTB in consultation with the experts.
- ☞ Association of EPTB with increasing BMI and urban residency in Bhutan can be further researched upon.

13. Ethical review (REBH) and approvals

Committee Name / Approving authority	Date		Person Responsible
	Submitted	Reviewed	
REBH (Research Ethical Board of Health), Ministry of Health, Bhutan and Amendments if required	Submitted	Approved	Dr Tandin Zangpo, PI
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14. Annexures

01. Sample size calculation using EpiInfo 7

The screenshot shows the EpiInfo 7 interface with the 'StatCalc - Sample Size and Power' window open. The window is titled 'Unmatched Case-Control Study (Comparison of ILL and NOT ILL)'. The following parameters are entered:

- Two-sided confidence level: 95%
- Power: 80%
- Ratio of controls to cases: 2
- Percent of controls exposed: 20%
- Odds ratio: 2.5
- Percent of cases with exposure: 38.5%

To the right of these inputs is a table comparing three methods: Kelsey, Fleiss, and Fleiss w/ CC.

	Kelsey	Fleiss	Fleiss w/ CC
Cases	67	69	77
Controls	134	138	153
Total	201	207	230

The bottom of the window shows the EpiInfo 7 logo and the text 'ANALYZE DATA', 'EPI INFO™ WEBSITE | ABOUT EPI INFO™', 'LANGUAGE: en-US', and 'VERSION: 7.2.1.0'.

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Prevalence and risk Factors of
Active Tuberculosis among
Diabetes Mellitus in Nepal
by STAC
2018



**Prevalence and Risk Factors of Active Tuberculosis
among Diabetic Mellitus Patients Attending
Selected Hospitals in Nepal, 2018**

Report

SAARC Tuberculosis and
HIV/AIDS Centre Kathmandu,
Nepal

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Title: Prevalence and Risk Factors of Active Tuberculosis among Diabetic Mellitus Patients Attending Selected Hospitals in Nepal, 2018

Abstract

Background:

In the developing countries, the burden of diabetes and tuberculosis (TB) are enormous and their interaction poses clinical as well as a public health challenge. In 2017, 15% of all notified tuberculosis cases could be attributable to diabetes. Researches implicated diabetes as independent risk factor for multi-drug resistant tuberculosis and unfavorable outcome of treatment. Thus we aim to assess the burden and demographics of active tuberculosis among diabetic patients attending tertiary care hospitals in Nepal.

Methods:

This cross sectional descriptive study included seven tertiary care hospitals representing five development region of the country. Data of socio demographic status, risk behaviors, knowledge about tuberculosis were collected from the eligible participants using a structured questionnaire. Two step laboratory screening was done. First, symptomatic and chest X-ray; second, sputum for microscopy and Gene-Xpert. Participants with positive first step screening went through the second step screening.

Result:

A total of 520 patients were enrolled in the study with 23 positive cases who were screened as tuberculosis positive. The prevalence calculated as 4.42%. The positive cases had older age group and the mean age of the positive TB cases were 59.73 ± 17.36 years with male predominance of 78% (18/23). Regarding the occupation, 43.47% were farmers, 23% house wife and 13% service holder and rest are with different other occupations. Among the 23 positive cases, only two (8.69 %) had extra pulmonary TB. Rest 23 (91.3%) were diagnosed to have pulmonary TB. Diabetic control had significant ($p=0.006$) relationship to develop Tuberculosis. The comorbid conditions e.g., Hypertension (OR 13, 95% CI: 4.54 to 37.14); diabetic nephritis (OR 9.25, 95% CI: 2.03 to 42.20); Diabetic neuropathy (OR 26.66, 95% CI: 5.16 to 137.71) and dyslipidemia (OR 2.77, 95% CI: 0.84 to 9.08) are significant risk factors to develop tuberculosis among the diabetes patients.

Average 60% of the participants had fair to good knowledge about tuberculosis its cause, symptoms, transmission and risk factors.

Conclusion:

The prevalence of tuberculosis among diabetic patients is low in Nepal. Poor diabetic control and comorbid conditions of diabetes poses threat to develop tuberculosis. This is the result of tertiary care hospital outdoor patients only, thus representativeness was compromised. We recommend to assess the magnitude of TB and associated risk factors among diabetic patients in all level and mandatory screening for co morbidities like tuberculosis, diabetes etc. is recommended. Mass advocacy program could help to increase the awareness.

Background

The burden of diabetes and tuberculosis are enormous, particularly in developing countries. The International Diabetes Federation has reported that there were 451 (95% CI 367 – 585) million cases of diabetes worldwide in the year 2017 and about 79% of people with diabetes live in low- and middle-income countries.¹ Meanwhile, the World Health Organization has reported that an estimated 10.4 million cases of tuberculosis occurred worldwide in the year 2016 and 87% of all the cases occurred in the 30 high-burden countries, many of which are low and middle income countries.² The interactions between these two diseases present additional clinical as well as a public health challenge. Diabetes has long been recognized as an important risk factor for tuberculosis. The widely quoted systematic review published in 2008 had found that DM was associated with an increased risk of TB by three-fold (Relative Risk 3.11, 95% CI 2.27-4.26).³ The more recent studies have however estimated this risk to be between 1.77 (95% CI 1.48–2.11) to 2 (95% CI 1.78 – 2.24).^{4,5} A systematic review and meta-analysis has also reported that diabetes confers a small but statistically significant risk of latent TB infection (pooled odds ratio 1.18 (95% CI, 1.06–1.30)).⁶ Diabetes also affects the natural history of tuberculosis and is associated with the worse treatment outcomes. It is associated with the delayed sputum conversion (aOR 1.51, 95% CI 1.09 to 2.10) and a higher probability of treatment failure (aOR 2.93, 95% CI 1.18 to 7.23).⁷ A systematic review has implicated diabetes as a risk factor for death (RR, 1.89; 95% CI, 1.52 to 2.36), and increased risk of relapse (RR, 3.89; 95% CI, 2.43 to 6.23) as well.⁸ In another meta-analysis, diabetes has also been identified as an independent risk factor for the multi-drug resistant tuberculosis (OR 1.71; 95% CI = 1.32, 2.22).⁹ Conversely, tuberculosis in itself can worsen glycemic control among the people with diabetes. The interactions between the drugs can further complicate the picture, leading to a reduction in the effectiveness of both tuberculosis and diabetes treatments, as well as potential worsening of drug side-effects.^{10,11}

Available studies have reported that the prevalence of active tuberculosis among the people with diabetes range from 0.38% to 14% with the overall median global prevalence as 4.1% (IQR 1.8%-6.2%). The median prevalence from Asia has been reported as 3.5% (IQR 0.9 – 10.5%).¹² A screening program in six diabetes clinics of tertiary healthcare centers in India

identified 18 cases of active tuberculosis as a result of the screening program.¹³ Another study at a single tertiary care hospital from Bangladesh identified 37 cases of active tuberculosis while screening the people with diabetes.¹⁴ A cross-sectional study in a tertiary care center in Eastern Nepal detected eight cases of tuberculosis among the 100 patients with established diabetes.¹⁵

Rationale

For optimal outcomes of both the conditions, therefore, people with diabetes should be considered for evaluation of tuberculosis and vice –versa aiming for early and intensified diagnosis followed by integrated management. Furthermore, it is of crucial importance that these activities are reflected in the policy and programmatic issues be addressed. Recognizing the unique challenges posted by tuberculosis and diabetes, the World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease launched the Collaborative Framework for the care and control of diabetes and tuberculosis in 2011.¹⁶ It provides a guidance to the policy makers and health-care professionals in combating the dual epidemic with an emphasis on operational and clinical research to build-up and strengthen the evidence base for action. In 2014, the new WHO End TB Strategy was approved which stresses the importance of addressing diabetes and other tuberculosis risk factors and comorbidities to reach its ambitious targets.¹⁰ It has been further emphasized in the Bali declaration in 2015 which declared the looming tuberculosis-diabetes co-epidemic as a public health priority and called for early and decisive action.¹⁷

Nevertheless, the adaptation and implementation of the collaborative framework under national programs have been slow. Several programmatic issues challenge the wide-scale implementation of the framework including the most appropriate strategy as well as timing for TB-Diabetes bidirectional screening and its cost-effectiveness, particularly in the resource limited settings.¹⁸ Additionally, the issues on the optimal duration of TB treatment in the diabetic population, drug-drug interactions leading to reduced drug concentrations, overlapping toxicities of drugs, adherence to medications, infection control as well as conflicting dietary recommendations need further evaluation. The lack of non-communicable disease

program or systematic and decentralized diabetes management centers in many developing countries make it more difficult to implement an effective collaborative model of care.¹⁹

Thus, it is imperative that the 115 with diabetes are screened for tuberculosis and vice-versa using the cost-effective screening strategies available, which are also feasible under the programmatic conditions. In this study, we aimed to estimate the prevalence of active tuberculosis among the people with established diagnosis seeking care at large public healthcare centers using the existing tools as per the national tuberculosis program protocols. We also aimed to assess the risk factors for developing active tuberculosis among the diabetic population to help identify the most appropriate individuals who will likely benefit from TB screening.

Since available evidence suggests that diabetes precedes tuberculosis, screening diabetic individuals for tuberculosis helps in early diagnosis and therefore, subsequent treatment of tuberculosis.²⁰ Furthermore, the End TB strategy recognizes the diabetic population as an important high-risk group and recommends TB screening among them as a component of pillar 1

- Integrated patient-centered care and prevention. The National TB Program in Nepal estimates that there were around 10000 cases undiagnosed or unreported and TB screening among people with diabetes will contribute in finding the ‘missing’ cases of active tuberculosis ultimately also contributing to the End TB targets.^{21, 22}

Objective

General

- To assess the burden of active tuberculosis among the people with diabetes and describe the risk factors for the same attending the selected diabetic clinics of the tertiary care centers across the country

Specific Objectives

- To determine the prevalence of active tuberculosis among the people with diabetes mellitus attending selected diabetic clinics of the tertiary care centers across the country
- To identify the common types of tuberculosis – pulmonary or extra-pulmonary, among those patients with tuberculosis and diabetes mellitus
- To identify the risk factors of developing active tuberculosis among the patients with diabetes mellitus
- To assess the baseline knowledge of tuberculosis among those patients with diabetes mellitus

Methods

Research design

It was a cross sectional descriptive study

Description of research design

This study aimed to assess the proportion of active tuberculosis among the people with established diabetes. Therefore, a cross-sectional research design had been applied.

Study site and its justification

The diabetes care in Nepal is mainly provided through tertiary care centers or specialized private centers by endocrinologists and internists. These centers are concentrated in more accessible cities and Kathmandu, the capital city, contributes to a significant proportion of patients who seek diabetes care. Accordingly, this study had identified one major city in each of the five development regions. The centers identified are all large public hospitals, preferably university hospitals with a high volume of patients and services for diabetes management. Due to the high volume of patients, three major university teaching hospitals had been identified from Kathmandu valley. The study centers selected were as follows –

SN	Region	Study Site	Address
1	Central	Institute of Medicine, Tribhuvan University Teaching Hospital	Kathmandu
2		National Academy of Medical Sciences, Bir Hospital	Kathmandu
3		Patan Academy of Health Sciences, Patan Hospital	Lalitpur
4	Eastern	BP Koirala Institute of Health Sciences	Dharan
5	Western	Pokhara Academy of Health Sciences, Gandaki Regional Hospital	Pokhara
6	Mid-Western	Bheri Zonal Hospital	Nepalgunj
7	Far-Western	Seti Zonal Hospital	Dhangadhi

Study Population

People with established diabetes mellitus, who were attending to above hospitals during study period (August to October, 2019)

Sampling unit

- Primary sampling units – Each of the study site with diabetes clinics selected for the study
- Secondary sampling units – patients with established diabetes mellitus attending the diabetes clinics of the selected study centers for their diabetes related care

Sample size

The sample size had been calculated by the formula $n = Z^2P(1-P)/d^2$ where Z is level of confidence taken as 1.96, P is expected the prevalence active TB among the people with diabetes taken as 3.5%¹² and d is the degree of precision taken as 2%. The sample size thus derived was

324. Since these 324 samples was taken from the seven different study centers (clusters), it was multiplied by the design effect of 1.25 to obtain the same degree of precision. An additional 20% non-response rate had been estimated and the final sample size was 490 so that there was 70 participants from each of the seven study centers for the total of 490 patients.

Sampling Technique

The sampling technique was employed as the multi stage sampling. First stage was done by selecting each center considered as a cluster. Within each cluster, second stage was by systematic random sampling. The first sample was selected randomly and then every 5th patient with diabetes were included in the study till the required number of participants was obtained.

Inclusion criteria

- Adults of 16 or more years of age
- Established diabetes mellitus according to ADA 2018 criteria for the diagnosis of Diabetes – whether diagnosed for the first time or previously diagnosed and now seeking diabetes care.

- Those agreeing to participate in the study and providing written informed consent
- Those who already have established diabetes, as well as tuberculosis (Bias) at the time of the study were also included.
- The diabetic ladies, who were diagnosed as diabetic beforehand and became pregnant afterwards and seeking care at the time of data collection were also included and underwent symptom screening and sputum examination. The Chest X-Ray was exempted
- Those patients who agreed to participate in the study but deny to undergo Chest X-Ray were also exempted from Chest X-Ray and will undergo symptom screening and sputum examination.

Exclusion criteria

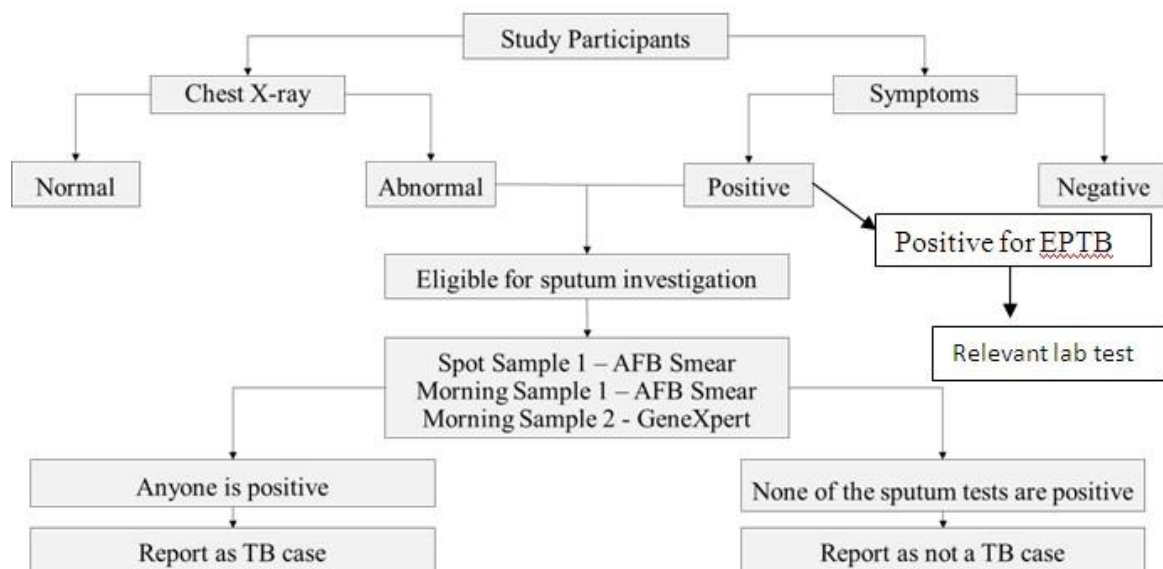
- Children less than 16 years of age, as the targeted laboratory sample (sputum) is difficult to collect from them
- Those who did not agree to participate in the study and providing written informed consent
- Patient who developed gestational diabetes during data collection time

Data Collection Technique

In each of the study centers, an endocrinologist or medicine consultant internist was identified as a research coordinator in close collaboration with the head of the institution. In addition, research assistant/s were identified in each of the study centers with the recommendation of the research coordinator. The research coordinator was responsible to identify the study participants as per the study protocol and send them to research assistant for evaluation and data collection. S/He was also be responsible for reimbursement of the costs related to chest X-ray, sputum examination and investigations for extra-pulmonary TB as requested whenever applicable. The research assistant received the study participants, evaluated for active tuberculosis and collect the information as asked in the data collection tool including the investigation reports.

A team from the SAARC TB and HIV/AIDS center comprising of the Principal Investigator and study team members were visiting each of the study sites. A consultative meeting was held in which the study protocol was presented, data collection tool and advance amount for reimbursement of the costs of the investigation was handed over to the research coordinator. The demographic information, as well as the other relevant information regarding diabetes treatment, were obtained in a data collection tool. These patients were evaluated for active tuberculosis by a comprehensive history and clinical examination. A parallel screening is

employed in which both symptom and Chest X-ray screening were done and people found to have symptoms, or abnormalities, or both on CXR were eligible for further bacteriological examination. The bacteriological examination were done by sputum smear microscopy as well as a GeneXpert assay. The eligible patients provided spot sputum sample for microscopy and two early morning samples – one each for microscopy and Gene Xpert. Those patients who had symptoms suggestive of extra-pulmonary TB were investigated appropriately.



Once all the required number of patients had been obtained along with the results of the investigations requested, the datasheets were collected by one of the study team from the SAARC TB and HIV/AIDS center.

Operational definitions

- Diabetes: Defined by ADA 2018 criteria – Fasting Sugar ≥ 126 mg/dL or 75 gm 2 hour post-prandial blood sugar or ≥ 200 mg/dL or HbA1C $\geq 6.5\%$ ²³
- Diabetes control – Depending upon whether or not the patient has met patient specific targets on FBS/PPBS/HbA1C as set by the consultant
- Cough – cough for any duration (not limited to cough of 2 weeks or more duration) will be included for increased sensitivity
- Weight loss – clinically significant weight loss or 10% or more in 6 months or 5% or more in 1 month, can be documented or undocumented
- Any other relevant symptom – any symptom which the consultant and/or the research assistant perceive as that of possible TB disease
- Symptom positive – any one or more of the symptoms enlisted positive
- Chest X-ray positive – grouped into any abnormality or abnormality possibly due to TB

Data collection tools

The structured data collection tool had been prepared that collects the baseline characteristics, details on diabetes status and treatment and TB symptoms and signs, and results of the sputum or other investigations if performed.

Validity and reliability of the tool

The strategy of screening for active TB and testing them for confirmation has been validated and recommended by the World Health Organization, including in the settings of TB prevalence surveys.^{25, 26}

Potential Biases

- Detection bias – since the research assistants and coordinators were from different health centers, there were bias in detection of TB symptoms and signs and differed in the threshold for requesting sputum and/or other relevant investigation. These were minimized by discussion and short training during the consultative meeting.
- Recall bias – the study participants might not able to recall all of the TB symptoms they were screened for. This was minimized by leading questions and giving hint.
- Response bias – There were some nonresponsive participants. This was minimized by counselling during data collection.

Data management and analysis

Data entry was done in Microsoft Excel 2016 and Analysis in IBM SPSS 23. Socio- demographic and other relevant characteristics of the study participants using mean, standard deviation, median and percentages as appropriate. Data was statistically analyzed with the significance level setting as two-tailed and at p value <0.05. Descriptive statistics for continuous variables were described as means $\pm$ standard deviation, while categorical data were reported as frequency and percentage. Differences between the two groups (males and females) were compared using Fisher's exact test for categorical variables, or independent t-test for continuous variables. Odds ratio were calculated to identify the risk factors associated with active tuberculosis.

Ethical approval

Ethical approval were taken from Nepal Health Research Council and appropriate local ethical board of Pokhara Academy of Health Sciences, Gandaki Regional Hospital, Patan Academy of Health Sciences, Patan Hospital.

Result

During the study period, a total of 520 eligible participants were enrolled in the study and went through the data collection and laboratory diagnosis. Among them, 23 were diagnosed as having Tuberculosis. The prevalence counted as 4.42%.

The mean age of the study subjects was 54.34 ± 12.67 years. 18 (3.5%) subjects were aged less than 30 years, 23 (4.4%) aged between 31–35 years, 34 (6.5%) of them were aged between 36–40 years, 62 (12%) subjects were aged between 41–45 years, 72 (13.8%) between 46–50 years, and 311 (59.8%) of them were aged >50 years. The mean age of the positive TB cases were 59.73 ± 17.36 years with a minimum 28 years, maximum 83 years and median age of 62 years.

The gender-wise demographic information of all participants is detailed in Table 1. Females were comparatively younger than male (53.57 ± 12.12 vs. 55.40 ± 13.17 years; $p = 0.001$). Gender difference were seen in educational status, occupation and personal habits. Majority of men were employed compared to women. Above 22% of men gave history of smoking or consuming tobacco in other form and about 15% consumed alcohol.

Table 1: Demographic details of the Study

Characteristics	Total (n=520)	Male (n=228)	Female (291)	P value (Male vs. Female)
Age (years) Values are mean ± SD	54.34±12.67	55.40±13.17	53.57±12.12	0.001
Educational Status				
Literate	293 (56.34 %)	172 (75.43 %)	121 (41.58%)	Not significant
Illiterate	219 (42.1%)	53 (23.24%)	166 (57%)	
Personal habit				
Smoking	77 (14.8%)	56 (24.56%)	21 (7.21%)	Not significant
Alcohol	59 (9.8%)	51 (22.36%)	8 (2.75%)	Not significant
Tobacco	45 (8.65%)	35 (15.35%)	10 (3.43%)	Not significant

** χ^2 test and Fisher's exact test were done

Among the positive TB cases, 78% (18/23) were male and 21% (5/23) were female. Figure 1 shows the sex differences of the positive cases who were positive to have TB.

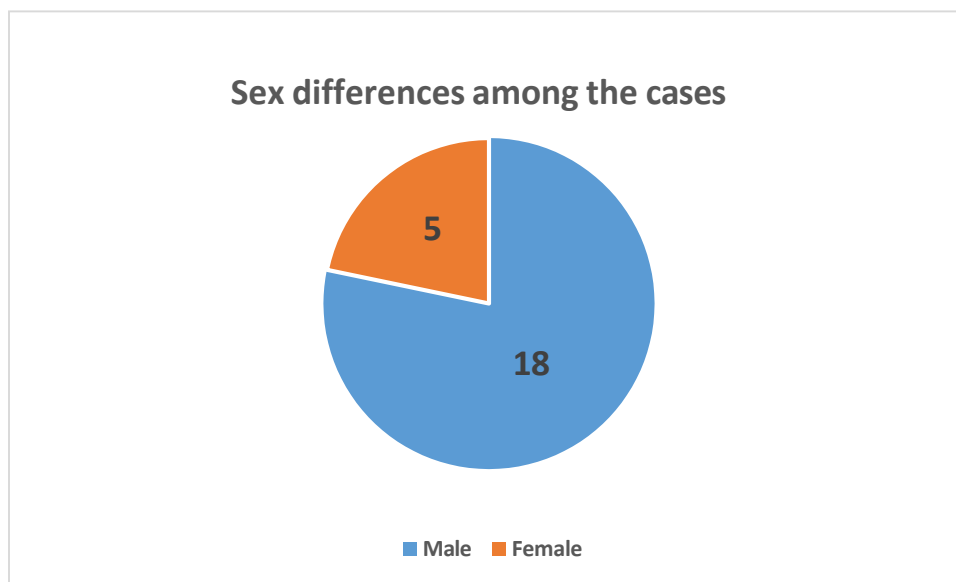


Figure 1: Sex differences among the positive cases who had tuberculosis along with diabetes, Nepal, 2018

Among the 23 positive cases, only two (8.69 %) had extra pulmonary TB. Rest 23 (91.3%) were diagnosed to have pulmonary TB.

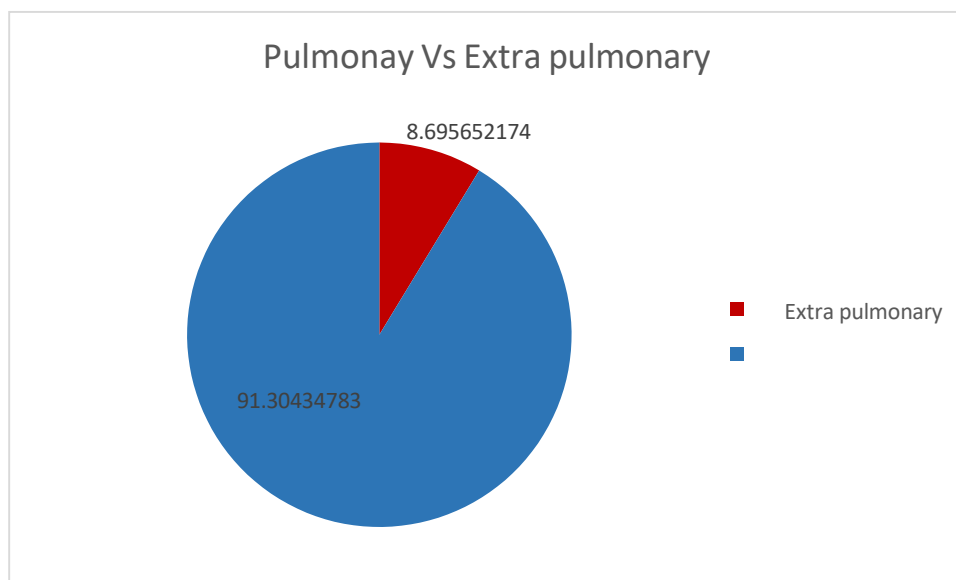


Figure 2: Common type of Tuberculosis among the diabetic patients, Nepal, 2018

While going through the demographic detail, none of the educational status, marital status shows any significant relation with the positive TB cases. The occupation of the Cases were classified in figure 3.

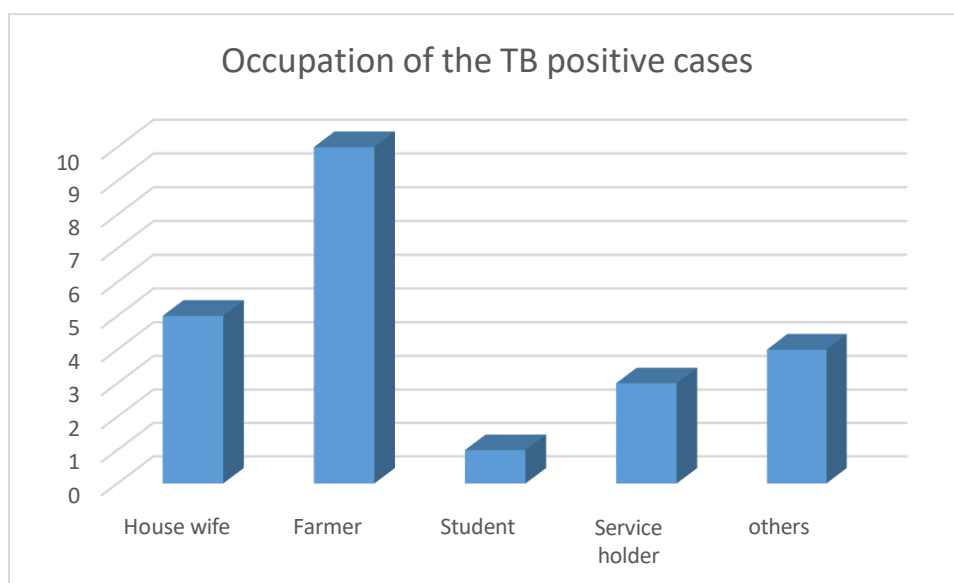


Figure 3: Occupation differences of TB patients among the diabetic patients, Nepal, 2018

Personal habit like tobacco and alcohol consumption or chewing of betel nuts or any other addictive substance didn't show any significant relationship to develop TB among the diabetic patients. However the other co morbidities associated with diabetes showed as a risk factor to develop Tuberculosis among the participants. Table 2 shows the relationship of the comorbidities which acted as a risk factor.

Table 2: Risk of associated co morbidities of diabetic patient to develop Tuberculosis, Nepal, 2018

Co morbidities	Odds ratio	95% CI	Significant level
Hypertension	13	4.54 to 37.14	P < 0.0001
Diabetic nephritis	9.25	2.03 to 42.20	P = 0.0040
Diabetic Neuropathy	26.66	5.16 to 137.71	P = 0.0001
Dyslipidemia	2.77	0.84 to 9.08	P = 0.0911

Among the positive twenty three cases, the duration of diabetes calculates as 11.48 ± 7.72 years (mean $\pm$ SD). They are taking treatment of diabetes for average 9.83 years. Diabetic control had significant ($p=0.006$) relationship to develop Tuberculosis.

We asked five questions to assess the baseline knowledge of tuberculosis among the patient with diabetes. The variables were fact tuberculosis and its cause, symptoms, transmission and risk factors and Tuberculosis. The response of these are tabulated in Table 3.

Table 3: Baseline knowledge of tuberculosis among patients with diabetes, Nepal, 2018

Sl.no.	Questions	Positive answers (%)
1.	Do you know, Tuberculosis is a curable disease or not?	60
2.	Tuberculosis is caused by?	52.17
3.	How Tuberculosis is transmitted?	60.86
4.	Do you know the risk factor for tuberculosis?	73.91
5.	Can you mention the symptoms for tuberculosis?	73.91

Discussion

In this study the overall prevalence of Tuberculosis among the diagnosed diabetes patient was seen as 4.42%, which is comparable with other studies which it shows that among the 78 studies, 48 (61.5%) studies were conducted in countries of Asia and showed prevalence rates ranging from 5.1% in Saluru-South India to 44% in Kerala-India¹². This meta-analysis also showed, overall median prevalence of DM among TB patients in Asia was calculated to be 17% (IQR 11.4%-25.8%).

Among the positive cases we see male predominance (78%). In many of the studies, diabetes was associated with a progressive shift of male predominance with tuberculosis²⁷. It might be because, male are more active in outside social activities then women and in Asian countries in middle and lower socio economic group. Though the other socio cultural variables were not evaluated in this study, different factors might play important role to explain this gender gap including biological differences in disease and disease presentation and different access to health care specifically in developing countries²⁸. Also the mean age of the positive TB cases were 59.73 ± 17.36 years with a minimum 28 years, maximum 83 years and median age of 62 years, which shows prevalence is more in older age group. Similar phenomena was seen in some other studies where they found diabetic group of TB was older in age with longer duration of cough²⁹.

Pulmonary tuberculosis were more (91.3%) among the study participants. This might be because of the diagnostic hurdle and unavailability of the diagnostic facilities among the study sites. On the other hand, pulmonary tuberculosis is predominant in these settings of Nepal. Most of the positive cases were farmer (43%). Other occupation poses minimal threat to develop Tuberculosis. The exact cause of this occupational relationship were not revealed in depth.

In many studies, Diabetes mellitus itself was identified as a risk factor for developing tuberculosis infection. In this study smoking, consumption of alcohol, educational level didn't pose any threat to develop tuberculosis among the diabetes patients. But tuberculosis developed most frequently in patients with poor diabetic control and associated co morbidities were significant risk factors to develop tuberculosis.

Similarly, poor glycemic control in Asian populations represents a potentially important risk factor for TB³⁰. This might be because, hyperglycemia could play a role to alter the immune responses to the causative organism of tuberculosis in diabetic patients. Some studies shown that poor Diabetes control (indicated by HbA1c level) was associated with differences in the innate and cellular cytokine responses to stimulation with purified protein derivative from *M. tuberculosis*, thus facilitating progression to active TB³¹. Another recent study, that recruited 4,690 elderly diabetic patients in Hong Kong, showed that the patients with greater HbA1c value (>7%) had a hazard risk of active TB that was 3 times increased compared with those who had HbA1c < 7% (HR 3.11; 95% CI 1.63–5.92, $p < 0.01$)³².

In this study, other co morbidities related to diabetes e.g., Hypertension, Nephritis, Neuropathy and Dyslipidemia came up as significant risk factors to develop tuberculosis. The reason might be same as hyperglycemia; compromised and poor immune response; and interaction of the microorganism etc.

The study participants had overall good conception regarding tuberculosis. Several questions were asked to reveal the baseline knowledge of tuberculosis. More than half of the participants came up to have good knowledge.

However there were some limitations of the study. As much as an effort has been made to take samples representative of the whole country, many of the health centers could not be included. The private diabetes centers, which also hold a significant number of patients with diabetes, have not been included in the study. Also all the patients have not been screened for microbiological analysis. Thus the study result could not be generalize to be representative of Nepal.

Thus more comprehensive study is recommended including the private sector, indoor patients. Also it is recommended to have mandatory tuberculosis screening to all indoor and outdoor patients who will be coming to take health care.

Conflict of interest

None

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Appendix

Appendix I: Budget details Budget breakdown details

SN	Particulars	No. of centers	No. per center	Rate	Total in NPR	Total in USD	Remarks
1	Personnel						
	Research Coordinator	7	1	27500	192500	1868.93	Physician or Endocrinologist
	Research Assistant	7	1	17500	122500	1189.32	Medical officer or Resident or Registrar (250/patient)
	Co-investigator honorarium		4	50000	200000	1941.75	
	Sub total				515000	5000.00	
2	Laboratory cost				15800	153.40	
3	Chest X Ray cost				150500	1461.17	Chest X-ray to all patients screened regardless of symptoms
4	Local Travel for consultative meeting 4 persons				387280	3760	1 PI + one of the technical study team members in each of the 4 centers for consultative meeting
4	Local Travel for datasheet collection 5 persons				242050	2350	The administrative study team members will collect the data sheets and make the payments to the research
							coordinators and assistants

5	Communications & Stationary				41200	400.00	
6	Consultative meeting and training				426900	4144.66	Each meeting will include PI, and 1 central team member, 3 local team members, and CoPI will participate in 2 centers, except in centers within Kathmandu where all the team members will participate
7	Fuel				51500	500.00	
8	Monitoring				154500	1500.00	
9	Dissemination				41200	400.00	
10	Publication				51500	500.00	
12	Contingency				4200	40.78	
13	Ethical clearance				40170	390.00	3% of the total in fees to NHRC taken as 13 000 USD
	Sub total				1606800	15600.00	
	GRAND TOTAL				2121800	20600	
	1 USD = 103 NPR						

Screening patients with diabetes mellitus for active tuberculosis in Nepal

Data collection form

Patient ID number:

1. Name (optional):
2. Age:
3. Sex: Male/Female
4. Education: Illiterate/Primary/Secondary/Higher Secondary/Bachelors/Masters
5. Occupation:
6. Smoking: Yes/No
7. Other tobacco use: Yes/No
8. Alcohol consumption: Yes/No
9. Associated comorbidities:
 - a. Hypertension: Yes/No
 - b. Diabetic Nephropathy: Yes/No
 - c. Diabetic Neuropathy: Yes/No
 - d. Dyslipidemia: Yes/No
 - e. Others: Yes/No Please specify: _____
10. Height:
11. Weight:

Diabetes Details

1. Duration of diabetes:
2. Duration of start of diabetes treatment:
3. Most recent values (including unit of measurements)
 - a. Fasting Blood Sugar: _____
 - b. Post Prandial Blood Sugar: _____
 - c. HbA_{1c}: _____

Diabetes control: Poor/Adequate

Current Treatment : Not on treatment

Oral anti-diabetics alone

Oral anti diabetics + Insulin (Insulin dose in units):

4. Knowledge on tuberculosis (multiple responses are acceptable)

a) Tuberculosis is caused by:

- i. Germs
- ii. Hard work
- iii. Weakness
- iv. Hereditary transmission
- v. Others: _____
- vi. Don't know

b) TB is transmitted by:

- i. Through air while coughing/sneezing/talking
- ii. Through blood
- iii. Through sexual intercourse
- iv. Through hands-shake with infected persons
- v. By sharing food with infected persons
- vi. Hereditary transmission
- vii. Others: _____
- viii. Don't know

c) Risk factor for tuberculosis

- i. Smoking
- ii. Air pollution (including indoor air pollution)
- iii. Diabetes
- iv. HIV/AIDS
- v. Other chronic diseases like Renal or liver diseases
- vi. Malnutrition
- vii. Overcrowding
- viii. Others: _____
- ix. Don't know

Symptoms for tuberculosis

- x. Cough more than 2 weeks
- xi. Blood in sputum
- xii. Loss of appetite
- xiii. Weight loss
- xiv. Fever
- xv. Night sweats
- xvi. Others: _____
- xvii. Don't know

d) Tuberculosis is curable

- i. Yes
- ii. No
- iii. Don't know

TB Details

1. Symptom Screening

- a. Cough: Yes/No
- b. Blood in sputum: Yes/No
- c. Fever: Yes/No
- d. Loss of appetite: Yes/No
- e. Weight loss: Yes/No
- f. Night sweats: Yes/No
- g. Any other relevant symptom: Yes/No
 - i. _____
 - ii. _____
 - iii. _____

2. Chest X-ray

- a. Normal
- b. Abnormal
 - i. TB likely
 - ii. TB unlikely

Sputum Examination

c. Not conducted

d. Conducted

- i. AFB Smear I: Negative/Positive
- ii. AFB Smear II: Negative/Positive
- iii. GeneXpert: MTB: Detected/Not detected

Rifampicin: Sensitive/Resistant

3. Other investigations (if available)

a. FNAC

- i. Site:
- ii. Results: Compatible with TB/Not compatible with TB

b. Biopsy

- i. Site:
- ii. Results: Compatible with TB/Not compatible with TB

c. Imaging evidence (Except Chest X-ray)

- i. Site: _____
- ii. Modality: _____
- iii. Results: _____

4. Final TB diagnosis

a. No TB

b. TB present

- i. Pulmonary: Bacteriologically Confirmed/Clinically diagnosed
- ii. Extra-Pulmonary Site: _____

Appendix III: IRB approval



Government of Nepal
Nepal Health Research Council (NHRC)



Ref. No.: 3223

11 July 2018

Dr. Ram Padarath Bichha
Principal Investigator
SAARC TB and HIV/AIDS Centre
Bhaktapur

Re: **Approval of research proposal entitled Prevalence and risk factors of tuberculous disease among diabetic mellitus patients attending selected hospitals in Nepal, 2018**

Dear Dr. Bichha,

It is my pleasure to inform you that the above-mentioned proposal submitted on **18 June 2018** [Reg. no. **340/2018**] please use this Reg. No. during further correspondence] has been approved by Nepal Health Research Council (NHRC) Ethical Review Board on **27 June 2018**.

As per NHRC rules and regulations, the investigator has to strictly follow the protocol stipulated in the proposal. Any change in objective(s), problem statement, research question or hypothesis, methodology, implementation procedure, data management and budget that may be necessary in course of the implementation of the research proposal can only be made so and implemented after prior approval from this council. Thus, it is compulsory to submit the detail of such changes intended or desired with justification prior to actual change in the protocol. Expiration date of this proposal is **November 2018**.

If the researcher requires transfer of the bio samples to other countries, the investigator should apply to the NHRC for the permission. The researchers will not be allowed to ship any raw/ crude human biomaterial outside the country; only extracted and amplified samples can be taken to labs outside of Nepal for further study, as per the protocol submitted and approved by the NHRC. The remaining samples of the lab should be destroyed as per standard operating procedure, the process documented, and the NHRC informed.

Further, the researchers are directed to strictly abide by the National Ethical Guidelines published by NHRC during the implementation of their project proposal and **submit progress report in between and full or summary report upon completion**.

As per your research proposal, the total research amount is \$ 12,000 and accordingly the processing fee amounts to \$ 360. It is acknowledged that the above-mentioned processing fee has been received at NHRC.

If you have any questions, please contact the Ethical Review M & E Section at NHRC.

Thanking you,

Prof. Dr. Anjani Kumar Jha
Executive Chairperson



Trends (Frequency and
Distribution patterns) of
opportunistic infections
associated with
HIV/AIDS Afghanistan
2019

Current trends in the spectrum of opportunistic infections in human immunodeficiency virus infected individuals: Afghanistan

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Background

The emergence and pandemic spread of Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) constitutes to be a major public health challenge that has claimed to kill more than 35 million lives to date. According to the World Health Organization (WHO) factsheet in the year 2017, 940 000 people died from HIV-related causes globally. It also claimed more than 35 million lives so far. Globally at the end of 2018, approximately 36.9 million people living with HIV with 1.8 million people becoming newly infected (WHO, 2019).

HIV targets the immune system of a person and weakens the natural defense systems which fight against infections and some types of cancer. As the virus destroys and impairs the immune cell function, infected individuals gradually become immune-deficient. Immunodeficiency results in increased susceptibility to a wide range of infections. Among the HIV-infected individuals, Opportunistic Infections (OI) are the major cause of morbidity and mortality and the most common complication (Kaplan, 2011). The most advanced stage of HIV infection is AIDS, which can take from 2 to 15 years to develop depending on the individual. AIDS is defined by the development of certain cancers, infections, or other severe clinical manifestations.

HIV causes progressive depletion of the CD4 T cells, which leads to life-threatening OIs or malignancies during the natural course of the disease and research says that more than 90% of OIs are responsible for the development of AIDS morbidities and mortalities (CDC, 2013).

Despite efforts to diagnose and treat HIV infection before patients develop advanced disease, Opportunistic Infections (OIs) continue to occur in the era of effective Antiretroviral Therapy (ART). The relative frequencies and pattern of OIs may vary widely in different countries and even in different areas within the same country (PR, 2014). The spectrum of OIs of a particular locality should be known to

prevent these infections by giving adequate prophylaxis (PR, 2014). ART has reduced the incidence of OI among HIV-infected individuals though the effectiveness of the treatment depends on the patient's adherence to the drugs, stage of the disease, drug resistance, toxicity, and other factors (PR, 2009). At present, the initiation of prophylactic therapies against opportunistic pathogens is mainly based on the absolute CD4 count, as CD4 count measures CD-4 T lymphocytes (T cells), generally accepted as the best indicator of the immediate state of immunologic competence of the patient with HIV infection (6-7).

There are many reports available regarding the pattern of OIs in HIV infected individuals, but as in today's era of continuous changes of pathogens and considering the antimicrobial-resistant, there is not much data of current trend of opportunistic infections in HIV, where we pose different religion, geographical patterns, and culture. Hence this multi centric study is planned to find out the current trend of opportunistic infections considering the AMR in the South Asian Agency for Regional Cooperation (SAARC) member countries Afghanistan, Nepal, and Bhutan.

HIV Situation in Afghanistan

Afghanistan is experiencing a low and concentrated HIV epidemic and HIV is a public health concern among the key affected and vulnerable populations. The drivers of the epidemic, being verified through a consultation process and research, are generally understood to include injecting drug use, partially intersecting with multiple and concurrent sexual partnerships, gender inequalities and violence, and stigma and discrimination. Determinants of vulnerability include a high level of tuberculosis and sexually transmitted infections; drug cultivation, trade, and use; low literacy level and poverty; poor HIV-related knowledge; and limited access to sexual and reproductive education. The Integrated Biological Behavioral Survey (IBBS) in 2012 shows an overall 4.4% HIV prevalence among people who inject drugs. The study also found 0.3%, 0.4%, and 0.7% among Women with High-Risk Behavior, Men with High-Risk Behavior, and Prisoners respectively. The prevalence among the general population 15-49 years of age is estimated at 0.04% (ANPASH, 2012). 11000 People are living with HIV (PHLIV) in Afghanistan. 1500 new infections occurred during 2019. 1044 people living with HIV are on ART throughout the country. The new infections and AIDS-related deaths are on the rise (UNAIDS, 2019).

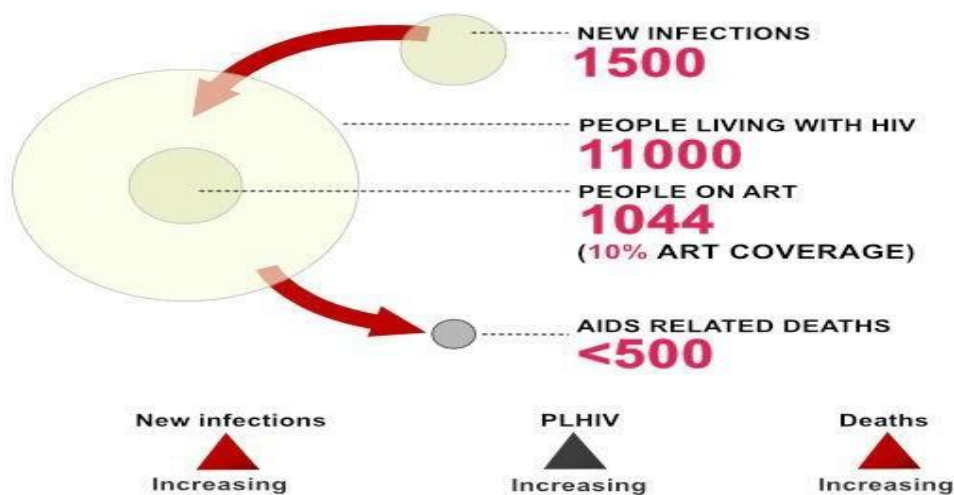
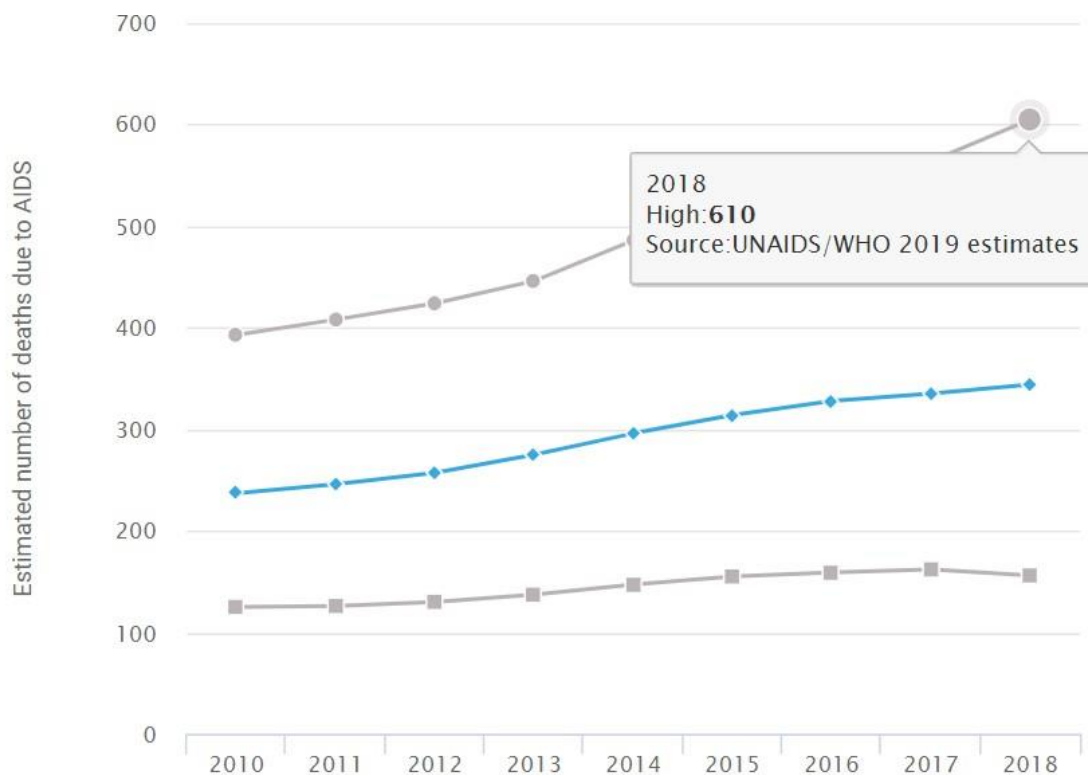


Figure 1 Estimated # of HIV, New Infections, and ART Coverage

ART is currently provided in five sites (Kabul, Herat, Mazar, Nangarhar and Khost) and six extension sites in existing Voluntary Counselling and Testing Centers (VCTCs). Coverage with ART in 2019 is only 13 % of registered HV cases. Women and children together represent only 32% of those currently on ART. Retention at 12 months after initiation of ART is reported at 97% for the PLHIV who initiated ART in 2018. There are data gaps with regards to analysis of retention rates at 24, 36 and 60 months. Infants born to HIV-infected mothers received ARV and co-trimoxizole prophylaxis within two months of birth. Estimated number of deaths due to AIDS has been on increase since last decade (ANPASH, 2019).



↑ Trend since 2010

Figure 2 A Decade Trend of AIDS-Related Deaths, Maximum, Medium, and Minimum

The program consultation reveals that a high number of AIDS-related deaths are due to starting treatment at the final stages of AIDS and OIs. There is no concrete data on the prevalence of various OIs among people living with HIV receiving ART. Therefore, this study intends to find out the current level of specified opportunistic infections in patients taking ART drugs in Kabul ART center. Specifically, the study intends to:

1. To assess the prevalence of active and latent Tuberculosis
2. To identify relative frequencies of certain clinically diagnosed OIs (Hepatitis and others).
3. To identify associated socio-demographic factors adjusting for the country of origin, age, sex, HIV-related risk behaviors, and immune status at the time of study

Methods:

Study design

This is a cross-sectional study. This study aims to assess the prevalence of active Tuberculosis (TB) along with some selected diagnosed opportunistic infections like HCV, HBV, and Syphilis among the people with established HIV.

Study population

People with established HIV infection who are on ART, who has been attending the ART treatment centers in Kabul city, Afghanistan.

Study period

July – December 2019

Sample size and sampling technique

All 541 patients attended the ART centers were screened and necessary lab examinations were conducted.

Inclusion criteria

- Patients ≥ 18 years who are currently on ART
- Visits the ART clinic
- Gives consent to be a part of the study

Exclusion criteria

- An unstable health condition or severe mental health problem
- With any other immune-compromised states e.g., malignancies, organ transplant, on steroids therapy or immunosuppressive therapy
- Non consented

Data collection technique:

The focal person identified 05 research assistants as per his/her need to collect the data. The research assistant were medical doctors. ART nurses and lab technicians were also involved to collect the data. The focal person provided 2 days of training to the research assistants regarding the clinically diagnosed OIs and referral of patients for further investigations. The focal person was responsible for time to time monitoring and give feedback regarding any confusion of the clinical diagnosis of listed OIs.

Study Variables

Age, sex, address, education, risk of transmission, treatment, and related variables, HBV, HCV status.

Data collection tools

The structured data collection tool was to collect the baseline characteristics, details on HIV infection and treatment status, TB symptoms and signs, and results of the sputum or other investigations that were performed.

Supervision and monitoring

10 percent of the data collected was validated by the field visit at least once a week by the focal person.

Ethical considerations

- Institutional approval (IRB) was obtained by the focal person.
- The study participants were briefed regarding the nature, objectives, and method of the study and informed that their participation is voluntary.

Data management and statistical analyses

The data was first entered in the Microsoft Excel 2016 and transferred /export to IBM SPSS (latest version) for analysis. Data were entered by three persons and after 30% of data entry, a validity checkup was done under the supervision of the focal person.

Descriptive statistics were used to summarize the sociodemographic and other relevant characteristics of the study participants using proportions, standard deviation, CI, and statistical tests as appropriate.

Results

There has been an estimated 11000 PLHV in Afghanistan. 1044 PLHV are on treatment. There is a treatment gap of 9956 PLHV which needs to be put on treatment.

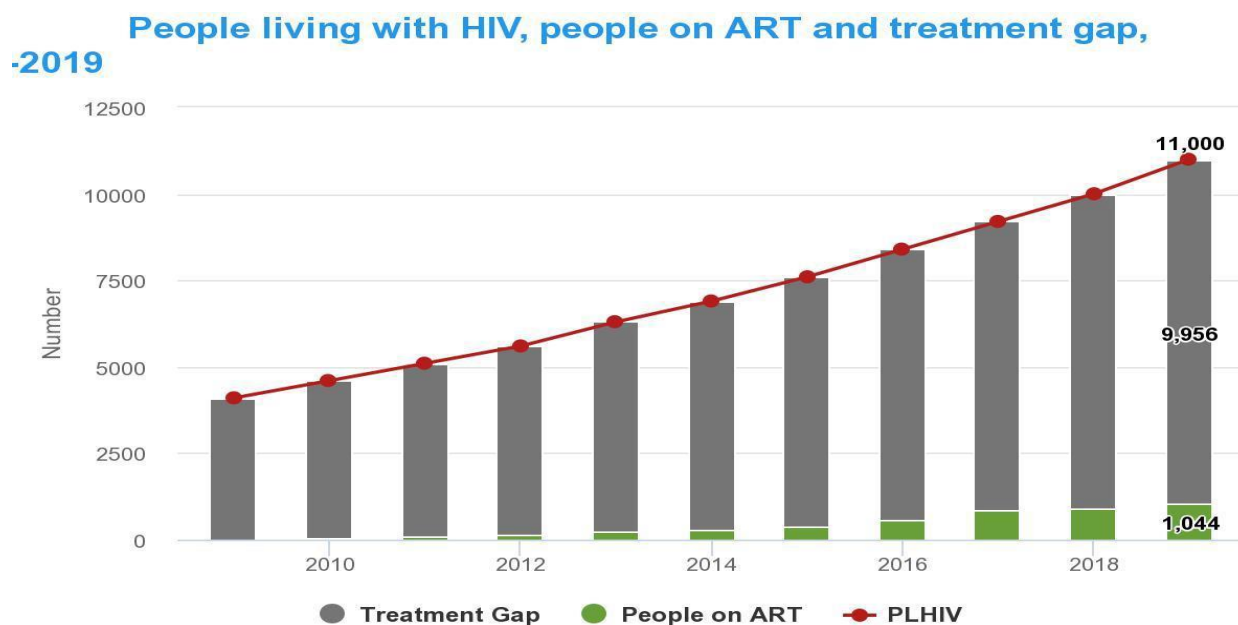


Figure 3 ART Coverage Gap

A total of 541 PLHIV were included in the study making a response rate of 99.9%. 128 (23.66 %) of the respondents were females. The mean age $\pm$ standard deviation (SD) of the study participants was 28 ± 10 years. 192 (35.49%) of the participants were in the age group 20-29 years and 166 (30.68%) were in the age group 30-39 years. 72.09% has not attended any education. 18% of primary education. 237 (56.6%) of the respondents were currently married.

Variable	#	%
Male	413	76.34%
Female	128	23.66%
0-9	19	3.51%
10-19	29	5.36%
20-29	192	35.49%
30-39	166	30.68%
40-49	78	14.42%
50-59	38	7.02%
60-70	19	3.51%
No education	390	72.09%
Primary School	98	18.11%
Secondary School	47	8.69%
High School	6	1.11%
Total	541	100.00%

Table 3 Participants Characteristics

Nearly all, 541 of the study participants reported that they were asked for cardinal signs of TB during their clinic visit. 28 (5.18 %) were found to be positive for active TB using a national algorithm. The statistical analysis showed that age, sex, marital status, educational status, and level of income were significantly associated with TB infection among PLHIV after possible confounders were controlled. Female clients were 1.8 times more likely to be positive for TB than males (0.00001) and those who attended formal education were less likely to have TB infection than those who had never attended formal education (0.00001). The PLHV with better income had significantly lower TB prevalence than PLHIVs with lower income.

Column1	Positive	Negative	The chi-square statistic	P-value	Significant at $p < .05$
Less than 25	3 (13.68) [8.33]	229 (218.32) [0.52]	17.31	0.000032	Yes
25 to 49 years	25 (14.32) [7.96]	218 (228.68) [0.5]			

Male	10 (20.96) [5.73]	395 (384.04) [0.31]	24.04	0.00001	Yes
Female	18 (7.04) [17.07]	118 (128.96) [0.93]			
Single	8 (20.86) [7.93]	395 (382.14) [0.43]	32.76	0.00001	Yes
Married	20 (7.14) [23.15]	118 (130.86) [1.26]			
Primary education	10 (21.39) [6.07]	372 (360.61) [0.36]	27.23	0.00001	Yes
No formal education	18 (6.61) [19.64]	100 (111.39) [1.17]			
Monthly income 5000 AFN Or Less	25 (20.08) [1.2]	363 (367.92) [0.07]	4.4925	0.034044	Yes
Monthly Income More than 5000 AFN	3 (7.92) [3.06]	150 (145.08) [0.17]			

Table 4 Factors Associated with TB and HIV Co-Infection

Almost half of all PLHVs on treatment at Kabul ART suffered from opportunistic infections. HCV has the highest prevalence rate (16 %) and is followed by Syphilis (10%). The multiple OIs were also reported high (11 %).

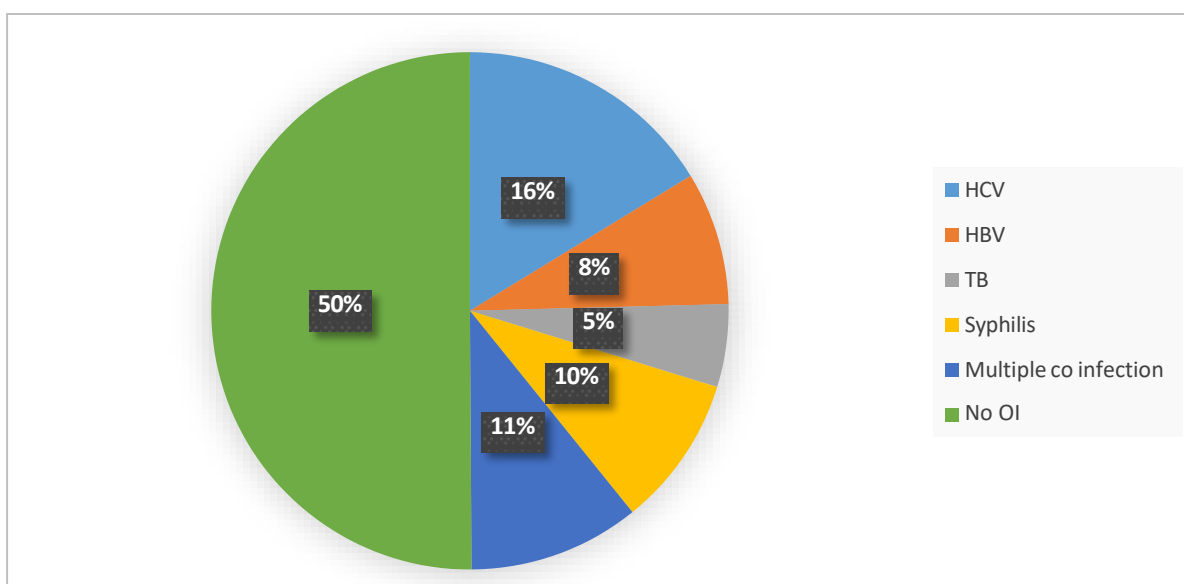


Figure 4 Snapshot of Prevalence of Various OIs in PLHVs

Discussion

HIV is the strongest risk factor for developing TB (Narasimhan, 2013). The risk of developing TB is between 20 and 37 times greater in PLHIV than among those who do not have HIV infection (Briggs, 2019). In response to the dual epidemics of HIV and TB, WHO has recommended several collaborative TB/HIV activities as part of core HIV and TB prevention (WHO, 2012).

In this study, 5.18 % have been reported as having active TB. This result higher than that of an earlier study conducted in Tehran, Iran which reported the TB in PLHIVs at 09 % in 2017 (Rezaeian, 2017).

Results of a study from northeast Ethiopia indicated higher TB/HIV co-infection 24.3%. The results of this study indicated significantly higher proportions of PLHIV were infected with TB when compared with the case of developed countries (Mekonnen, 2015)

A study conducted in Germany among a cohort of 11,693 patients, 233 (~2%) were diagnosed with TB, 62 at enrollment, and 171 during follow-up, respectively. A study conducted in Georgia in 2008 also showed a higher prevalence of TB among PLHIV where 22% of HIV positive peoples have active TB.

In this study, age, sex, marital status, educational status, and level of income were some of the factors that were significantly associated with TB infection among PLHIV. The same factors were reported from the study conducted in Brazil in 2008. Being female sex of the respondent and lower education levels are some of the factors that have a significant association with TB infection in the Brazil study.

In this study, the prevalence of HBV in PLHIV has been reported 8 % which is lower than India (13.7%) and higher than a study conducted in Iran (7.8%) in 2014 (ASKARI, 2014). The burden of HCV among PHLV in Afghanistan (16 %) which is lower than HCV in PLHV in India (50 %) (Kermode, 2016) .

Limitation of the study

The study couldn't determine the latent TB as there was not any policy in place to undertake latent TB analysis.

The study was conducted only in Kabul, so the findings cannot be generalized at national level.

Conclusion

It can be concluded the TB among PLHIV in Kabul is lower when compared to other studies conducted in different countries. However, the prevalence of TB among PLHIV seems to be higher compared to previous findings from the routine data.

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Trends (Frequency and
Distribution patterns) of
opportunistic infections
associated with
HIV/AIDS Bhutan
2019

Assessment of Current Trends in the Spectrum of Opportunistic Infections in Human Immunodeficiency Virus Infected Individuals

A Multi-Centric Study

(Afghanistan, Bhutan & Nepal)

Jointly conducted by the NTCP, DoPH,
Ministry of Health, Bhutan & STAC with



The funding support of the SAARC
Tuberculosis & HIV/AIDS Centre (STAC)
Kathmandu,
Nepal



Afghanistan



Bangladesh



Bhutan



India



Maldives



Nepal



Pakistan



Sri Lanka



SAARC Tuberculosis and HIV/AIDS Centre (STAC)

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List of Abbreviations

AIDS	Acquired Immune Deficiency Syndrome
AMR	Antimicrobial Resistance
ART	Anti-retroviral Therapy
CD4 T cells	Clusters of differentiation 4 Cells
CID	Citizenship Identity Card
CST	Care Support and Treatment Unit, JDWNRH, Thimphu
HISC	Health Information and Service Centre
HIV	Human Immunodeficiency Virus
HPV	Human Papillomavirus
IBBS 2016	Integrated Biological and Behavioural Surveillance 2016
LTBI	Latent Tuberculosis
NACP	National HIV/AIDS Control Program
NTCP	National TB Control Program
OI	Opportunistic Infections
PLHIV	People Living with HIV
PSE 2019	Population Size Estimation of Transgender Women and Men in Bhutan 2019
REBH	Research Ethics Board of Health
SAARC	South Asian Association for Regional Cooperation
STAC	SAARC TB and HIV/AIDS Centre
TB	Tuberculosis
TST	Tuberculin Skin Test
UNAIDS	Joint United Nations Programme on HIV/AIDS
VCT	Voluntary Counseling and Testing
WHO	World Health Organization

Executive summary

We have conducted a cross-sectional bio-behavioral multicentric study to find out the current trend of opportunistic infections in people living with human immunodeficiency virus on antiretroviral therapy, considering the growing antimicrobial resistance in the SAARC member countries of Afghanistan, Bhutan and Nepal. The study also aimed to identify the associated socio-demographic risk factors adjusting for the country of origin, age, sex, HIV-related risk behaviors and immune status of the HIV patients who are on antiretroviral therapy. The findings from this study will provide the required epidemiological information on Active Tuberculosis and Latent Tuberculosis in Bhutan, Nepal and Afghanistan and help us develop appropriate local guidelines on prophylaxis and management of these infections. The study which was implemented in three Regional Referral Hospitals (Thimphu, Gelephu and Mongar), and Health Information and Service Centre of Phuentsholing and Gelephu presents the findings from Bhutan only. The study included Patients ≥ 18 years who were on ART and visited the ART centres regularly. However, HIV patients with other health condition that are likely to compromise their participation in the study due to chronic debilitating medical conditions and those not consenting were not included. The Principal Investigators oriented and provided training to the research assistant and data collectors on interviewing the study participants taking into consideration the sensitivity of the case and document clinically diagnosed opportunistic infections accurately. The data collectors/interviewers have followed the protocol for screening for opportunistic infections and ensured data quality by seeking clarifications on clinical diagnosis of the opportunistic infections listed for this study from the PIs and experts when in doubt. The database was designed and entered into EpiData 3.1 database and descriptive statistical analysis was performed in STATA Corp (Version 14.1) software. The main findings of this study include TB as the main opportunistic infections prevalent among the people living with HIV (PLHIV). The study also describes socio-demography, risk behaviours, preventive therapy, common comorbidity conditions, outcomes of the screening tests and other opportunistic infections besides TB. The discussion mainly stays close to the current study findings and programmatic recommendations based on the key findings are explicitly penned down for a handy reference. Further inferential analysis of the combined data of the multicentric study sites is required to explore the associated socio-demographic risk in consultation with the SAARC TB and HIV/AIDS Centre.

Background and rationale

The emergence and pandemic spread of human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) constitutes to be a major public health challenge which has claimed more than 32 million lives so far. About 770 000 people died from HIV-related causes globally, according to the WHO factsheet in the year 2018¹. There were approximately 37.9 million people living with HIV at the end of 2018 with 1.7 million people becoming newly infected in 2018 globally¹.

The virus that causes AIDS was discovered by scientists in France in 1983 and the routes of transmission were confirmed. The virus which eventually came to be known as the human immunodeficiency virus are of two types, the **HIV-1** the most common type found worldwide, and **HIV-2** found mostly in West Africa².

The HIV targets the immune system of a person and weakens the natural defense systems which fight against infections and some types of cancer. As the virus destroys and impairs the immune cell function, infected individuals gradually go into immunodeficiency state. Immunodeficiency results in increased susceptibility to a wide range of infections. Among the HIV infected individuals, Anti-Retroviral Therapy (OIs) are the major cause of morbidity and mortality and most common complication³. The most advanced stage of HIV infection is AIDS, which can take from 2 to 15 years to develop depending on the individual age (less than 5 years, or over 40 years), other infections, and possibly genetic (hereditary) factors. As HIV infection progresses and immunity declines, people become more susceptible to OIs. AIDS is defined by the development of certain cancers, infections, or other severe clinical manifestations.

HIV causes progressive depletion of the CD4 T cells, which leads to life-threatening OIs or malignancies during the natural course of the disease. Research says that more than 90% of OIs are responsible for the development of AIDS morbidities and mortalities⁴. Despite efforts to diagnose and treat HIV infection before patients develop advanced disease, OIs continue to occur in the era of effective antiretroviral therapy (ART). The relative frequencies and pattern of OIs may vary widely in different countries and even in different areas within the same country⁵. The spectrum of OIs of a particular locality should be known to prevent these infections by giving adequate prophylaxis⁵. The ART has reduced the incidence of OI among HIV infected individuals though the effectiveness of the treatment depends on the patient's

¹ <https://www.who.int/news-room/fact-sheets/detail/hiv-aids>

² https://www.who.int/hiv/about/hiv/fact_sheet_hiv.htm

³ Preventing opportunistic infections in human immunodeficiency virus-infected persons: implications for the developing world. Kaplan JE, Hu DJ, Holmes KK, Jaffe HW, Masur H, De Cock KMAm J Trop Med Hyg. 1996 Jul; 55(1):1-11.

⁴ Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents Guidelines for the prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America 2013. [Accessed November 8, 2014]. Available http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf.

⁵ Shahapur PR, Bidri RC. Recent trends in the spectrum of opportunistic infections in human immunodeficiency virus infected individuals on antiretroviral therapy in South India. *Journal of natural science, biology, and medicine*. 2014;5(2):392-6

adherence to the drugs, stage of the disease, drug resistance, ¹⁵⁴ and other factors⁶. At present, the initiation of prophylactic therapies against opportunistic pathogens is mainly based on the absolute CD4 count, as CD4 count measures CD-4 T lymphocytes (T cells), generally accepted as the best indicator of the immediate state of immunologic competence of the patient with HIV infection^{7,8}.

There are many reports available regarding the pattern of OIs in HIV infected individuals, but as in today's era of continuous changes of pathogens and considering the antimicrobial resistant, there is not much data of current trend of opportunistic infections in HIV in SAARC member countries, where we practice different religion and tradition, varied culture and practice and live in a mix of geo-political situations in the face of ever growing economy in the region. Hence this multi-centric study is planned to find out the current trend of opportunistic infections considering the ever-increasing antimicrobial resistance (AMR) in the SAARC member countries of Afghanistan, Bhutan and Nepal.

Study objectives

General objective

1. To find out the current trend of specified opportunistic infections in patients taking ART drugs.

Specific objectives

1. To assess the prevalence of active Tuberculosis (TB) and Latent Tuberculosis Infections (LTBI) among the HIV patients taking ART drugs.
2. To identify relative frequencies of certain clinically diagnosed OIs such as bacterial, fungal, parasitic and other viral infections.
3. To identify associated socio-demographic risk factors adjusting for country of origin, age, sex, HIV-related risk behaviors, and immune status at the time of study.

Study design and Setting

Study design

A cross-sectional integrated bio-behavioral study was conducted with the aims to assess the prevalence of active and latent tuberculosis infections along with some common clinically diagnosed opportunistic infections among the people with established HIV.

⁶ Effect of "HAART" on CD4+ T lymphocyte counts in HIV seropositive south-Indian individuals--a follow up study. *Shahapur PR, Bairy I, Shivananda PG Indian J Pathol Microbiol. 2005 Apr; (2):270-2.*

⁷ CD4 and CD8 reference counts in normal healthy south-Indian adults. *Shahapur PR, Bairy I, Shivananda PG Indian J Med Microbiol. 2008 Jul-Sep; 26(3):280-1.*

⁸ Ying R, Granich RM, Gupta S, Williams BG. CD4 Cell Count: Declining Value for Antiretroviral Therapy Eligibility [published correction appears in *Clin Infect Dis.* 2016 Jun 15;62(12):1621]. *Clin Infect Dis.* 2016;62(8):1022–1028. doi:10.1093/cid/civ1224

Study setting

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The study was conducted in the following health centers; JDWNRH, Thimphu, CRRH, Gelephu, ERRH, Mongar, General Hospital, Phuentsholing, HISC, Phuentsholing, District Hospital Paro, District Hospital Punakha and District Hospital Wangdue. These study sites cater to higher number of People Living with HIV attending ART treatment. The study was conducted from October 2021 to February 2022.

Study participants / Eligibility criteria

People with established HIV infection who are on ART, who will be attending the ART treatment centers during study period. We have considered ≥ 18 years as the study respondents because it is the legal age for HIV testing as per the current national guidelines for HIV Counseling and Testing. The national guidelines are based on the WHO and UNAIDS recommendations.

a. Inclusion criteria

- Patients ≥ 18 years who are currently on ART
- Regularly on ART
- PLHIV (People living with HIV) on ART with TB

b. Exclusion criteria

- Unstable health condition or severe mental health problem
- With any other immune compromised states e.g., malignancies, organ transplant, on steroids therapy or immunosuppressive therapy.
- Non-compliant for ART/Refuse to ART/Non-adherence to ART
- Non consented
- Reason for refusal to do the Tuberculin Skin Test (TST) were as follows, which disqualified the participants study from taking up TST.

- Worried about injection
- Concerns about skin reaction
- Anxious about positive reaction
- Requirement of two visits
- Did TST in past 3 months

Sample size

Considering the estimated prevalence of TB and HIV co-infection in the Asia-Pacific region at 6.3% (10) with 95% confidence interval and 3% degree of precision, the sample calculated was 252 using the formula.

$n = Z^2P(1-P)/d^2$ Where Z is level of confidence taken as 1.96; P is prevalence, and d is the degree of precision.

An additional 20% non-response rate was estimated and the final sample size of 300 was targeted. Out of 741 cumulative HIV cases detected from 1993 until Nov 2015, there were about 539 PLHIV on ART. The systematic random sampling (SRS) method was used to minimize the sampling variance.

Recruitment and data collection

The Voluntary Counseling and Testing (VCT) focal person managing the PLHIV were identified as the research assistants or data collectors and they have collected all the required data including the sample collection to rule out the active and latent TB besides screening and examination of other OIs. At the national level the study team comprised of one public health expert, one medical doctor and one microbiologist provided all the required technical guidance to the research assistants. The confidentiality of the data was maintained in such a way that the Principal Investigators (PIs) and CO-PIs were not accessible to the PLHIV directly nor met in person. The questionnaire was administered and information were collected by the research assistants with no identifiers of the individual such as names and national Citizenship Identity card (CID), for which the existing patient code numbers were used to identify the study participant.

The PI and his Co-Investigators provided one day training on the theoretical aspects of the study followed by field simulation exercises. The training focused on the core principles and methodology of the study protocol including the knowledge and skills required to collect the data and the examination of the OIs. In the event of any confusion in ascertaining the diagnosis, the decision of the focal person in consultation with the experts was regarded as final.

Study participants were selected by simple random sampling methods using random number generator mobile app. We used the list of registered PLHIV on ART maintained at identified health centers and accordingly generate the random numbers to recruit the study respondents from that sample frame. The list of PLHIV were accessed through the concerned VCT focal person who in turn was the field data collectors for the study. At present the VCT focal person are responsible for carrying out HIV Counseling and Testing including the overall management of HIV cases. Written informed consent were obtained from the study participants before asking any question or taking the samples by the field data collectors (VCT Focal Person). By using a structured, pre-tested questionnaire; socio-demographic data (age, sex, socio economic status, profession, HIV-related risk behaviors, and immune status CD4 count) were collected from the consented patient and patient record in face-to-face interview.

For TB symptom analysis following symptoms was regarded as presumptive tuberculosis:

- Cough for ≥ 2 weeks on its own, or cough of any duration, with any other of fever, weight loss or night sweats (4S)
- Documented or undocumented fever
- Documented or undocumented unexplained weight loss (more than 1.5 Kg in last one month)
- Hemoptysis, chest pain, breathlessness
- Night sweats, tiredness, loss of appetite

Laboratory tests was done as per the existing standard protocols of the country specific laboratory and national program.

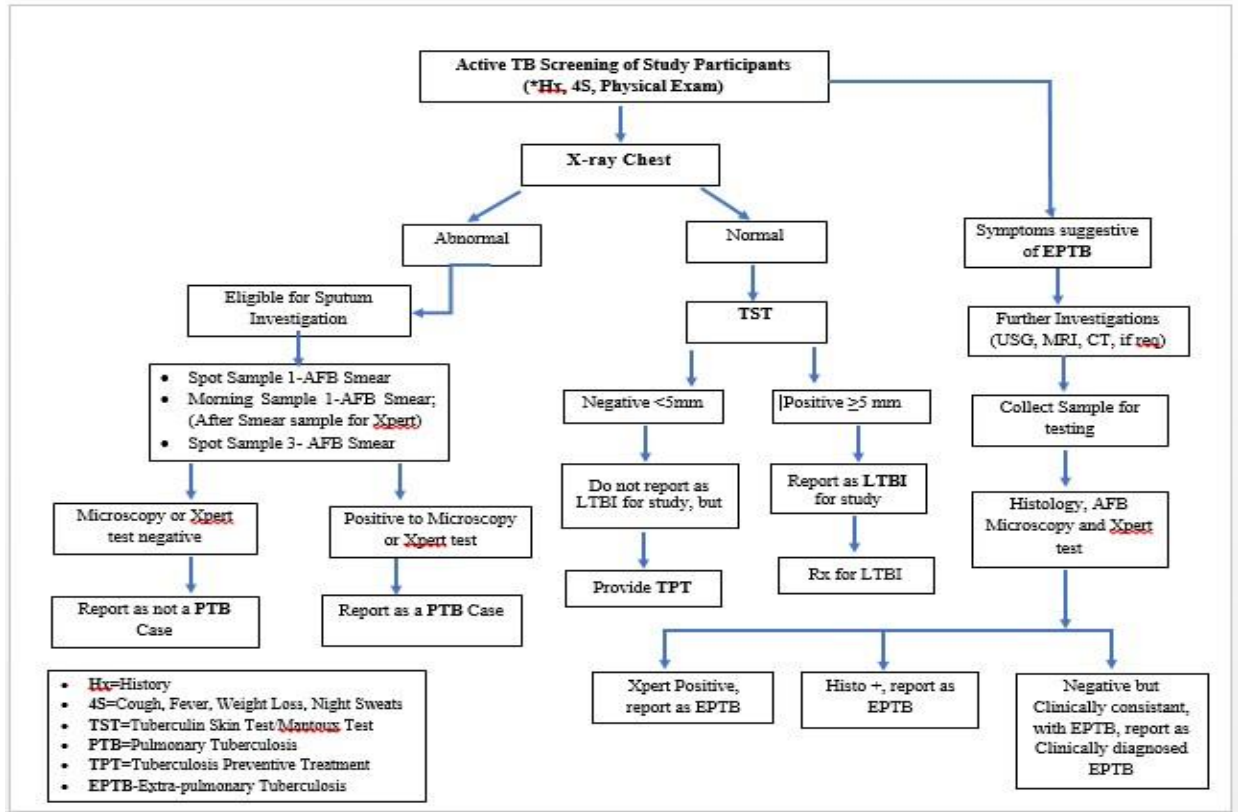


Figure 1 Algorithm for screening study participants for active TB (PTB & EPTB) and LTBI.

Other than active and latent TB following clinically diagnosed infections would be ruled out (Operational definition in Annex 1; Broad category of OIs with classification for reference is in Annex 2).

1. Fungal diseases
 - Oral candidiasis
 - Esophageal candidiasis
 - Vaginal candidiasis
2. Pneumocystis jiroveci pneumonia (PCP)
3. Viral diseases
 - Herpes simplex
 - Herpes zoster (varicella-zoster)
 - Molluscum Contagiosum
 - Genital warts (HPV)

Data collection tools

The structured data collection tool was developed based on the past studies like IBBS 2016, PSE 2019, HIV vulnerability study conducted in 2015 by the National HIV/AIDS Control Program (NACP) and then the existing TB register which collects the baseline characteristics, details on HIV infection and treatment status, TB symptoms and signs, results of the sputum examination and HIV related risk behavior. Pretesting was done in first 15 (5% of the sample size) patients one week before the implementation of the study and necessary changes were made accordingly. The participants involved in pretesting were not included in the main study.

Study Variables

Study variables included age, sex, address, occupation, education, risk behaviour and risk of transmission, hepatitis, herpes infection, TB screening and testing in the past and during the study, treatment, and related variables and priority Opportunistic Infections.

Potential Biases and steps taken to minimize the biases

- Selection and Detection bias: Since the research assistants were from different health centers, there may be a bias in detection of clinically diagnosed OI's and TB symptoms. To minimize this bias and maintain the uniformity, research assistants were trained thoroughly, and a medical specialist consulted in case of a doubtful OIs being diagnosed for confirmation.
- Recall bias – the study participants (patients) may have failed to recall all the symptoms they are screened for. Some probe would/ might be used to help them to recall. E.g., for weight loss, we may ask that whether his/her dress became loose in past one month.
- Response bias – only those symptomatic patients would have been more concerned about their health may participate in the study, but we have followed SRS to reduce it.

Data management and statistical analyses

The database was designed and entered in EpiData 3.1 database and descriptive statistical analysis was performed in STATA Corp (Version 14.1) software. Further inferential analysis of the combined data of the multicentric study sites is required to explore the associated socio-demographic risk in consultation with the STAC.

Supervision and monitoring

Field supervision for data collection was not done but it was remotely monitored in WhatsApp by the investigators although the initial plan was to perform on site data validation through field visits at least once during study time by the Investigators to the ART centers. The reason cited by the program for this was budget insufficiency.

Expected utilization of research findings

- This study report provides advances in scientific knowledge of the epidemiology of active and latent Tuberculosis among the PLHIV on ART in the SAARC countries.
- The report also shows some light on burden of certain specific clinically diagnosed OI's among the HIV infected patients.
- This study can provide recommendations and guidance for developing local guidelines on prophylaxis and management of OIs.
- To generate baseline data to guide the National TB Control Program (NTCP) and National HIV/AIDS Control Program (NTCP) policy in introducing bi-directional active and latent TB screening 159 m among the people infected with HIV in all public and government hos, i SAARC region.

Research ethical clearance

- The research ethical approval was sought from the REBH (Research Ethics Board of Health) for Bhutan vide approval letter *Ref. No. REBH/Approval/2021/024* Dated 4th May 2021;
- The study participants were briefed regarding the nature, objectives and method of the study and their participation was purely voluntary;
- The study participants had the right to withdraw from the study at any point of time;
- Total confidentiality of study participants was assure during and after the study;
- Administrative approval was sought from the Department of Public health and PPD (Policy and Planning Division) of the Ministry of Health;
- All positive patients were referred for standard treatment free of charge at nearest government clinic;
- Hard copies of all the data will be retained with the country focal person with confidentiality under lock and key for at least 5 years;
- Data has been entered giving a unique patient code and analyzed without any identifier such as name and address;
- Soft copy of the entered data will be shared with STAC for final compiling. No patients will be directly contacted or re-examined for this study;

Informed consent

Informed consent for the study participants were obtained by the data collectors of this study. They have explained to the participants about the purpose of the study, the procedures, the potential harms, potential benefits (including incentives), who is the PI and investigators, and whom to contact if they have complaints. Sufficient time was be given to answer participant questions. Participants are also given options to have access to the final report of the study.

Confidentiality

We undertook several measures to maintain participant confidentiality:

1. The study was entirely anonymous. At no time did we ask or collect any personally identifying information (e.g., name, phone number, address, CID) or information that can be used to link identifying information (e.g., medical record number, exact date of clinic visit).
2. Ensured interview and sample collection privacy at a place.
3. Dissemination materials will not name or show their locations.
4. Computer based files will only be made available to personnel involved in the study using access privileges and passwords.
5. Records will be kept in a secured location and only accessible to PI of the study.
6. Before collecting or accessing to any study-related information, personnel must sign statements agreeing to protect the security and confidentiality of participants interviewed or persons seen in the field.
7. Data collector were briefed on ethical practice during data collection, including protections for key populations and maintaining confidentiality at all times.

Results – Findings of the study

Sociodemographic characteristics

A total of 301 people living with HIV (PLHIV) has participated in this study, out of which 144 (47.84%) were males and 157 (52.16%) were females. The mean age of the study participants was 40.67 years (range 19-73 years) with slight right skewness with moderately normal age distribution (Figure 1) and (Table 1). Maximum participants were interviewed at JDWNRH in Thimphu followed by Phuentsholing Hospital and CRRH, Gelephu (Figures 2 & 3).

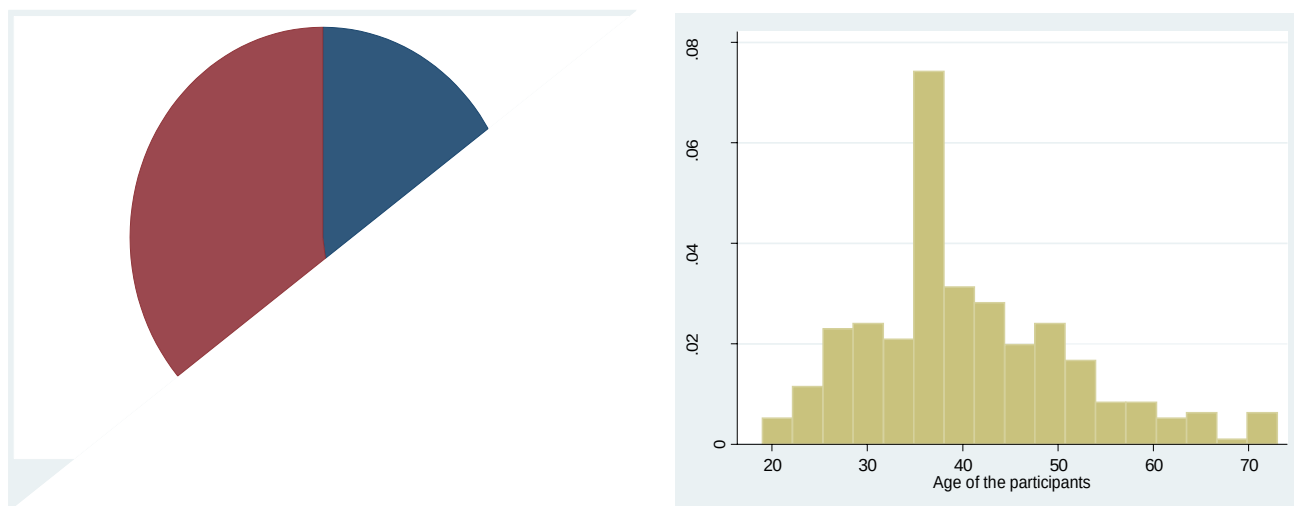


Figure 1 Gender and age distribution of HIV OI study, Bhutan 2021-2022

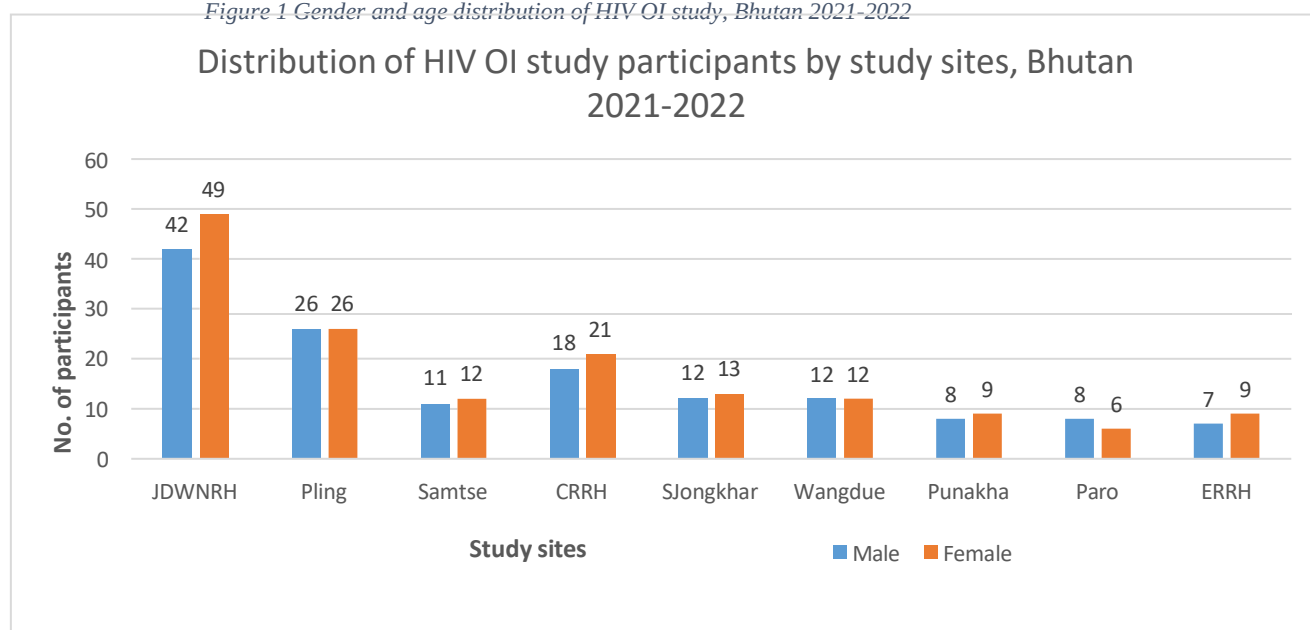


Figure 2 Distribution of HIV OI study participants by gender at study sites, Bhutan 2021-2022

Study sites for HIV Opportunistic Infection 2021-2022, Bhutan

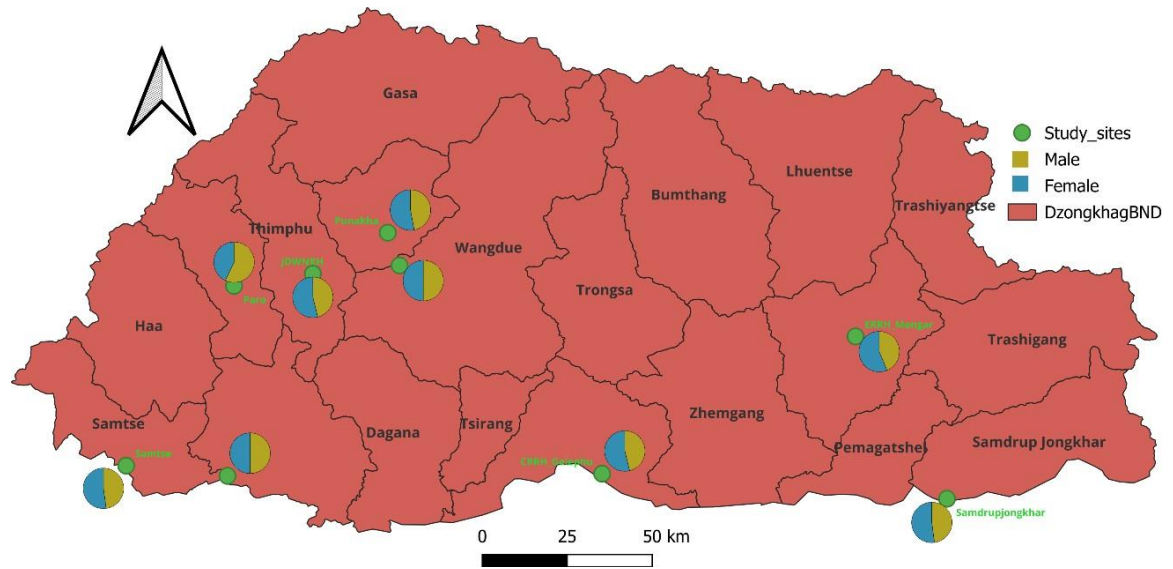


Figure 3 Distribution of HIV OI study participants by gender at each study site, Bhutan 2021-2022

The largest number of participants belong to productive and active age groups of 25 to 39 years with slight female predominance and 40-59 years with slight male predominance, respectively within these two age groups. Most of the study participants were uneducated 32.56% (98/301) with maximum cases seen among the corporate and private business group 31.56% (95/301) and were mostly married currently (Table 1).

Table 1 Sociodemographic characteristics of the HIV OI study participants, 2021-2022

Variables	Male (n=144)	Female (n=157)	Total (n=301)	Overall Percentage(%)	P- value
Age	<i>n (%)</i>	<i>n (%)</i>			0.013
0-17 years	0 (0.00)	0 (0.00)	0	0	
18-24 years	5 (3.47)	7 (4.46)	12	3.99	
25-39 years	50 (34.72)	82 (52.23)	132	43.85	
40-59 years	74 (51.39)	59 (37.58)	133	44.19	
>=60 years	15 (10.42)	9 (5.73)	24	7.97	
Sex					0.000
Male	144 (47.84)		144	47.84	
Female		157 (52.16)	157	52.16	
Education					0.000
No education	33 (22.92)	64 (40.76)	98	32.56	
NFE	1 (0.69)	12 (7.64)	14	4.65	
Primary (PP-VI)	35 (24.31)	23 (14.65)	58	19.27	
LSS (VII-VIII)	18 (12.50)	12 (7.64)	30	9.97	
MSS (IX-X)	16 (11.11)	21 (13.38)	37	12.29	

HSS (XI-XII)	14 (9.72)	16(10.19)	30	9.97	
Tertiary (Degree & above)	13 (9.03)	8 (5.10)	19	6.31	
Monastic	14 (9.72)	1 (0.64)	15	4.98	
Religion					0.326
Buddhist	125 (86.81)	145 (92.36)	270	89.7	
Muslim	1 (0.69)	0 (0)	1	0.33	
Hindu	16 (11.11)	10 (6.37)	26	8.64	
Christian	2 (1.39)	1 (0.64)	3	1	
Others	0 (0.00)	1 (0.64)	1	0.33	
Occupation					0.00
Civil service	8 (5.56)	11 (7.01)	19	6.31	
Corporate & Private business	54 (37.50)	40 (25.48)	95	31.56	
Uniform personnel	8 (5.56)	0 (0.00)	8	2.66	
Religious body	2 (1.39)	0 (0.00)	2	0.66	
Farmer	25 (17.36)	35 (22.29)	61	20.27	
Driver	14 (9.72)	0 (0.00)	14	4.65	
Dependent (Student & home makers	26 (18.06)	67 (42.68)	92	30.56	
Migrants & labours	7 (4.86)	4 (2.55)	10	3.32	
Average monthly family income					0.000
Less than 7000	27 (18.75)	38 (24.2)	65	21.59	
7001 to 14000	38 (26.39)	33 (21.02)	71	23.59	
14001 to 35000	64 (44.44)	37 (23.57)	101	33.55	
More than 35000	10 (6.94)	7 (4.46)	17	5.65	
Dependent	5 (3.47)	42 (26.75)	47	15.61	
Marital status					0.077
Currently married	96 (66.67)	103 (65.61)	199	66.11	0.043
Never married	15 (10.42)	5 (3.18)	20	6.64	
Divorced or separated	25 (17.36)	35 (22.29)	60	19.93	
Widow or Widower	6 (4.17)	13 (8.28)	19	6.31	
Living together	1 (0.69)	1 (0.64)	2	0.66	
No answer	1 (0.69)	0 (0.00)	1	0.33	

Risk behaviour

About 11% of the study participants reported smoking while many of them who have said they have used alcohol have used it 1-3 times a week in the last one month. Only one PLHIV has reported using psychotropic substance (Table 2).

Table 2 Risk behaviour of the HIV OI study participants in Bhutan, 2021-2022

Variables	Male (n=144)	Female (n=157)	Total (n=301)	Overall Percentage(%)	P- value
Smoking	<u>n (%)</u>	<u>n (%)</u>			0.014
Yes	23 (15.97)	11 (7.01)	34	11.3	
No	121 (84.03)	146 (92.99)	267	88.7	
Alcohol use					0.003
Yes	62 (43.06)	42 (26.75)	104	34.55	
No	82 (56.94)	115 (73.25)	197	65.45	
Alcohol use frequency, past 4 weeks					0.942
5 to 6 days a week	11 (17.74)	5 (11.9)	16	15.38	
1 to 4 days a week	6 (9.68)	4 (9.52)	10	9.62	
1 to 3 days a week	25 (40.32)	19 (45.24)	44	42.31	
Once a month	19 (30.65)	13 (30.95)	32	30.77	
Don't want to reveal	1 (1.61)	1 (2.38)	2	1.92	
Taken any psychotropic substance/SUD?					0.367
Yes	1 (0.69)	0 (0.00)	1	0.33	
No	143 (99.31)	156 (99.36)	299	99.34	
Don't know	0 (0.00)	1 (0.64)	1	0.33	
Shared Inj. needle in last 12 months?					
Yes	0	0 (0.00)	0	0 (0.00)	
No	144(100)	157 (100)	301	1000	

Sexual behavioural risk assessment

The most preferred mode of sexual intercourse expressed by the study participants was vaginal which was about 97%. While condom usage with different sexual partners varied, about 2% to 3% reported that they have never used condom with casual, paying, or transactional partners (Table 3).

Table 3 Sexual behavioral risk assessment of the HIV OI study participants, Bhutan 2021-2022

Variables	Male (n=144)	Female (n=157)	Total (n=301)	Overall Percentage(%)	P- value
Type of sex	<u>n (%)</u>	<u>n (%)</u>			0.239
Vaginal	138 (95.83)	153 (97.45)	291	96.68	
Anal	3 (2.08)	0 (0.00)	3	1	
Both vaginal and anal	0 (0.00)	1 (0.64)	1	0.33	
Dont want to reveal	3 (2.08)	3 (1.91)	6	1.99	
Used condom with spouse					0.986
Always	44 (30.56)	45 (28.66)	89	29.57	
Not always	15 (10.42)	17 (10.83)	32	10.63	
Never	33 (22.92)	36 (22.93)	69	22.92	
Not applicable	52 (36.11)	59 (37.58)	111	36.88	
Used condom with regular partner					0.815
Always	10 (6.94)	8 (5.1)	18	5.98	
Not always	2 (1.39)	1 (0.64)	3	1	
Never	5 (3.47)	5 (3.18)	10	3.32	
Not applicable	127 (88.19)	143 (91.08)	270	89.7	
Used condom with casual partner					0.093
Always	13 (9.03)	11 (7.01)	24	7.97	
Not always	5 (3.47)	0 (0.00)	5	1.66	
Never	5 (3.47)	4 (2.55)	9	2.99	
Not applicable	121 (84.03)	142 (90.45)	263	87.38	
Used condom with paying partner (Sex workers)					0.407
Always	2 (1.39)	1 (0.64)	3	1	
Not always	1 (0.69)	0 (0.00)	1	0.33	
Never	6 (4.17)	3 (1.91)	9	2.99	
Not applicable	135 (93.75)	153 (97.45)	288	95.68	
Used condom with transactional partners					0.121
Always	0 (0.00)	1 (0.64)	1	0.33	
Never	7 (4.86)	2 (1.27)	9	2.99	
Not applicable	137 (95.14)	154 (98.09)	291	96.68	

CD4 counts, TPT and CPT

During the time of the study, most of the participants have shown their latest CD4 count higher than 200 cells/mm³ whereas TPT and CPT courses completed was 32% and 25% respectively. About 13% of the participants have also reported comorbidity conditions such as diabetes, heart, lungs, and kidney diseases (Tables 4 & 5).

Table 4 Latest CD4 count, TB Preventive Treatment (TPT) and Co-trimoxazole (CPT) status of the HIV OI study participants, Bhutan 2021-2022

Variables	Male (n=144)	Female (n=157)	Total (n=301)	Overall Percentage(%)	P-value
What is the latest CD4 count (cells/mm ³)	<u>n (%)</u>	<u>n (%)</u>			0.26
Less than 200	13 (9.03)	14 (8.92)	27	8.97	
201 to 500	86 (59.72)	80 (50.96)	166	55.15	
More than 500	45 (31.25)	63 (40.13)	108	35.88	
IPT/TPT completed					0.296
Yes	45 (31.25)	51 (32.48)	96	31.89	
No	93 (64.58)	104 (66.24)	197	65.45	
Currently on IPT/TPT	6 (4.17)	2 (1.27)	8	2.66	
		165			
CPT course completed				0.481	
Yes	35 (24.31)	41 (26.11)	76	25.25	
No	91 (63.19)	103 (65.61)	194	64.45	
Currently on CPT	18 (12.50)	13 (8.28)	31	10.3	

IPT=Isoniazid Prophylaxis Treatment

Comorbidity conditions

Table 5 comorbidity status of the HIV OI study participants, Bhutan 2021-2022

Variables	Male (n=144)	Female (n=157)	Total (n=301)	Overall Percentage(%)	P-value
Chronic illness	<u>n (%)</u>	<u>n (%)</u>			0.821
Yes	18 (12.50)	21 (13.38)	39	12.96	
No	126 (87.50)	136 (86.62)	262	87.04	
Diabetes					0.638
Yes	5 (3.47)	4 (2.55)	9	2.99	
No	139 (96.53)	153 (97.45)	292	97.01	
Heart diseases					0.95
Yes	18 (12.50)	20 (12.74)	38	12.62	
No	126 (87.50)	137 (87.26)	263	87.38	
Respiratory diseases					0.605
Yes	8 (5.56)	11 (7.01)	17	5.65	
No	136 (94.44)	146 (92.99)	284	94.35	
Renal disease					0.951
Yes	1 (0.69)	1 (0.64)	2	0.66	
No	143 (99.31)	156 (99.36)	299	99.34	

TB symptoms analysis

Figures 4 & 5 shows Pulmonary and Extra-pulmonary TB symptom analysis among the participants. A very low percentage of the participants have shown a few symptoms of PTB and EPTB respectively and maximum of the participants have not shown symptoms of TB disease.

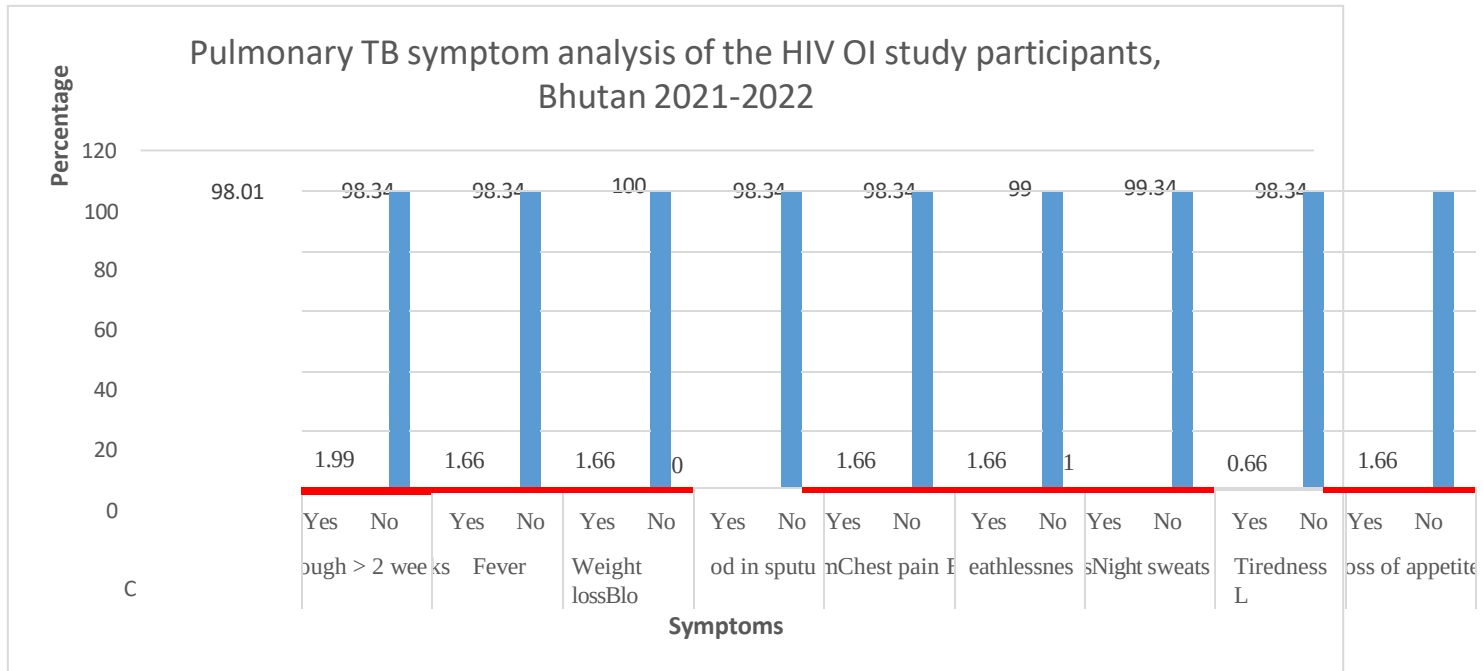


Figure 4 Pulmonary TB symptom analysis of the HIV OI study participants, Bhutan 2021-2022

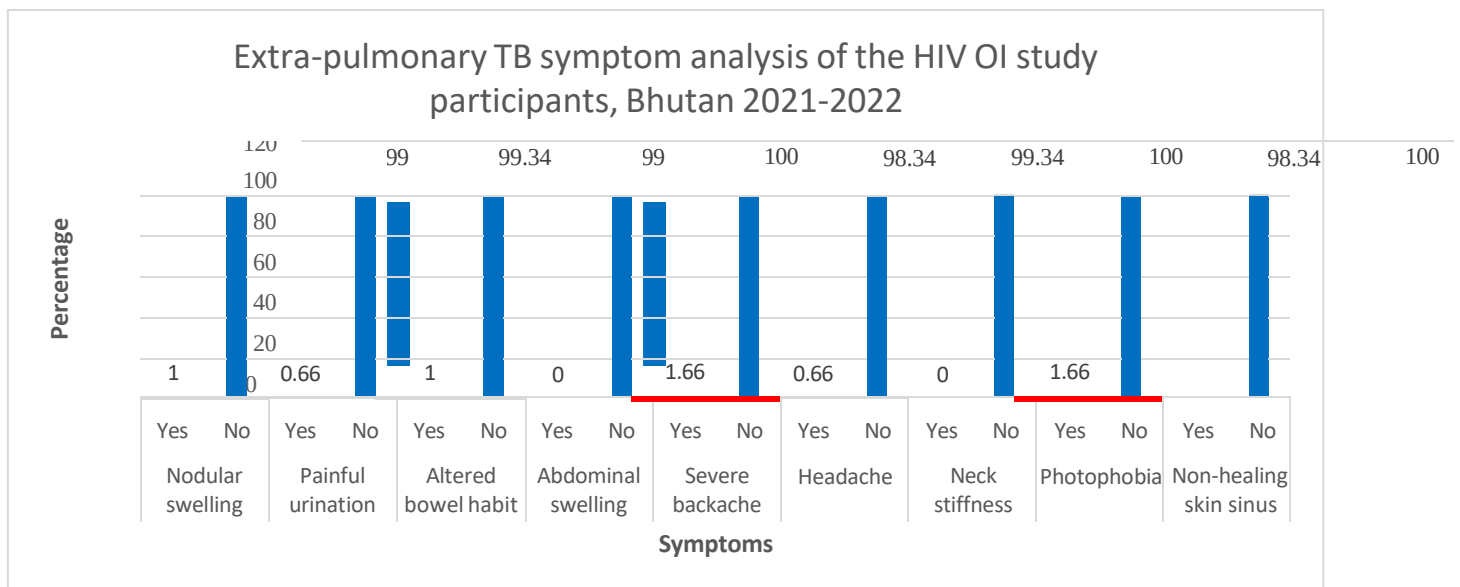


Figure 5 Extra-pulmonary TB symptom analysis of the HIV OI study participants, Bhutan 2021-2022

Result of the TB tests performed

As shown in (Figure 6), nine (9/301) has showed X-ray chest findings suggestive of TB and five (5/301) was found to be sputum positive. Two out of five (2/5) sputum positives have tested positive to Xpert MDR/RIF TB test. TST was positive in 28 of the participants (28/301).

Frequency of positive and negative TB diagnostic tests among HIV OI study participants, Bhutan 2021-2022

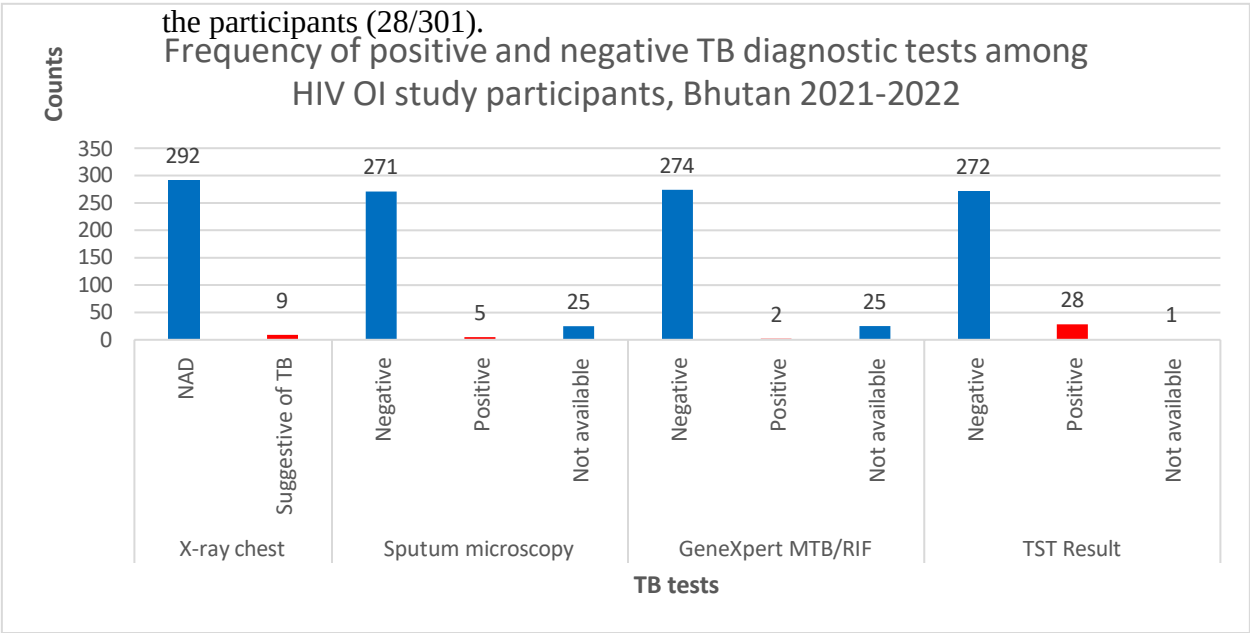


Figure 6 Frequency of positive and negative TB diagnostic tests among HIV OI study participants, Bhutan 2021-2022

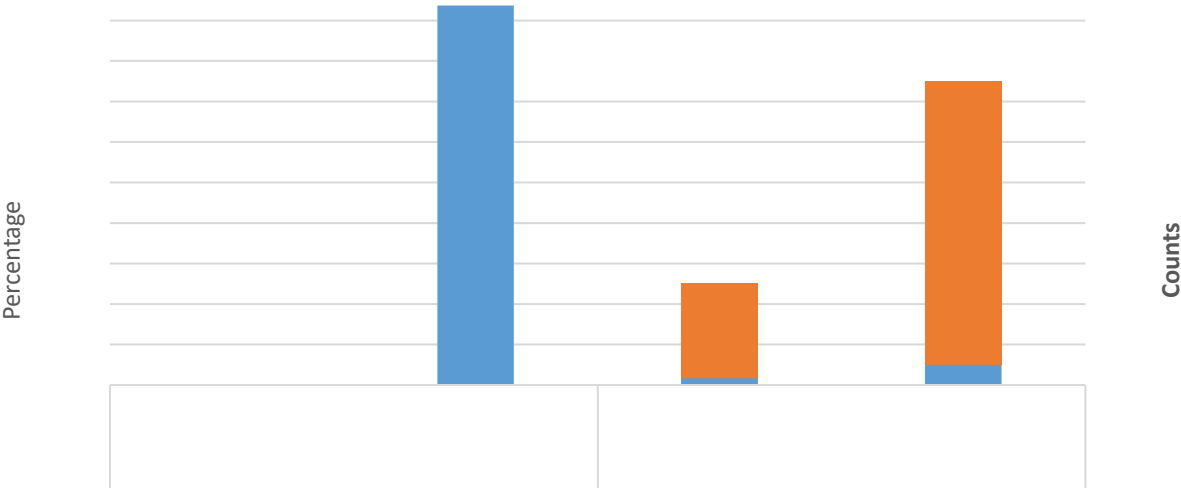


Figure 7 Distribution of PTB and EPTB among the HIV OI study participants, Bhutan 2021-2022

TB prevalence among the PLHIV on ART

As the main findings of the study, the overall TB prevalence as opportunistic infection among PLHIV on ART was at 7% (20/301) with EPTB predominance of three-fourths or 75% (15/20). Pulmonary TB comprised of one-quarter or 25% (5/20) (Figure 7). Most of the PLHIV have suffered from TB as co-infection prior to the conduct of the study while on ART. Data collectors have verified through documentary evidence in the field and the investigators have further validated with the records maintained at the Care Support and Treatment Unit (CST) of JDWNRH, Thimphu.

Besides TB, other opportunistic infections detected were herpes simplex in about 2% (6/301) followed by oral candidiasis, vaginal candidiasis and genital warts of 0.66% each (Figure 8).

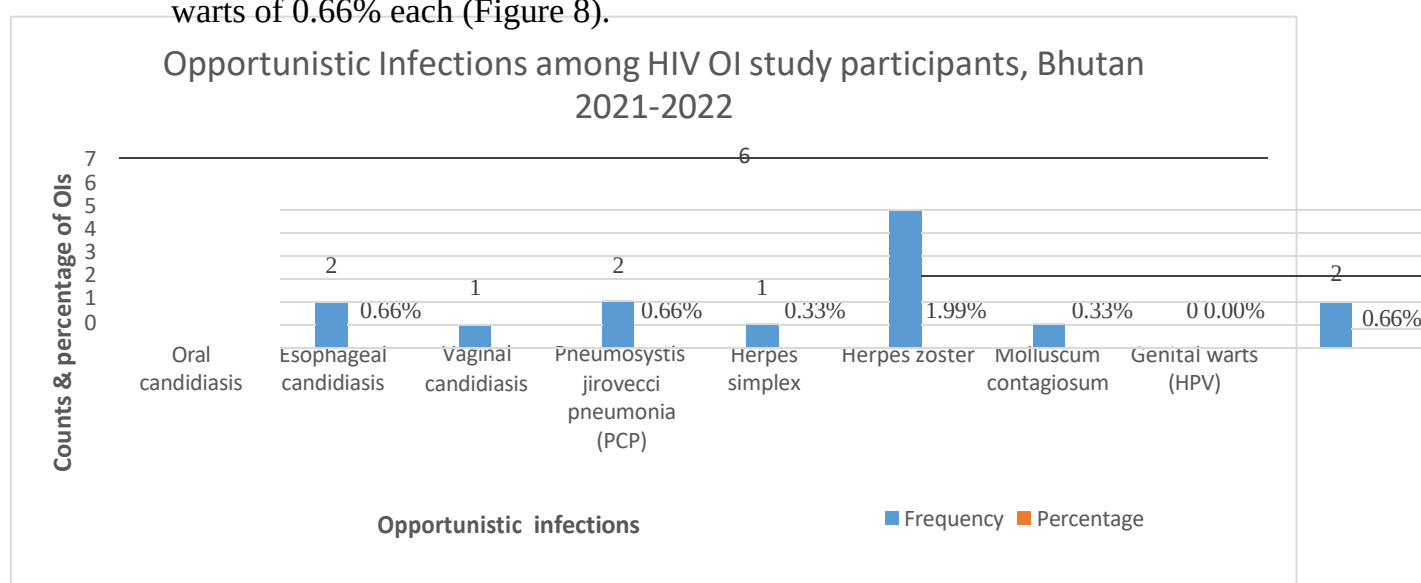


Figure 8 Opportunistic Infections among HIV OI study participants, Bhutan 2021-2022

Key Findings of the Study

Socio-demography

- Of 301 PLHIV who participated in this study, female participants 52% were slightly higher than male participants 48%;
- The largest number of participants belong to productive and active age group of 25 to 39 years (44%) with slight female predominance;
- A growing number of PLHIVs were also seen in the group of 40-59 years (44%) with slight male predominance, indicating a shift in age group.
- Most of the study participants were uneducated 33% (98/301);
- Maximum cases were seen among the corporate and private business group 32% (95/301);
- PLHIV participants who were currently married was 66%;

Risk behaviours

- About 11% of the study participants has reported of current smoking habits;
- About 35% reported to have used alcohol, out of which, 42.31% said they have used it 1-3 times a week in the last one month;
- Only one PLHIV has reported of using psychotropic substance;
- The most preferred mode of sexual intercourse expressed by the study participants was vaginal which was about 97%.
- While condom usage with different sexual partners varied, about 2% to 3% reported that they have never used condom with casual, paying or transactional partners.

IPT/TPT & CPT – Preventive Therapy

- TB preventive treatment in the form of Isoniazid Preventive Therapy (ITP) or TB Preventive Treatment (TPT) was quite low at 32%;
- Co-trimoxazole Preventive Therapy (CPT) was at 25%, much lower than TPT during this study period;

Comorbidity conditions

- About 13% of the participants have also reported comorbidity conditions such as diabetes 2.99% (9/301), cardiovascular 12.62 (38/301), respiratory 17 (5.65/301), and kidney diseases 0.66 (2/301).

Outcome TB screening tests during this study

- Nine (9/301) participants showed X-ray chest findings suggestive of TB;
- Five (5/301) participants were detected positive in sputum microscopy;
- Two out of five (2/5) sputum positives have tested positive to Xpert MDR/RIF TB test.
- Tuberculin Sensitivity Test (TST) was positive in 28 of the participants (28/301).

Opportunistic Infection (OI) prevalence among PLHIV on ART

- The overall TB prevalence as an opportu 170 ifection among PLHIV on ART was 7% (20/301) with EPTB predominance of three-fourths or 75% (15/20);
- Besides TB, other opportunistic infections detected was herpes simplex in about 2% (6/301) followed by oral candidiasis, vaginal candidiasis and genital warts of 0.66% each;

Discussions

In our study to identify and explore the epidemiology of OIs among HIV-infected individuals who are currently on ART in Bhutan, we report a concerning prevalence of 7% TB as an opportunistic infection. Of the 20 TB cases detected among PLHIV on ART, EPTB predominated, highlighting the need for increased vigilance in detecting and managing EPTB in PLHIV.

HIV infection continues to pose significant challenges worldwide, particularly in low-resource settings where access to healthcare and preventive measures may be limited. Understanding the socio- demographic characteristics, risk behaviors, preventive therapy uptake, comorbidity conditions, and prevalence of OI among PLHIV is crucial for informing targeted interventions and improving health outcomes. This discussion delves into the key findings of a recent study on HIV opportunistic infections and their implication.

The prevalence of TB-HIV co-infection varies widely. In a meta-analysis based on systematic review of published articles, the estimates of TB prevalence among HIV-positive patients ranged from 2.9% to 64.5%, with pooled prevalence of TB/HIV co-infection of 23.51%. A substantial heterogeneity was noticed with prevalence of TB/HIV co-infection of 31.25% in African countries, 17.21% in Asian countries, 20.11% in European countries, 25.06% in Latin America countries and 14.84% in the USA⁹.

In a study conducted in eastern India, the prevalence of HIV/TB co-infection was noted at 12.3%¹⁰ while another study from India showed that 17% of HIV infected persons had tuberculosis co-infection¹¹.

According to the 2020 Global TB Report¹², PLHIV are 18 times more likely to develop active TB disease than people without HIV. In 2019, of the estimated 10 million (range, 8.9–11.0 million) people who developed TB worldwide, 8.2% were PLHIV. Even when on ART, PLHIV are 3 times more likely to die during TB treatment and continue to suffer disproportionately from this preventable and curable disease¹³.

Most of the PLHIV have suffered from TB as co-infection prior to the conduct of the study while on ART. The data collectors have verified TB diagnosis and treatment cards through documentary evidence in the field and the investigators have further validated with the records maintained at the Care Support and Treatment Unit (CST) of JDWNRH, Thimphu.

⁹ Gao J, Zheng P, Fu H. Prevalence of TB/HIV co-infection in countries except China: a systematic review and meta- analysis. *PLoS One*. 2013;8:e64915.

¹⁰ Prevalence of HIV infection among tuberculosis patients in Eastern India <https://www.sciencedirect.com/science/article/pii/S1876034113000671>

¹¹ Giri PA, Deshpande JD, Phalke DB. Prevalence of Pulmonary Tuberculosis Among HIV Positive Patients Attending Antiretroviral Therapy Clinic. *N Am J Med Sci*. 2013 Jun;5(6):367-70. doi: 10.4103/1947-2714.114169. PMID: 23923111; PMCID: PMC3731868. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3731868/>

¹² [2020 Global TB Report](#)

¹³ <https://www.who.int/teams/global-hiv-hepatitis-and-stis-programmes/hiv/treatment/tuberculosis-hiv>

In addition to TB, other opportunistic infections, including herpes simplex, oral candidiasis, vaginal candidiasis, and genital warts, were reported among PLHIV. These infections underscore the importance of comprehensive HIV care, including routine screening, prophylaxis, and treatment of opportunistic infections, to improve the quality of life and reduce morbidity among PLHIV.

Socio-Demography

In 2021 when we conducted this study, there were an overall of 795 HIV cases detected with 599 PLHIV. The study sampling frame consisted of HIV cases detected from as early as 2002 till 2021. The study sample consisted of 301 PLHIV, with a slight female predominance (52%). Assessing the demographic characteristics of the study population is critical to understanding Bhutan's HIV epidemic. The study found almost a balanced gender distribution among participants, which suggests that both men and women are affected by HIV/AIDS in Bhutan. Notably, the largest proportion belonged to the productive age group of 25 to 39 years as well as among the age group of 40-59 years indicating a shift in age distribution towards older age groups. This trend underscores the importance of addressing HIV prevention and treatment across all age groups, including older individuals. Additionally, a significant proportion of participants were uneducated (33%) and engaged in corporate or private business (32%), highlighting potential socioeconomic barriers to accessing healthcare and adherence to treatment.

This highlights the need for gender-sensitive interventions that are tailored to the unique socio-cultural context of the country. Therefore, it is essential to develop and implement interventions that are sensitive to gender differences and effectively target the distinct challenges faced by both men and women in accessing HIV prevention, detection, treatment, and care services.

To address this, enhancing sexual health education programs and ensuring easy access to preventive measures such as condoms are crucial steps towards mitigating HIV transmission risks within Bhutanese communities. Establishing comprehensive sexual health education programs and promoting the accessibility of preventive measures, can effectively reduce HIV transmission rates and empower individuals to make informed decisions regarding their sexual health.

Risk behaviour

Concerningly, a notable proportion of PLHIV reported engaging in risky behaviours, such as smoking (11%) and alcohol use (35%). Substance abuse, although reported by only one participant, remains a concern due to its potential impact on treatment adherence and disease progression. The identification of risky behaviours such as smoking and alcohol consumption among participants emphasizes the urgent need for targeted public health interventions. Although both the risk factors are important but higher prevalence of alcohol intake by the diagnosed PLHIV requires immediate attention. These behaviours are known to be the key contributors to HIV/AIDS progression and increased susceptibility to opportunistic infections, requiring comprehensive strategies to address them effectively. Therefore, establishing targeted

interventions and counseling is essential for comprehensive HIV care, tailored treatment adherence support programs that address substance use disorders, counseling services, and promote healthy behaviours are essential to optimize treatment outcomes and reduce the risk of opportunistic infections among the diagnosed PLHIV in Bhutan's setting.

Additionally, the reported low condom usage with casual, paying and transactional partners is one of the risky behaviours among the diagnosed PLHIV in Bhutan. Therefore, it is paramount to enhance sexual health education programs and ensure easy access to preventive measures among PLHIV communities and other high-risk individuals to mitigate HIV transmission risks within Bhutanese communities and beyond. Promoting the accessibility of preventive measures such as greater accessibility of condoms, can effectively reduce HIV transmission rates and empower individuals to make informed decisions regarding their sexual health.

Preventive therapy

The uptake of preventive therapies, including TB TPT and CPT, was suboptimal, with only 32% and 25% of participants receiving these interventions, respectively. Given the high burden of TB among PLHIV and the benefits of preventive therapy in reducing morbidity and mortality, efforts to improve access and adherence to these interventions are warranted.

Assessment of clinical parameters, including CD4 counts and completion of preventive therapy offers crucial insights into the overall health status and treatment adherence among PLHIV in Bhutan. While relatively high CD4 counts suggest positive ART outcomes, suboptimal completion rates for TPT and CPT highlight the importance for tailored adherence support programs. These programs should be designed to address our unique challenges faced by PLHIV in Bhutanese settings, ensuring optimal health outcomes and reducing the risk of opportunistic infections.

Until 2012, the NTCP followed the earlier Guidelines wherein clients were put on ART treatment based on CD4 count thresholds (CD4 count < 200; 2005-2008 Guidelines, CD4 count <350; 2012 Guidelines). The Program had initiated ART irrespective of CD4 count from 2016 onwards (*Personal communication, Jurmi Drukpa, Care Support and Treatment Unit (CST), JDWNRH, Thimphu*).

Comorbidity conditions

Comorbidity conditions, including diabetes, cardiovascular disease, respiratory diseases, and kidney diseases, were reported by a subset of participants. These conditions may complicate HIV management and increase the risk of adverse outcomes. Comprehensive care for PLHIV should include screening, management, and integrated treatment approaches for comorbidities to optimize health outcomes.

Outcome of TB screening tests

Furthermore, the analysis of tuberculosis (TB) symptoms and diagnostic tests underscores the persistent burden of TB among PLHIV in Bhutan, despite the availability of uninterrupted supply of ART. The prevalence of TB, EPTB highlights the need for strengthened TB/HIV collaborative efforts both at all levels of care and integrated TB screening and treatment services within HIV care settings. Such initiatives are essential to effectively address the co-occurrence of tuberculosis and HIV among PLHIV in Bhutan and improve the overall patient treatment outcomes. The utilization of advanced diagnostic tools, such as

Xpert MDR/RIF TB test, can enhance the accuracy and timeliness of TB diagnosis, facilitating prompt initiation of treatment and thus interrupting the spread of the disease.

Opportunistic Infection prevalence

While TB remains as the main co-infection among PLHIV (7% prevalence), the presence of other OIs highlights the need for comprehensive healthcare services tailored to address a spectrum of infectious diseases among the PLHIV. The detection of herpes simplex in about 2% of the study population suggests the importance of addressing viral infections alongside HIV/AIDS management. Herpes simplex can exacerbate HIV progression and increase the risk of transmission, emphasizing the necessity for integrated care approaches that encompass both HIV and viral infection management. Similarly, the occurrence of oral candidiasis, vaginal candidiasis, and genital warts, though at lower frequencies, underscores the vulnerability of PLHIV to fungal and viral infections. These OIs not only impact the quality of life of PLHIV but also signify potential gaps in preventive measures and in routine healthcare service delivery.

Integrating screening, prevention, and treatment services for a range of OIs within HIV care settings is important to addressing the comprehensive healthcare needs of PLHIV in Bhutan. This approach requires collaboration between the HIV/AIDS Control programs, TB Control Programs, Medical, Surgical, Dermatology, Orthopedic, ENT, Paediatrics, Gastroenterology, Obstetrics and Gynaecology, Psychiatry, Radiology, Pathology and Laboratory Departments and other allied healthcare services to ensure timely detection, management, and prevention of OIs among PLHIV.

Conclusion

This study indicates that tuberculosis is the commonest opportunistic infection among the HIV-infected patients receiving ART in Bhutan. The findings accentuate the urgent need for comprehensive public health strategies to tackle the complex challenges faced by diagnosed PLHIV. Such strategies should include gender-sensitive interventions, ensure availability and accessibility of the services, improved sexual health education programs, and establishing adherence to ART medication and tuberculosis preventive therapy that must be strengthened to further reduce the burden of OIs. Furthermore, continuous assessment of HIV patients for risk factors such as smoking, alcohol use, unprotected sexual encounters and for signs and symptoms of OIs by physicians is crucial to prevent, timely diagnosis and treat the OIs to ultimately reduce morbidity and mortality. There is a need for further research to study and evaluate the key drivers of higher prevalence of TB-related OIs, amongst all other OIs. The programmatic intervention should aim to detect, treat, prevent, and reduce OIs and its complications among the PLHIV on ART in the long run in the country. By addressing these key findings and translating study recommendations into policy actions by the policymakers, supported by the healthcare providers, and stakeholders, we are optimistic that NTCP can collaborate with other programs, institutions, agencies and work together to further improve HIV care and outcomes in the key affected communities, targeting – a very low or PLHIV free of opportunistic infections on ART, which is a lifelong treatment.

Recommendations

Recommendations based on the key study findings:

Sn	Key Findings	Recommendations	Intervention approach proposed
1	Socio-demography		
	• Of 301 PLHIV who participated in this study, female participants 52% were slightly higher than male participants 48%; • The largest number of participants belong to productive and active age group of 25 to 39 years (44%) with slight female predominance; • A growing number of PLHIVs were also seen in the group of 40-59 years (44%) with slight male predominance, indicating a shift in age group. • Most of the study participants were uneducated 33% (98/301); • Maximum cases were seen among the corporate and private business group 32% (95/301); • PLHIV participants who were currently married was 66%;	• The program is recommended to equally focus on the elderly age group shift as reported in this study; • Reach out to the uneducated lot as well as corporate and private business groups in terms of HIV/AIDS awareness and campaigns; • Establish comprehensive sexual health education in schools, community centres, healthcare facilities, VCT HICS centres;	• <i>Develop and distribute culturally tailored educational materials and outreach programs to raise awareness among PLHIV about the signs, symptoms, and preventive measures for common OIs, promoting self-care and proactive healthcare-seeking behaviours.</i> • <i>Highlight TB symptoms, including EPTB among the PLHIV across all ages;</i> • <i>Provide pictorial information and images of the OIs in PLHIV across all ages;</i> • <i>Implement comprehensive sexual health education programs through advocacy in schools, community centres, ORCs, VHWs, healthcare facilities, VCT centres, and HISCs;</i>

2	Risk behaviours		
	- About 11% of the study participants has reported of current smoking habits; - About 35% reported to have used alcohol, out of which, 42.31% said they	The program is recommended to draw a plan for smoking and alcohol cessation and educate PLHIV on	<i>Collaborate with Mental Health Program to implement smoking cessation and alcohol harm reduction among the PLHIVs;</i>
	have used it 1-3 times a week in the last one month; - Only one PLHIV has reported of using psychotropic substance; - The most preferred mode of sexual intercourse expressed by the study participants was vaginal, which was about 97%. - While condom usage with different sexual partners varied, about 2% to 3% reported that they have never used condom with casual, paying or transactional partners.	harmful effects of substance use disorder; - Enhance sexual health education programs and ensure access to condoms to mitigate HIV transmission risks, particularly among individuals engaging in casual sexual encounters; - Promote condom usage among the PLHIV, high risk or key populations and among the discordant couples/partners.	<i>Educate PLHIV on adverse effected of substance use disorder and risk of HIV spread among the injection drug users;</i> <i>Provide detailed information on HIV/AIDS prevention, safe sex practices, and the importance of condom use through both print and broadcast media and social media platforms.</i> <i>Ensure easy access to free or subsidised condoms through distribution outlets in high-risk areas and venues frequented by individuals engaging in casual sexual encounters.</i> <i>A strongly recommended paradigm shifts, by this study group:</i> <i>Make condoms available in “Pan-shops”,</i> <i>Hotels & Lodge – Revive it</i> <i>Pubs & bar and</i> <i>High time for online condom delivery app!</i>

3	IPT/TPT & CPT – Preventive Therapy		
	• TB preventive treatment in the form of Isoniazid Preventive Therapy (ITP) or TB Preventive Treatment (TPT) was quite low at 32%;	• The program is recommended to collaborate with National TB Control Program to further enhance IPT and CPT preventive therapy;	• <i>Collaborate with TB Control Program to initiate TPT and CPT among the PLHIV;</i> • <i>Collaborate with the TB Control Program to for bi-directional TB</i>

	• Co-trimoxazole Preventive Therapy (CPT) was at 25%, much lower than TPT during this study period;	• Conduct bi-directional screening of TB in PLHIV and HIV in people detected for TB disease; • Establish tailored adherence support programs to improve completion rates of TPT and CPT among PLHIV in Bhutan. • Develop adherence support programs that incorporate adherence counselling within VCT centres, HISCs, and Care Support and Treatment Centers (CST) like JDWNRH.	<i>and HIV screening among the populations at risk;</i> • <i>Utilise peer counselling services by engaging community-based organizations like Lhaksam, where feasible for TPT and CPT uptake.</i> • <i>Strengthen existing reminder systems, such as telephone call reminders, to enhance medication adherence. These programs should be implemented through VCT/HISC/CST with trained HIV/AIDS counsellors.</i> • <i>Consider collaborating with HIV- related NGOs and community- based organisations to provide services based on clients' feasibility and acceptability.</i> • <i>Perform mid-year in-house and annual review of TPT and CPT program performance in collaboration with the TB Control Program;</i>

4	Comorbidity conditions		
	About 13% of the participants have also reported comorbidity conditions such as diabetes 2.99% (9/301), cardiovascular 12.62 (38/301), respiratory 17 (5.65/301), and kidney diseases 0.66 (2/301).	The program is recommended to include screening of co-morbid conditions and make timely referral to the concerned speciality for holistic management of PLHIV;	<i>Collaborate with the NCD program for screening of the co-morbidity conditions among the PLHIV;</i> <i>Reach out this collaborative effort to health centres in the</i>
		177	<i>form of executive orders for thorough screening;</i>
5	Outcome TB screening tests during this study		
	- Nine (9/301) participants showed X-ray chest findings suggestive of TB; - Five (5/301) participants were detected positive in sputum microscopy; - Two out of five (2/5) sputum positives have tested positive to Xpert MDR/RIF TB test. - Tuberculin Sensitivity Test (TST) was positive in 28 of the participants (28/301).	- The program is recommended to collaborate with National TB Control Program for TB testing among the PLHIV; - TST is not recommended in PLHIV but for this study we have performed to study the response to TST. PLHIV with or without TST should receive TPT.	<i>Collaborate with TB Control Program to screen PLHIV for TB disease;</i> <i>Reach out this collaborative effort to health centres in the form of executive orders for thorough screening;</i> <i>Perform mid-year in-house and annual perform review of TB screening among PLHIV program performance in collaboration with the TB Control Program;</i>

6	Opportunistic Infection (OI) prevalence among PLHIV on ART		
	- The overall TB prevalence as an opportunistic infection among PLHIV on ART was 7% (20/301) with EPTB predominance of three-fourths or 75% (15/20); - Besides TB, other opportunistic infections detected was herpes simplex in about 2% (6/301) followed by oral candidiasis, vaginal candidiasis and genital warts of 0.66% each;	Enhance integrated healthcare services for PLHIV to address a spectrum of OIs beyond tuberculosis (TB), including herpes simplex, oral candidiasis, vaginal candidiasis, and genital warts.	<i>Integrate care models within existing facilities such as VCT/HISC/CST and hospitals on HIV/AIDS by providing comprehensive screening, diagnosis, and treatment services for a range of OIs;</i> <i>Train healthcare providers on the recognition, management, and prevention of common OIs among PLHIV, ensuring a multidisciplinary approach to care delivery;</i> <i>Implement regular OI screening protocols for PLHIV during routine healthcare visits, utilizing</i>
			<i>clinical examinations and laboratory tests to detect infections early;</i>

7	Operational research and program review <i>(not as key findings but inspired by current study findings!)</i>		
	- Operational research (OR) should be conducted to monitor the evolving epidemiology of OIs among the PLHIV; - OR to understand the causes of OIs among PLHIV; - OR to assess the effectiveness of proposed interventions methods - Mid-year inhouse and formal annual program performance reviews	- The program is recommended to conduct screening of TB and HIV - Program review on TPT and CPT coverage, annually; - OR on HIV OI among PLHIV for continuous monitoring process from the baseline is established by this study to garner more evidence for towards changing epidemiology detection, prevention and treatment of the OIs; - OR to assess the effectiveness of interventions such as community-based support groups, stigma reduction initiatives, <i>and innovative health communication strategies;</i>	<i>Collaborate with academic institutions such as MECRIT under KGUMSB, RCDC, government agencies, and community organizations to conduct research studies in evaluating the impact of culturally tailored interventions on prevention, detection and treatment outcomes of OIs among PLHIV in Bhutan.</i> <i>May collaborate with HIV/AIDS and TB Program experts nationally or internationally for program performance reviews within the purview mitigating the challenges of OIs among PLHIV in the country.</i>

Limitation of the study

- The participants who did not attend the ART clinic during the study period would have been missed, altogether with OIs if at all they suffered from one;
- Participants with clinically diagnosed OIs were not subjected through any laboratory confirmatory tests;
- Study results may need to be generalized with caution as the full cohort of PLHIV on ART were not covered;
- Some of the limitations concerning the findings of this study are firstly, the study was cross- sectional and was not able to accurately show the cause-effect relationship between the factors and the outcome of interest;
- The study may also have suffered from recall bias and socially desirable bias because variables related to risk factors are based on self-reported information with regards to OIs besides TB;
- The trained field investigators from the VCT and HISC centres used face-to-face interview techniques, resulting in easy access to study respondents, which could be one of the key strengths of this study.
- The current findings of the study did not explore the associated risk factors with regards to HIV OI owing to small sample of PLHIV with OIs. This is recommended to be conducted at multi- centric level along with the other countries – Afghanistan and Nepal data.

Acknowledgements

The National HIV/AIDS Control Program would like to deeply thank all PLHIV participants who agreed to take part in this study and we also acknowledge the SAARC Tuberculosis and HIV/AIDS Center, Kathmandu, Nepal for the funding support. The program also would like to extend our warm appreciations and thank all the data collectors, comprised of VCT focal person, TB focal person, HISC staffs and staffs from the CST JDWNRH for their dedicated data collection and timely submission to the program. We would also like to sincerely thank the study investigators who dedicated their time and energy from inception of the study till completion of this report.

Declaration of interest

All investigators listed in this protocol declare they have no conflicts of interests, financial or other competing interests, for the overall study and each study site.

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Annexures

- Annex 1. Operational definitions.
- Annex 2. Broad category of opportunistic infections for reference.
- Annex 3. Structured Questionnaires
- Annex 4. Guidelines for procedure and Reading the Tuberculin Skin Test (TST)

Annex 1. Operational definitions.

1. **Oral candidiasis:** Multiple creamy white, easily removable patches or pseudo membraneous plaques on the tongue, gums, buccal mucosa, and palate, anterior surface of tonsils, posterior wall of throat. Treatment: Fluconazole 100–150 mg per day or Ketoconazole 200 mg b.i.d for 7 days.
2. **Esophageal candidiasis:** painful swallowing Diagnosis mainly clinical. Treatment: Fluconazole 200–300 mg/day x 14 days or Itraconazole 400mg/day x 14 days or Ketoconazole 200 mg b.i.d x 14 days
3. **Vaginal candidiasis:** Itching and burning sensation; creamy white vaginal discharge with cheese like plaques. Vulvovaginal area is erythematous, swollen and painful. Recurrence is common. Treatment: Fluconazole 150– 200 mg orally, single dose: higher dose and more prolonged duration of treatment in case of severe immunodeficiency; or: Itraconazole 100 mg orally, b.i.d x 3 consecutive days; or Clotrimazole 100 mg or miconazole 100 mg as vaginal suppositories, 1suppositorium/day x 7 days, or Clotrimazole 500 mg single vaginal suppositorium, or Nystatin 100.000 units, 1 vaginal suppositorium/day x 14 days.
4. **Pneumocystis jiroveci pneumonia (PCP):** Cough, dyspnea, fever, night sweat Subacute onset of symptoms over 1– 2 weeks. Treatment: Co-trimoxazole: 15mg TMP/kg/day in 4 divided. doses x 21 days; patients weighed < 40 kg: TMP/SMX 480 mg, 2 tablets x 4 times/day; patients weighed > 40 kg: TMP– SMX 480 mg, 3 tablets x 4 times/day. In case of respiratory failure: prednisone (orally or intravenously) 40mg x 2 times/day x 5 days, then 40mg x once daily x 5 days, then 20 mg x once
5. **Herpes simplex:** Clusters of typical blisters usually in genital area or face. Systemic involvement (HSV encephalitis) is possible. Treatment: Acyclovir 200mg 5 times (or 400 mg 4-5 times) daily for 7 days Local care with gentian violet or chlorhexidine.
6. **Herpes zoster:** Typical painful blisters in clusters within a dermatome. Eye can be involved. Treatment: Acyclovir 800 mg 5 times daily for 7 days Local care with gentian violet or chlorhexidine. Ophthalmic herpes zoster: acyclovir eye ointment
7. **Molluscum contagiosum:** Pedunculated nodular lesions, usually on face, genital area and neck, armpits. Removal by enucleating or cryotherapy, prick the centre and apply phenol. Responsive to ART.

8. **Genital warts (HPV) Manifestation:** Warts present as, Topical soft, moist, pink, cauliflower like papules with peduncle, painless easily bleeding. In men warts are found most frequently at the coronal sulcus, prepuce and penis shaft and occasionally at urethral meatus. In women, warts often occur at clitoris, minor labia, around urethral meatus, perineum. Genital HPV infection increases the risk of genital cancer. Mainly relied on clinical features. Consultation with Dermatologist. Treatment under specialized supervision with podophyllin, trichloroacetic acid; cryotherapy with liquid nitrogen, carbonic laser or electrosurgery. Responsive to ART.

Annex 2: Broad category of opportunistic infections for reference.

1. Bacterial Opportunistic Infections
2. Fungal Opportunistic Infections
3. Parasitic Opportunistic Infections
4. Others Opportunistic Infections

1. Bacterial Opportunistic Infections

- a. *Mycobacterium*
- b. *Rhodococcus*
- c. *Nocardia*
- d. *Salmonella*
- e. Others:
 - *Haemophilus*
 - *S.pneumoniae*

2. Fungal Opportunistic Infections

- a. *Cryptococcus Neoformans*
- b. *Histoplasma Capsulaum*
- c. *Tularomysis Marneffe*
- d. Others:
 - PCP

3. Parasitic Opportunistic Infections

- a. *Cryptosporidium*
- b. *Microsporidium*
- c. *Strongyliodes*
- d. *Cystoisospora*
- e. *Cyclospora*

4. Others Opportunistic Infections

- a. Tzanck's "Multi Nucleated Giant cells"
- b. Molluscum "Molluscum bodies"
- c. Scabies "*Sarcotisscabii*, adult"

Annex 3. Structured Questionnaires

Assess Current Trends in the Spectrum of Opportunistic Infections in Human Immunodeficiency Virus Infected Individuals

A Multi-Centric Study

(Version-1.3: 13/08/2021)

TP-Number (Code No.): Name

of facility: Date of interview:

...../...../

.....(DD/MM/YY

YY)

Date of Detection:/...../..... (DD/MM/YYYY);

Date put on ART:/...../ (DD/MM/YYYY);

Age at detection (Years)

*****Instruction to fill up the questionnaire:**

Fill up the document fully. In case the given options don't match, write down the response of the

responder. In case of any confusion, please contact with focal person to complete the document.

1. Socio-demographic characteristics:

1.1 How old are you (in completed years) (probe with best estimate)?

1.2 Sex ☐ Male 1

☐ Female 2

☐ Transgender person 3

1.3 What is the level of your education?

☐ No education..... 1

☐ Non-formal education..... 2

☐ Primary (PP-VI)..... 3

☐ Lower Secondary (Cl VII-VIII). 3

☐ Middle Secondary (Cl IX-X)... 4

☐ Higher Secondary (Cl XI-XII). 5

☐ Tertiary (Degree & above)..... 6

☐ Monastic education.....7

1.4 What is your religion?

☐ Buddhist..... 1

☐ Muslim.....2

- ☐ Hindu 3
- ☐ Christian..... 4
- ☐ No religion.....5
- ☐ Other 6

1.5 What is your occupation? [PROBE: What kind of work do you do most of your time? record verbatim and then code]

- ☐ Civil Service 1
- ☐ Corporate 2
- ☐ Private employee 3
- ☐ Independent business owner 4
- ☐ Uniform personnel 5
- ☐ Religious body... 6
- ☐ Corporate employees 7
- ☐ Farmer..... 8
- ☐ Driver.....9
- ☐ Student.....10
- ☐ Home maker 11
- ☐ Employee of entertainment centers... 12
- ☐ Migrant workers 13
- ☐ National labour 14
- ☐ Other, specify..... 15

1.6 What is your average monthly family income?

- ☐ Nu. < 7000 1
- ☐ Nu. 7001-14000... 2
- ☐ Nu. 14001-35000 3
- ☐ Nu. >35000... 4
- ☐ Dependent..... 5

1.7 What is your current marital status?

- ☐ Currently married 1
- ☐ Never married 2
- ☐ Divorced/Separated.....3
- ☐ Widow/ Widower 4
- ☐ Living together..... 5
- ☐ No answer.....6

2. Behavioural characteristics:

2.1. Do you smoke cigarettes?

- ☐ Yes.....1 If yes ask 2.2
☐ No... 2 if no skip to Q2.3

2.2. If yes how many sticks per day?

2.3. Do you drink alcohol?

- ☐ Yes.....1 If yes ask 2.4
☐ No... 2 if no skip to Q2.5

2.4. In the past 4 weeks, how often have you had drinks containing alcohol?

- ☐ 5-6 days a week..... 1
☐ 1-4 days a week 2
☐ 1-3 days a week 3
☐ Once a month..... 4
☐ Not at all... 5
☐ Don't want to reveal 6

2.5. Have you taken any psychotropic substances that were not prescribed by a health professional in the past 12 months? (This can include orally, sniffing, injection, other locally common methods for using drugs) *** Note: A health professional does not include traditional medical practitioner.

- ☐ Yes..... 1
☐ No... 2
☐ No answer.....3
☐ Don't know 4

2.6. Have you injected any drugs that were not prescribed by a health professional in the past 12

months by yourself or friends? *** Note: A health professional does not include traditional medical practitioner.

- ☐ Yes..... 1
☐ No... 2
☐ No answer.....3
☐ Don't know 4

2.8 In the last 12 months did you share needles to inject drugs?

- ☐ Yes... 1
- ☐ No... 2
- ☐ No answer.....3
- ☐ Don't know 4

2.9. Have you ever heard of condoms?

- ☐ Yes... 1
- ☐ No... 2
- ☐ No answer.....3
- ☐ Don't know 4

2.10. Type of sex: Please tick (☐) all that apply:

- ☐ Vaginal sex... 1
- ☐ Anal sex... 2
- ☐ Oral sex..... 3
- ☐ Don't want to reveal 4

2.11. Did you always use condom with your sexual partners each time you had sex with them in the last 30 days?

2.11.1. Spouse:

- ☐ Always..... 1
- ☐ Not always..... 2
- ☐ Never 3
- ☐ NA... 4

2.11.2. Regular partners:

- ☐ Always 1
- ☐ Not always 2
- ☐ Never.....3
- ☐ NA.....4

2.11.3. Casual partners:

- ☐ Always 1
- ☐ Not always 2
- ☐ Never.....3
- ☐ NA.....4

2.11.4. Paying partners (Sex Workers):

- ☐ Always 1
- ☐ Not always 2
- ☐ Never.....3
- ☐ NA.....4

2.11.5. Transactional partners (Transaction of good/kinds):

- ☐ Always 1
- ☐ Not always 2
- ☐ Never.....3
- ☐ NA.....4

3. Clinical history:

*****Note: Some data would be collected and matched with the clinical register. To rule out the clinically diagnosed OI's, data collector will ask the question and at the same time do the clinical examination according to the case definition and finally mark as “Yes” or “No”.**

3.1. How long you have been diagnosed as HIV positive? (in month)

3.2. How long you have been taking ART? (in month)

3.3. What is the latest CD4 count?

- ☐ < 200.....1
- ☐ 201-500.....2
- ☐ > 500.....3

3.5. Has the participant completed IPT/TPT course?

- ☐ Yes..... 1
- ☐ No... 2
- ☐ Currently on IPT/TPT..... 3

3.6. Has the participant complete CPT course?

- ☐ Yes... 1
- ☐ No... 2
- ☐ Currently on CPT.....3

3.7. Do you have any chronic illness (illness for >6 months) for which you have to take medication? ☐

Yes.....1

If Yes ask Q 3.8

- ☐ No.....2 if No skip to Q 3.9
- 3.8. If Yes to 3.7, specify (Tick ☐ more than one as appropriate)
- ☐ Diabetes... 1
- ☐ Heart disease..... 2
- ☐ Respiratory disease (Asthma, COPD etc)..... 3
- ☐ Renal disease 4
- ☐ Others, specify:.....5
- 3.9. Have you ever been diagnosed with TB (Tick (☐) as appropriate)
- ☐ Yes... 1 (☐ Pulmonary or ☐ Extra-pulmonary TB)
- ☐ No... 2
- ☐ If Yes, DD/MM/YYYY:

3.10. Symptom analysis for Tuberculosis: Please tick (☐) the options that apply:

- Cough > 2 weeks ☐ Yes; ☐ No
- Fever ☐ Yes; ☐ No
- Weight loss ☐ Yes; ☐ No
- Blood in sputum ☐ Yes; ☐ No
- Chest pain ☐ Yes; ☐ No
- Breathlessness ☐ Yes; ☐ No
- Night sweats ☐ Yes; ☐ No
- Tiredness ☐ Yes; ☐ No
- Loss of appetite ☐ Yes; ☐ No
- Others, Specify:

**3.11. To rule out EPTB, besides the 4S (Cough, Fever, night sweats and weight loss):
Please ask the following questions and if present, consult with the clinicians:**

- Nodular swellings on the body ☐ Yes; ☐ No
- Red or painful urination ☐ Yes; ☐ No
- Altered bowel habit ☐ Yes; ☐ No
- Abdominal swelling (Ascites) ☐ Yes; ☐ No
- Severe backache limiting your movements ☐ Yes; ☐ No
- Headache ☐ Yes; ☐ No
- Neck stiffness ☐ Yes; ☐ No
- Difficulty in looking at bright light (photophobia) ☐ Yes; ☐ No
- Non-healing sinus on the skin ☐ Yes; ☐ No
- Others, specify:

Laboratory reports, to be filled by the interviewer (Tick the correct option).

- 3.1 Chest ☐ X ¹⁸⁸ AD; ☐ Positive finding suggestive of TB
- 3.2 Sputum microscopy ☐ Negative; ☐ Positive
- 3.3 GeneXpert ☐ Negative; ☐ Positive
- 3.4 Tuberculin skin test ☐ Negative; ☐ Positive (in HIV, Positive ≥ 5 mm): TST:....mm
- 3.5 TST will be correlate with CXR to make final diagnosis about LTBI as per the revised, 7th edition of National Guidelines for Management of Tuberculosis, 2019.

4. Opportunistic infections detected (circle the best option): PAST OIs as per the documentary evidence.

- 4.1 Active Tuberculosis ☐ Yes; ☐ No (☐ PTB; ☐ EPTB)
- 4.2 Latent Tuberculosis ☐ Yes; ☐ No
- 5.3 Oral candidiasis ☐ Yes; ☐ No
- 5.4 Esophageal candidiasis ☐ Yes; ☐ No
- 5.5 Vaginal candidiasis ☐ Yes; ☐ No
- 5.6 Pneumocystis jirovecii pneumonia (PCP) ☐ Yes; ☐ No
- 5.7 Herpes simplex ☐ Yes; ☐ No
- 5.8 Herpes zoster ☐ Yes; ☐ No
- 5.9 Molluscum contagiosum ☐ Yes; ☐ No
- 5.10 Genital warts (HPV) ☐ Yes; ☐ No

5. Opportunistic infections detected (circle the best option): CURRENT OIs as per the clinical evaluation and diagnostic/laboratory investigations.

- 5.1 Active Tuberculosis ☐ Yes; ☐ No (☐ PTB; ☐ EPTB)
- 5.2 Latent Tuberculosis ☐ Yes; ☐ No
- 5.3 Oral candidiasis ☐ Yes; ☐ No
- 5.4 Esophageal candidiasis ☐ Yes; ☐ No
- 5.5 Vaginal candidiasis ☐ Yes; ☐ No
- 5.6 Pneumocystis jirovecii pneumonia (PCP) ☐ Yes; ☐ No

5.7 Herpes simplex	☐ Yes;	☐ No
5.8 Herpes zoster	☐ Yes;	☐ No
5.9 Molluscum contagiosum	☐ Yes;	☐ No
5.10 Genital warts (HPV)	☐ Yes;	☐ No

For clarification or assistance during data collection in the field, please contact:

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Annex 4. Guidelines for procedure and Reading the Tuberculin Skin Test (TST)

The TST is used to determine if a person is infected with *M. tuberculosis*. If a person is infected, a delayed-type hypersensitivity reaction is detectable 2 to 8 weeks after infection. The skin test is administered intradermally by injecting 0.1ml of 5 TU purified protein derivative (PPD) solution. The reading and interpretation of TST reactions should be conducted within 48 to 72 hours of administration. Results reported as “positive” or “negative” are not acceptable.

Educate

- Explain the significance of a positive skin test. For example, a positive skin test result means latent infection with the TB germ. A negative skin test result means there is no TB infection
- Direct the participant for second TST test.
- Answer the participants questions

Supplies needed

- Small, plastic ruler marked in millimeters
- Ball point pen to mark edges of the induration
- Alcohol pad to clean off pen marks
- Mantoux Tuberculin Skin Test Record Form

Preparation

- Verify that the correct patient (considering the inclusion and exclusion criteria of the study participants) for TST reading
- Explain the procedure to the patient to put him/her at ease
- Wash your hands

Procedure

- Inspect the arm in good light and on a firm surface
- Locate the site of injection on the palm-side-up surface of the forearm with the patient's arm supported and slightly flexed at the elbow
- Clean the area with an alcohol swab
- Check expiration date on vial and ensure vial contains tuberculin (5 TU per 0.1 ml)
- Use a single-dose tuberculin syringe with a ¼- to ½-inch, 27-gauge needle with a short bevel
- Fill the syringe with 0.1 ml of tuberculin
- Insert slowly, bevel up, at a 5- to 15-degree angle, needle bevel can be seen just below skin surface. Check that after injection, a tense, pale wheal should appear over the needle
- Wheal should be 6 to 10 mm in diameter. If not, repeat test at a site at least 2 inches away from original site
- Record all the information required for documentation (e.g., date and time of test administration, injection site location, lot number of tuberculin)

Reading (Figure 1)

- After 48 and 72 hours of introducing the injection, the palpable skin induration diameter will be recorded (in millimeters) by the laboratory technologist
- Since the induration is not always visible, you must palpate with your fingertips to determine induration at the injection site
- Touch the area lightly with the pads of your fingertips to find the margins of induration
- Without lifting, move your fingertips from the outer edge towards the induration
- Rest one fingertip firmly against the induration margin on one side before marking the margin. The fingertip should remain in contact with the skin at all times
- Use a ball point pen to mark lightly with a fine dot at the widest edge of the induration

- Repeat the procedure from the other side of the patient's forearm and place the second mark on the margin of induration
- If the margins are not equally clear all the way around the induration, it is still necessary to mark the margins on each side of the induration. For irregular margins of induration, mark and measure the longest diameter across the forearm
- Measure only the area of induration, a hard, dense, raised formation
- Do not measure erythema, reddening of the skin that can also have swelling
- Use the millimeter ruler to measure the diameter of the induration perpendicular to the long axis of the forearm
- Place the zero-ruler line inside the left dot edge and read the ruler line inside the right dot edge
- If the measurement falls between the two divisions on the millimeter scale, record the lower mark. If unsure, ask a co-worker

Note: Reactions to the tuberculin skin test at the injection site will vary. If there is blistering, palpate the induration gently as it may be painful. Measure only the induration. Only the margins of the induration are significant, redness and swelling should not be measured.

Record

- Record the exact measurement in millimeters of induration on the Tuberculin Skin Test Record Form. Do not record the interpretation of the results as "positive" or "negative."
- Record the date and time the test was read, the name and signature of the person who read the skin test, and the presence or absence of adverse effects (i.e., blistering, redness, and swelling)
- If there is no induration, this measurement should be recorded as 0 mm of indurations. **Interpretation** (Refer the following Box 4): Ref: 7th Edition, revised National Guidelines for Management of Tuberculosis in Bhutan, 2019.

The participants of this study are considered as persons at risk for developing TB disease, a TST

reaction of ≥ 5 mm of induration will be considered positive.

BOX 4: Tuberculin skin test procedure

The TST is performed by injecting 0.1 ml of tuberculin purified protein derivative (PPD) into the inner surface (volar aspect) of the forearm. The injection should be made with a tuberculin syringe, with the needle bevel facing upward. The TST is an intradermal injection. When placed correctly, the injection should produce a pale elevation of the skin (a wheal) 6 to 10 mm in diameter.



Figure 11.1 TST Intradermal procedure, producing a "wheal"

TST reading

The skin test reaction should be read between 48 and 72 hours after administration. A patient who does not return within 72 hours will need to be rescheduled for another skin test. The reaction should be measured in millimetres of the induration (palpable, raised, hardened area or swelling). The reader should not measure erythema (redness). The diameter of the indurated area should be measured across the forearm (perpendicular to the long axis).

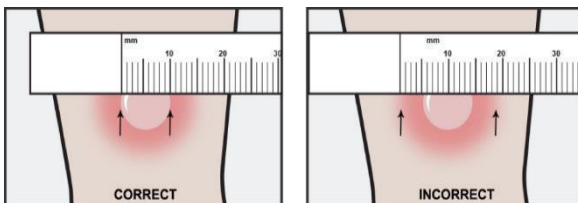


Figure 11.2 Correct way of reading TST

Figure 11.3 Correct way of reading using calliper ruler

TST Interpretation

Skin test interpretation depends on two factors:

- Measurement in millimeters of the induration
- Person's risk of being infected with TB and of progression to disease if infected

An induration of ≥ 10 mm is considered positive.

However, an induration of ≥ 5 mm is considered positive in:

- HIV-infected persons
- Patients with organ transplants
- Persons who are immunosuppressed for other reasons (e.g., taking the equivalent of >15 mg/day of prednisone for 1 month or longer, taking TNF- α antagonists)

False-Positive Reactions

Some persons may react to the TST even though they are not infected with *M. tuberculosis*. The causes of these false-positive reactions may include, but are not limited to, the following:

- Infection with non-tuberculosis mycobacteria
- Previous BCG vaccination
- Incorrect method of TST administration
- Incorrect interpretation of reaction
- Incorrect bottle of antigen used

False-Negative Reactions

Some persons may not react to the TST even though they are infected with *M. tuberculosis*. The reasons for these false-negative reactions may include, but are not limited to, the following:

- Cutaneous anergy (anergy is the inability to react to skin tests because of a weakened immune system)
- Recent TB infection (within 8-10 weeks of exposure)
- Very old TB infection (many years)
- Very young age (less than 6 months old)
- Recent live-virus vaccination (e.g., measles and smallpox)
- Overwhelming TB disease
- Some viral illnesses (e.g., measles and chicken pox)
- Incorrect method of TST administration
- Incorrect interpretation of reaction

Who Can Receive a TST?

It is not contraindicated for any other persons, including infants, children, pregnant women, persons who are HIV-infected or persons who have been vaccinated with BCG.

Contraindication or precaution in live vaccine recipient

TST is contraindicated only for persons who have had a severe reaction (e.g., necrosis, blistering, anaphylactic shock, or ulcerations) to a previous TST

Vaccination with live viruses may interfere with TST reactions. For persons scheduled to receive a TST, testing should be done as follows:

- Either on the same day as vaccination with live virus vaccine or 4-6 weeks after the administration of the live-virus vaccine
- LTBI testing by TST/IGRA is however not a requirement in PLHIV and contacts ≤ 5 years.

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Assessment of Compliance of
Shorter Regimen of MDR-TB
Treatment in Bhutan
2019

**Assessment of Treatment
Compliance and Outcomes of
Multidrug-Resistant Tuberculosis
(MDR-TB) in Bhutan, 2019**

A Retrospective Study

Jointly conducted by the NTCP, DoPH, Ministry of Health, Bhutan & STAC with



**The funding support of the SAARC
Tuberculosis & HIV/AIDS Centre (STAC),
based in Kathmandu, Nepal**



Afghanistan



Bangladesh



Bhutan



India



Maldives



Nepal



Pakistan



Sri Lanka



SAARC Tuberculosis and HIV/AIDS Centre (STAC)

EXECUTIVE SUMMARY

Tuberculosis (TB) still remains a major public health concern, although Bhutan is considered a relatively low TB burden country in the region. TB has taken a toll of several lives yearly and according to the NTCP in 2020 alone, there were 474 (52%) females and 445 (48%) males diagnosed with TB and there were 65 people diagnosed and registered for MDR-TB treatment, out of which 35 (56%) were females and 30 (44%) were males. Among the total MDR-TB notified cases, about 85% were concentrated in eight high TB burden districts of Chukha, Mongar, Paro, Samdrupjongkhar, Samtse, Sarpang, Thimphu and Wanduephodrang.

We have conducted a retrospective study with the major objective to assess MDR-TB treatment compliance and outcomes and to identify additional factors associated with MDR-TB in the country. The study recruited all MDR-TB cases diagnosed and provided treatment from 2016 to 2019 at JDWNRH in Thimphu, CRRH in Gelephu and ERRH in Mongar. The study found that both treatment adherence and treatment outcomes were exceptionally high and some factors (What are the factors?) were associated with early culture conversion and late conversion. The findings and recommendation from this study significantly influenced the programmatic management of MDR-TB (PMDT). Some of the most important ones are; targeted awareness among the congregate setting such as schools and institutions, targeted contact tracing and outbreak investigations, mandatorily perform baseline X-ray chest and sputum smear and culture, regular monthly smear and culture follow up, the requirement of both 1st line and 2nd line LPA or DST before the start of the MDR-TB treatment initiation, taking a detailed history of co-morbid conditions and assigning correct treatment outcomes based on the laboratory smear and culture follow up records and national TB guidelines definitions.

Some of the limitations of the study were incomplete patient information records, a small sample size, (249) and several missing data due to poor record-keeping keeping for the MDR-TB patients, both in the MDR-TB registers and in the patient treatment cards.

LIST OF ABBREVIATIONS

AFB	Acid Fast Bacilli
BMI	Body Mass Index
COPD	Chronic Obstructive Pulmonary Disease
CP	Continuation Phase
CRRH	Central Regional Referral Hospital
CXR	Chest X-ray
DR-TB	Drug-Resistant Tuberculosis
DST	Drug Susceptibility Testing
EPTB	Extrapulmonary Tuberculosis
ERRH	Eastern Regional Referral Hospital
FLD	First Line Drugs
FL-LPA	First Line-Line Probe Assay
FLQ	Fluoroquinolone
HIV	Human Immunodeficiency Virus
HSS	Higher Secondary School
IP	Intensive Phase
IQR	Interquartile Range
JDWNRH	Jigme Dorji Wangchuck National Referral Hospital
LSS	Lower Secondary School
RR/MDR-TB	Rifampicin Resistant / Multi Drug Resistant TB
MTB/RIF	Mycobacterium TB/Rifampicin
NTCP	National Tuberculosis Control Program
PMDT	Programmatic Management of Drug-Resistant TB
PTB	Pulmonary Tuberculosis
REBH	Research Ethics Board of Health
SAARC	South Asian Association for Regional Cooperation
SD	Standard Deviation
SLD	Second Line Drugs
STAC	SAARC Tuberculosis and HIV/AIDS Centre
STATA	Software for statistics and data science
STR	Standardized Treatment Regimen
TB	Tuberculosis
TbISS	Tuberculosis Information and Surveillance System
VCT	Voluntary Counselling and Testing
WHO	World Health Organization
XDR-TB	Extensively Drug-Resistant Tuberculosis

List of abbreviations for TB Drugs

Am	Amikacin
Bdq	Bedaquiline
Cfz	Clofazimine
Cs	Cycloserine
E	Ethambutol
Eto	Ethionamide
H	Isoniazid
Km	Kanamycin
Lfx	Levofloxacin
Lzd	Linezolid
Mfx	Moxifloxacin
Pto	Prothionamide
S	Streptomycin
Z	Pyrazinamide

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Assessment of treatment compliance and outcomes of Multidrug-resistant tuberculosis (MDR-TB) in Bhutan, 2019

BACKGROUND

Tuberculosis (TB) is a communicable disease that is a major cause of ill health, one of the top 10 causes of death worldwide, and the leading cause of death from a single infectious agent. In 2019, about 10 million people developed TB and 1.4 million died. TB is caused by the bacillus *Mycobacterium tuberculosis*, which is spread when people who are sick with TB expel bacteria into the air; for example, by coughing. The disease typically affects the lungs (pulmonary TB/PTB) but can also affect other sites (Extrapulmonary TB/EPTB). TB can affect anyone anywhere, but most people who develop the disease (about 90%) are adults; there are more cases among men than women; and of those who fell sick with TB in 2019, 87% were in 30 high TB burden countries¹.

The SAARC region, with an estimated incidence of 3.7 million TB cases, carried 37% of the Global burden of TB in 2019. Three of the eight Member Countries in the Region are among the 30 high burden countries (Bangladesh, India and Pakistan). These three countries together notified 96% of the cases in the region in 2019. India alone accounted for 77% of all notifications in the SAARC region in 2019. The SAARC region had 0.15 million total number of estimated MDR/RR-TB incidence cases in the year 2019 (STAC-2020)². According to the WHO Global TB Report 2020, the estimated all forms of TB and MDR/RR-TB incidence rates in Bhutan were 165/100, 000 and 25/100, 000 respectively, mostly affecting the productive age group of 15-49 years¹.

MDR-TB is defined as resistance to rifampicin (RR-TB) and isoniazid, the two most effective first-line TB drugs. Drug-resistant TB continues to be a public health threat worldwide in 2019, close to half a million people developed rifampicin-resistant TB (RR-TB), of which 78% had multidrug-resistant TB (MDR-TB). The three countries with the largest share of the global burden

¹ Global Tuberculosis Report 2020; <https://apps.who.int/iris/bitstream/handle/10665/336069/9789240013131-eng.pdf>

²SAARC Epidemiological Response on Tuberculosis 2020; <https://www.saarctb.org/wp-content/uploads/2021/08/SAARC-Epidemiological-response-on-TB-2020.pdf>

were India (27%), China (14,%) and the Russian Federation (8%). Globally in 2019, 3.3% of new TB cases and 17.7% of previously treated cases had MDR/RR-TB¹. The SAARC region had 0.15 million total number of estimated MDR/RR-TB incidence cases in the year 2019 (STAC-2020)².

TB Epidemiology in Bhutan

Tuberculosis still remains a major public health concern, although Bhutan is considered a relatively low TB burden country in the region. In 2020, as per the data maintained with the National TB Control Program (NTCP), there were 474 (52%) females and 445 (48%) males diagnosed with TB. Among the total notified cases, about 85% are concentrated in 8 high TB burden districts (Chukha, Mongar, Paro, Samdrupjongkhar, Samtse, Sarpang, Thimphu and Wanduephodrang districts) in the country.

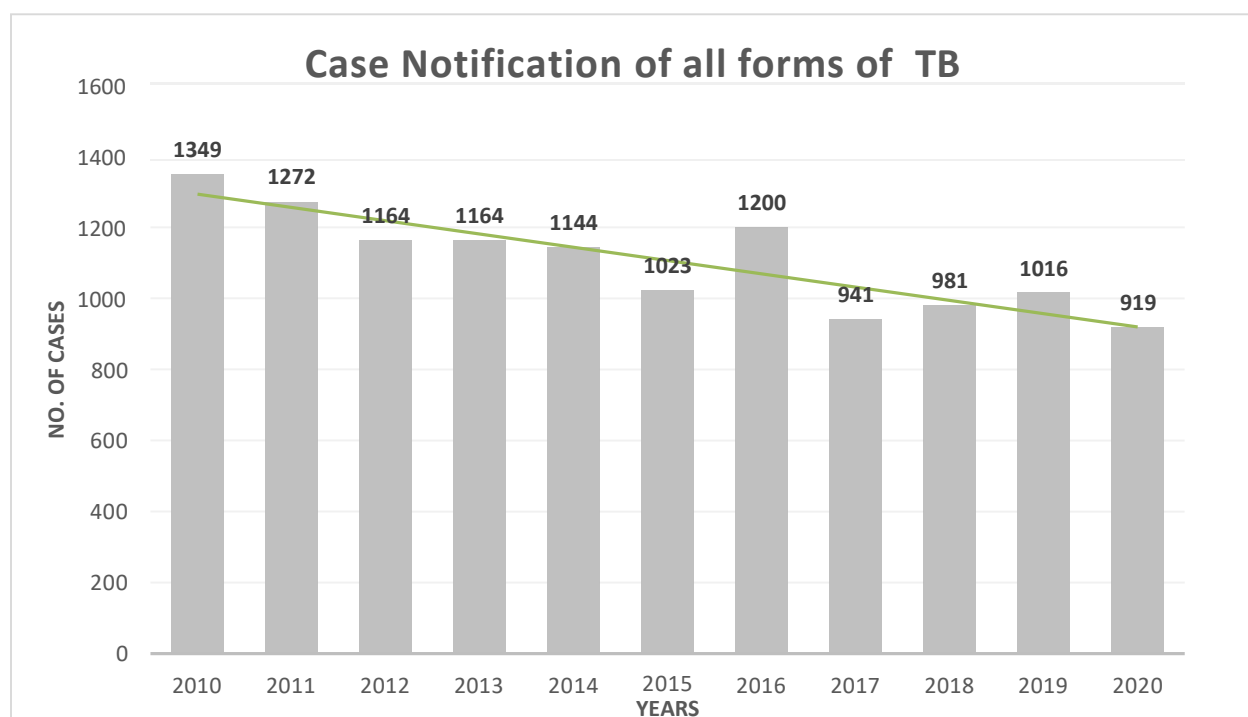


Figure 1: Time-series trend of national TB notifications, 2010-2020.

There has been increasing notification of MDR-TB for the last decade.

The time series MDR-TB case notification in Bhutan can be seen in figure 2.

According to the NTCP, in 2020, there were 65 people diagnosed and registered for MDR-TB treatment, 35 (56%) females and 30 (44%) males.

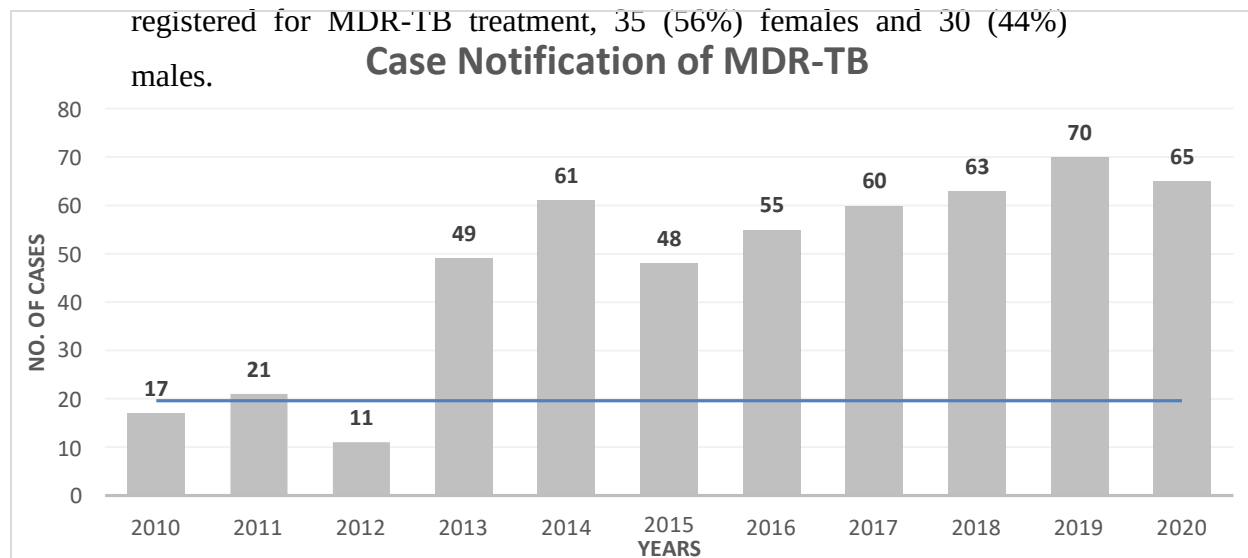


Figure 2: MDR/RR-TB notifications from 2010 to 2020.

The figure shows the MDR-TB trend among new cases from 2004 to 2020 in Bhutan. The proportion of MDR-TB among new TB cases constituted the highest proportion since 2012. Till the availability of Xpert MTB/RIF in the country (2016), most cases of MDR-TB were treated based on the suspected cases not responding to the first-line anti-tubercular regimen. From 2017 onwards, Rifampin resistant could be diagnosed directly using the Xpert MTB/RIF test and the RR/MDR-TB patients were directly enrolled for DR-TB treatment. The increasing trend of primary MDR-TB indicates that the disease is being actively transmitted as primary infection, indicating persistent burden in the community.

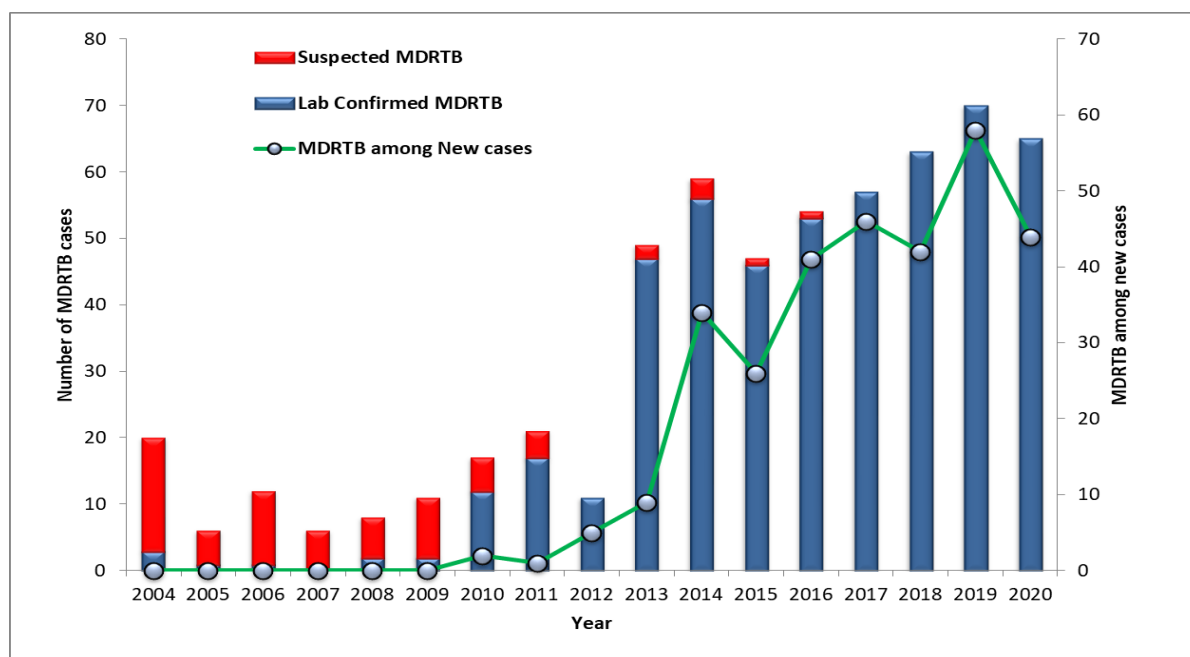


Figure 3: MDR-TB trend among new cases (2004-2020)

Standardized Treatment Regimen

Three referral hospitals in the country are identified as MDR-TB treatment sites for the initiation of treatment based on the laboratory confirmation results.

The Ministry of Health had followed the regimen of MDR-TB treatment as recommended by the WHO as mentioned in the table 1. From 2011 to 2016 the standardized treatment regimen followed was 6 to 9 months for intensive phase (IP) and 18 months for continuation phase (CP). In between 2017 and 2019, the two regimens were followed; shorter regimen (4 to 6 months intensive phase and 5 months for continuation phase) and longer regimen (8 and 12 months for IP and CP respectively).

Table 1: Standardized Treatment Regimen (STR) for RR/MDR-TB

Sl. No.	Intensive phase	Continuation phase	References
1	6/9 Z+Km+Lfx+Eto+Cs	18 Z+Lfx +Eto+Cs	Guidelines for MDR-TB, first Edition 2011 ³ .
2	Longer regimen		National Guidelines for DR- TB, 2017 Update ⁴ .
	8 Z+Ka+Lfx+Eto+Cs	12 Z+Lfx+Eto+Cs	
	Shorter regimen		
	4-6 Km+Mfx+Pto+Cfz+Z+H _{high dose} +E	5 Km+Mfx+Cfz+Z+E	
3	Longer regimen		Integrated National Guidelines for Management of TB in Bhutan. 7 th Edition, 2019 ⁵ .
	6 Bdq+Lfx+Lzd+Cfz	14 Lfx+Lzd+Cfz	
	Shorter regimen		
4	4-6 Am+Mfx+Cfz+Eto+Z+H _{high dose} +E	5 Mfx+Cfz+Z+E	

Despite the tremendous progress made in TB prevention, diagnosis and treatment and sustaining the treatment success rates of 90%, the increasing trend of MDR-TB cases has posed a challenge in TB control strategies. Moreover, detailed microbiological outcomes and treatment adherence has not been evaluated in Bhutan. Therefore, it is essential to understand and evaluate the microbiological outcomes and treatment compliance along with risk factors for poor outcomes among the MDR-TB patients initiated on treatment.

The outcome of this study is expected to evaluate microbiological and treatment outcomes along with risk factors for poor outcomes among the MDR-TB patients initiated on treatment for the cohorts of 2016 to 2019. The findings of this study is also expected to provide informed policy

³ Guidelines for the management of MDR-TB, first Edition 2011, Page no. 17.

⁴ National Guidelines for the Management of DR-TB, 2017 Update, Page no. 33.

⁵ Integrated National Guidelines for Management of TB in Bhutan, 7th Edition, 2019, Page no. 44 & 45.

decisions for programmatic management of drug-resistant tuberculosis (PMDT) in particular MDR-TB in Bhutan.

OBJECTIVES

1. To assess treatment compliance; and
2. To determine Treatment outcomes of Multidrug-resistant tuberculosis (MDR-TB) in Bhutan, 2019

MATERIALS AND METHODOLOGY

Study design, sites, sample size & source population

Study Design

A retrospective cohort study was conducted for the laboratory diagnosed MDR-TB patient treated using standard MDR-TB regimen as per the NTCP treatment guideline from 2016 to 2019 to determine the treatment compliance and outcome of MDR-TB in Bhutan.

Study sites

This study was conducted at three MDR-TB treatment centres; Jigme Dorji Wangchuck National Referral Hospital (JDWNRH) in Thimphu, Eastern Regional Referral Hospital (ERRH) in Mongar and Central Regional Referral Hospital (CRRH) in Gelephu.

Sample size

A total of 55 cases in 2016, 60 cases in 2017, 63 in 2018 and 72 cases in 2019 were diagnosed and registered for MDR-TB as per the NTCP report. The census of all 250 MDR-TB cases from 2016 to 2019 were included for this study.

Source Population

All the MDR-TB patients diagnosed in the year 2016 to 2019 with treatment outcome as cured, treatment completed, death, loss to follow up and treatment failure formed the source population.

Case definition: A case of MDR-TB was defined as a patient of any age diagnosed as having MDR- TB based on laboratory confirmation using rapid molecular test (GeneXpert and FL-LPA) or conventional tests such as liquid/solid culture and DST and found to be resistant to at least rifampicin or to both rifampicin and isoniazid. All these MDR-TB cases should have been diagnosed from January 2016 & December 2019 at the three identified study sites.

Inclusion criteria:

- All MDR-TB cases diagnosed & registered for treatment in Bhutan from 2016 to 2019.

Exclusion criteria:

- MDR-TB patients diagnosed and treatment undertaken outside Bhutan.

Study Duration

The study was conducted from October 2019 to October 2021 where data collection was done in April to May 2021.

Data Variables

The independent variables included for the study were basic demographic characteristics; type of patient during registration of initial treatment; treatment episodes in the past exposure to re- treatment regimen; clinical and microbiological data like co-morbidities (HIV and Diabetes) accessed from patients' baseline pre-treatment evaluation; resistance patterns to all first line drugs (Streptomycin [S], Isoniazid [H], Rifampicin [R], Ethambutol [E]); chest radiographs suggestive of TB or not; treatment delay by DST method; weight change at 6, 12 18 and at the end of treatment months against baseline; poor treatment adherence defined using 7 missed doses during IP and CP as a dichotomous classification similar to those used in previous studies (Dave et al., 2013; Liu et al., 2015; Parmar et al., 2018).

The dependent variables for all MDR-TB patients included initial culture conversion defined as two consecutive negative culture results more than 30 days apart, culture reversion defined as two consecutive positive culture results more than 30 days apart after conversion and, final treatment outcomes classified as favorable (cured, treatment completed) and unfavorable (deaths, lost to follow up and treatment failure) using standard WHO definitions (2008) (Falzon et al., 2011). Additional outcome definitions as in the 6th Edition National Guidelines for Management for Tuberculosis of Bhutan 2016, like switched to XDR-TB regimen and still on treatment beyond 24±27 months depending on monthly extension of Intensive Phase up to a maximum of 9 months were counted in treatment failures while those whose treatment was stopped due to adverse drug reaction or other medical conditions were counted in loss to follow- up. While those variables without data were categorized under unknown category.

Data Collection Plan

The required data variables were extracted from the MDR-TB registers/MDR-TB treatment cards/MDR-TB patient's card maintained at the three MDR-TB treatment centers and Tuberculosis Information and surveillance system (TbISS). Further an individual patient, relative and family members were called to obtain the required missing data that were not mentioned/recorded in registers. The data thus collected were transferred to a standard data questionnaire proforma by the trained data collectors in the field. The Data were entered and managed in Epi Info Version 7.2.

Statistical Analysis

A descriptive statistic was used to analyze the variables related to demography, clinical profile / comorbidity and microbiological data. Survival analysis was used to determine the risk factor for time to culture conversion and revision. A univariate analysis (Kaplan-Meier curves) for time to culture conversion and time to reversion was explored to test the proportional hazards assumptions across independent variable categories. The assumption of proportion hazard was verified using the Cox-models. Initially, the variables was included in a bivariate Cox-Model and those with a p-value of ≤ 0.20 were further subjected to the multivariate analysis.

Log-rank test and Cox-proportional hazard ratio logistic models were used to determine the key factors significantly associated with the unfavourable outcomes. For Cox-model, outcomes of treatment failed, death, loss to follow-up, not evaluated and switched to pre-XDR treatment were combined as a single unfavorable outcome and Cured and treatment completed as the favourable reference category. For all analyses, STATA version 14 (Stata Corp, USA) was used.

Ethical Consideration

Since there was no direct involvement of patients for interview, a fast-tracked ethical approval seeking waiver for consent was sought from the Research and Ethics Board of Health (REBH) of the Ministry of Health, Bhutan prior to the conduct of the study. Other necessary administrative and study site clearance were sought from the Ministry of Health along with other relevant permission from the study sites.

DESCRIPTIVE STATISTICS

Section A: Socio-demographic characteristics

A total of 255 study participants were collected from three study sites, namely JDWNRH in Thimphu, CRRH in Gelephu and ERRH in Mongar for years 2016, 2017, 2018 and 2019, which included retrospective data retrieval of the last four years from the above three sites.

Considering some ongoing treatment course of MDR-TB cases of 2019, all the cases who completed treatment at the time of data entry were considered for the study and ultimately 249 out of 255 data set were retained for final data analysis.

The median (IQR) age of the study participants was 29.09 (14-69) years, the youngest being a 14 days old male neonate with congenital MDR-TB diagnosed in 2017 and the oldest of 74 years (range <1-74 years). The study participants included 146 (58.63%) females and 103 (41.37%) males. The mean (SD) BMI of 153 records available were 19.80 (3.57%). MDR-TB cases were seen maximum among students/trainees 86 (34.5%) and majority of the cases, 185 (74.3%) resided in urban areas compared to rural, 64 (25.7%) residence (Table 1).

Table 1. Socio-demographic characteristic of the MDR-TB study participants from 2016-2019, Bhutan.

	Year No (%)`	2016 n(%)	2017 n(%)	2018 n(%)	2019 n(%)	Total n(%)	Mean ± SD	Median (IQR)
Total		54	59	68	68	249		
Age							29.09	29.09 ±12.79
0-4			0(0)	1(1.7)	0(0)	0(0)	1(0.4)	
5-14			1(1.9)	1(1.7)	2(2.9)	1(1.5)	5(2)	
15-24			24(44.4)	25(42.4)	30(44.1)	31(45.6)	110(44.2)	
25-34			9(16.7)	21(35.6)	24(35.3)	21(30.9)	75(30.1)	
35-44			6(11.1)	9(15.3)	2(2.9)	7(10.3)	24(9.6)	
45-54			8(14.8)	0(0)	6(8.8)	5(7.4)	19(7.6)	
55-64			4(7.4)	2(3.4)	3(4.4)	2(2.9)	11(4.4)	
65+			2(3.7)	0(0)	1(1.5)	1(1.5)	4(1.6)	
Sex								
Female		34(63)		30(50.8)	41(60.3)	41(60.3)	146(58.6)	
Male		20(37)		29(49.2)	27(39.7)	27(39.7)	103(41.4)	
BMI							(3.57)	19.8 (14-28)
Underweight		16(47.1)	13(38.2)	11(44)	23(38.3)	63(41.2)		
Normal		18(52.9)	20(58.8)	14(56)	37(61.7)	89(58.2)		
Overweight		0(0)	1(2.9)	0(0)	0(0)	1(0.7)		
Education								
None/NFE		18(34)	13(22.4)	16(26.2)	18(26.9)	65(27.2)		
Primary/LSS		11(20.8)	5(8.6)	12(19.7)	7(10.4)	35(14.6)		
HSS/Diploma		19(35.8)	26(44.8)	21(34.4)	30(44.8)	96(40.2)		
Degree and above		5(9.4)	13(22.4)	12(19.7)	12(17.9)	42(17.6)		
Minor		0(0)	1(1.7)	0(0)	0(0)	1(0.4)		
Occupation								
Business/Private		8(14.8)	15(25.4)	21(30.9)	13(19.1)	57(22.9)		
Civil Servant		3(5.6)	7(11.9)	5(7.4)	7(10.3)	22(8.8)		
Minor/Student/Trainee		17(31.5)	22(37.3)	20(29.4)	27(39.7)	86(34.5)		
Uniformed Personnel		3(5.6)	1(1.7)	1(1.5)	2(2.9)	7(2.8)		
Monastic		0(0)	1(1.7)	3(4.4)	2(2.9)	6(2.4)		
Farmer		11(20.4)	2(3.4)	9(13.2)	10(14.7)	32(12.9)		
Housewife		12(22.2)	11(18.6)	9(13.2)	7(10.3)	39(15.7)		

Marital Status					
Married/Living together	35(66)	29(55.8)	28(48.3)	32(47.8)	124(53.9)
Single/Separated	18(34)	23(44.2)	30(51.7)	35(52.2)	106(46.1)
Ethnicity					
Lhotshampa	6(11.1)	6(10.2)	14(20.6)	5(7.4)	31(12.4)
Ngalop	16(29.6)	15(25.4)	18(26.5)	28(41.2)	77(30.9)
Sharchop	32(59.3)	38(64.4)	36(52.9)	35(51.5)	141(56.6)
Religion					
Buddhist	51(96.2)	57(96.6)	52(89.7)	63(94)	223(94.1)
Hindu	2(3.8)	1(1.7)	5(8.6)	2(3)	10(4.2)
Others	0(0)	1(1.7)	1(1.7)	2(3)	4(1.7)
Location of Residence					
Rural	15(27.8)	14(23.7)	16(23.5)	19(27.9)	64(25.7)
Urban	39(72.2)	45(76.3)	52(76.5)	49(72.1)	185(74.3)

Spatial distribution of cumulative number of cases from 2016-2019 shows highest concentration of the cases in western region followed by central Bhutan. Thimphu 105 (42.2%) had the highest case notification of MDR-TB cases followed by Chukha 32 (12.9%), Samtse 20(8%), Sarpang 15(6%), Samdrup Jongkhar 13 (5.2%) and Wangdue 12 (4.8%) districts (Figure 1).

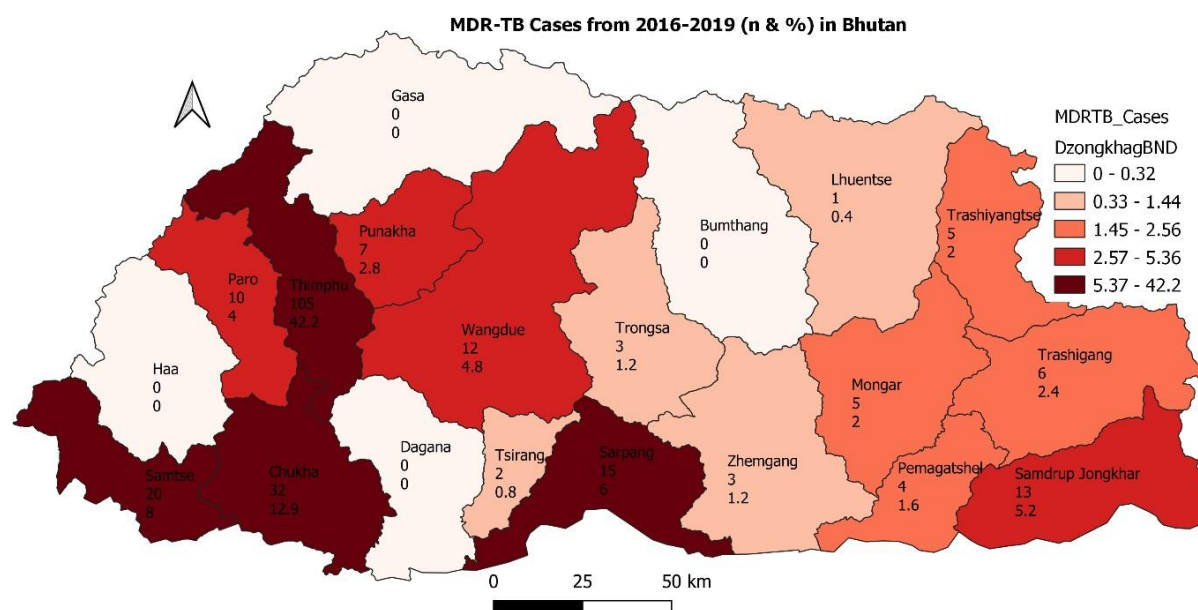


Figure 1 Spatial distribution of MDR-TB Cases from 2016 to 2019 showing number and percent of cases by districts in Bhutan.

Section B: Clinical characteristics

By type of case, Pulmonary TB (PTB) 212 (85.1%) dominated over Extra-pulmonary TB (EPTB) 37(14.9%) with 205(82.3%) initially registered as New MDR-TB and 44(17.7%) were among Retreatment category. Among the Retreatment category, 22(57.00%) of the cases have Relapsed and 14(31.82%) were cases among Treatment after failure (treatment failure). MDR-TB patients were screened 243/249 (97.6%) for HIV and only two positive cases, one each in 2016 and 2019 were detected through the Voluntary Counselling and Testing (VCT) method. Of the 10 who responded to have comorbid conditions, five had diabetes, two had hypertension and rest of them had COPD, cancer and autoimmune disorder each.

Table 2. Clinical characteristics of MDR-TB study participants from 2016-2019, Bhutan.

Year No (%)	2016 n(%)	2017 n(%)	2018 n(%)	2019 n(%)	Total n(%)
Total	54	59	68	68	249
Case Type					
PTB	47(87)	52(88.1)	56(82.4)	57(83.8)	212(85.1)
EPTB	7(13)	7(11.9)	12(17.6)	11(16.2)	37(14.9)
Initial registration					
New	50(92.6)	49(83.1)	44(64.7)	62(91.2)	205(82.3)
Retreatment	4(7.4)	10(16.9)	24(35.3)	6(8.8)	44(17.7)
Retreatment type					
Relapse	1(25)	6(66.00)	14(53.33)	1(16.7)	22(57.00)
Treatment after failure	3(75)	3(30.00)	4(16.67)	4(66.67)	14(31.82)
Loss To Follow Up	0(0)	0(0)	0(0)	1(16.67)	1(2.27)
Others	0(0)	0(0)	1(4.17)	0(0)	1(2.27)
Missing	0(0)	1(10.00)	5(20.83)	0(0)	6(13.64)
Retreatment twice					
No	3(5.6)	3(5.1)	12(17.6)	5(7.4)	23(9.2)
Missing	50(92.6)	50(84.7)	52(76.5)	62(91.2)	214(85.9)
Yes	1(1.9)	6(10.2)	4(5.9)	1(1.5)	12(4.8)
VCT done					
Yes	54(100)	56(94.9)	65(95.6)	68(100)	243(97.6)
No	0(0)	3(5.1)	3(4.4)	0(0)	6(2.4)
HIV status					
Negative	29(53.7)	52(92.9)	51(78.5)	48(70.6)	180(74.1)
Positive	1(1.9)	0(0)	0(0)	1(1.5)	2(0.8)
Unknown	24(44.4)	4(7.1)	14(21.5)	19(27.9)	61(25.1)
Comorbidity					
No	51(96.2)	54(98.2)	58(93.5)	65(95.6)	228(95.8)
Yes	2(3.8)	1(1.8)	4(6.5)	3(4.4)	10(4.2)

Section C: Diagnostic findings and drug resistance pattern

84.8% (201/237) of the MDR-TB cases were AFB positive indicating infectiousness while those cases with chest X-ray results, 44.4% (96/216) of cases were suggestive of pulmonary TB.

The diagnosis of MDR-TB was established in 247 (99.2%) patients with genotypic method with

>94% (233/247) cases being diagnosed using GeneXpert technology. Two case was diagnosed using phenotypic method while 129(51.81%) cases had both genotypic and phenotypic results available for at least isoniazid and rifampicin. More than 80% (194/247) of the cases were confirmed as MDR-TB by FL-LPA. In MDR-TB patients with H and R resistance, additional resistance was also observed in 28.8% (34/118) cases to ethambutol while higher proportion 96.8% (120/124) of resistance was observed to streptomycin. In a subgroup of 72 MDR-TB patients with additional baseline 2nd line LPA results available, fluoroquinolones (FLQs) resistance was present in 4 (1.7%), 2nd line injectable resistance was seen in 1 (0.4%) and 1 (0.4%) was resistant to both FLQs and 2nd line injectable (Table 3).

Table 3: Baseline diagnosis (AFB, CXR) and drug resistance pattern – FLD & SLD of study participants from 2016-2019, Bhutan.

Year No (%)	2016 n(%)	2017 n(%)	2018 n(%)	2019 n(%)	N	N(%)
Total	54	59	68	68	249	
AFB Positive					237	
Yes	51(100)	45(78.9)	43(69.4)	62(92.5)		201(84.8)
No	0(0)	12(21.1)	19(30.6)	5(7.5)		36(15.2)
X-ray Chest PTB					216	
Yes	15(29.4)	23(52.3)	37(61.7)	21(34.4)		96(44.4)
No	36(70.6)	21(47.7)	23(38.3)	40(65.6)		120(55.6)
DST method					249	
Genotypic	31(57.41)	34(57.63)	28(41.18)	25(36.76)		118(47.39)
Phenotypic	0(0)	1(1.69)	1(1.47)	0(0)		2(0.8)
Both	23(42.59)	24(40.68)	39(57.35)	43(63.24)		129(51.81)

Resistant to R (GeneXpert)				235	
Yes	50(100)	52(96.3)	64(100)	67(100)	233(99.15)
No	0(0)	2(3.7)	0(0)	0(0)	2(0.85)
Resistant to Ethambutol				118	
Resistant	5(27.8)	1(4.3)	14(41.2)	14(32.6)	34(28.8)
Susceptible	13(72.2)	22(95.7)	20(58.8)	29(67.4)	84(71.2)
Resistant to Streptomycin				124	
Resistant	19(95)	22(95.7)	38(100)	41(95.3)	120(96.8)
Susceptible	1(5)	1(4.3)	0(0)	2(4.7)	4(3.2)
First line DST (LPA)				216	
HR resistant	43(86)	49(98)	45(84.91)	57(90.48)	194(89.81)
H resistant only	3(6)	1(2)	0(0)	0(0)	4(1.85)
R resistant only	4(8)	0(0)	8(15.09)	6(9.52)	18(8.33)
Second line DST (LPA)				235	
All Sensitive	0(0)	6(10.9)	16(25.8)	44(64.7)	66(28.1)
FLQ resistant only	0(0)	0(0)	0(0)	4(5.9)	4(1.7)
2 nd line Injectable resistant only	0(0)	0(0)	0(0)	1(1.5)	1(0.4)
FLQ & 2 nd line Injectable resistant	0(0)	0(0)	1(1.6)	0(0)	1(0.4)
Results unavailable	50(100)	49(89.1)	45(72.6)	19(27.9)	163(69.4)

Prior to MDR-TB treatment initiation, baseline sputum smear was done in 87.6% (218/249) and culture in 85.1% (212/249) of MDR-TB patients. 12.4% (31/249) sputum smear and 14.9% (37/249) culture were not performed as baseline test among MDR-TB patients (Table 4).

Table 4. Baseline MDR-TB smear & culture follow-up (month 0) of study participants from 2016- 2019, Bhutan.

Year	2016	2017	2018	2019	Total
No (%)	n(%)	n(%)	n(%)	n(%)	n(%)
Total	54	59	68	68	249
Smear					
Positive	45(83.3)	38(64.4)	37(54.4)	43(63.2)	163(65.5)
Negative	4(7.4)	18(30.5)	19(27.9)	14(20.6)	55(22.1)
Not done	5(9.3)	3(5.1)	12(17.6)	11(16.2)	31(12.4)
Culture					
Growth	44(81.5)	45(76.3)	42(61.8)	48(70.6)	179(71.9)
No Growth	2(3.7)	9(15.3)	13(19.1)	6(8.8)	30(12)
Contamination	1(1.9)	0(0)	0(0)	2(2.9)	3(1.2)
Not done	7(13)	5(8.5)	13(19.1)	12(17.6)	37(14.9)

Of 179 patients who were sputum culture positive at baseline, 94.4% (169/179) converted their culture in a median of 91 days (IQR 59-184; range 52-212 days) and 3 (1.7%) patients did not have culture conversion. 88.1% (149/169) patients have had initial sputum culture conversion within three to four months after second line anti-TB treatment initiation. Only 5 (3.0%) of 169 patients with sputum culture positive at treatment initiation had culture conversion at ≥ 6 months (Table 5) & Figure (3).

Table 5: Combined (Longer & Shorter treatment regimen) Initial time to sputum culture conversion among the MDR-TB study participants from 2016-2019, Bhutan.

Month of initial culture conversion	2016 n(%)	2017 n(%)	2018 n(%)	2019 n(%)	Total n(%)
2 Month	19(47.5)	16(38.1)	15(37.5)	19(40.4)	69(40.8)
3 Month	13(32.5)	15(35.7)	14(35)	13(27.7)	55(32.5)
4 Month	3(7.5)	8(19)	6(15)	8(17)	25(14.8)
5 Month	3(7.5)	2(4.8)	4(10)	6(12.8)	15(8.9)
6 Month	2(5)	0(0)	1(2.5)	1(2.1)	4(2.4)
7 Month	0(0)	1(2.4)	0(0)	0(0)	1(0.6)

Median=91 (IQR 59-184; range 52-212) & Mean=92.87 ($\pm$ SD 32.66) days.

Figure 3 (A) shows culture conversion which has started at about 60th day (2 months approx.) and thereafter gradually increased in conversion and peaked at around 90th day (3 months approx.) and noticed a gradual decline in culture conversion after 200 days. Almost all have converted by few days after 120th day.

Figure 3(B) shows Kaplan-Meier curve which explains that culture conversion has begun at about 60th day (2 months approx.), peaked around 90-100th days by achieving about 75% conversion of the cases and thereafter declined till all converted by few days after 200th day.

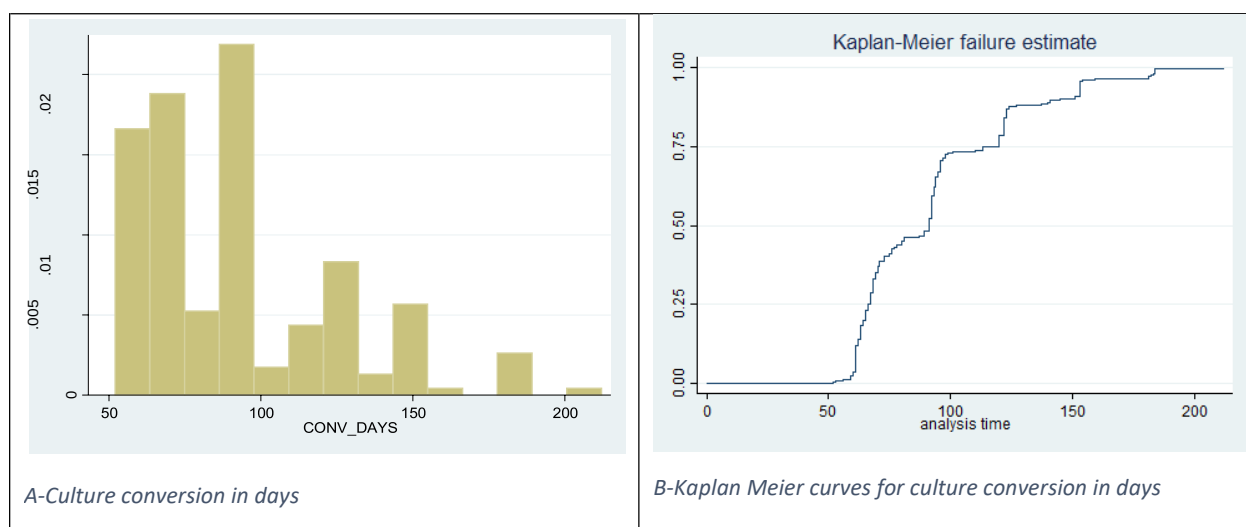


Figure 2 Combined culture conversion days

Culture reversion

There were three patients who did not culture convert. These cases are described case by case as follows;

- First patient had baseline smear positive and culture growth before initiation of MDR-TB treatment and attained initial culture conversion at 2nd month. Follow up smear and culture was done at 3rd & 5th month and both were negative. However, at 7th month, smear was negative and culture growth. At 8th, 9th, 10th 11th, 14th months, follow up smear and culture were both negative. Again at 16th month, culture was positive. Thereafter, two more cultures were done at 17th & 18th and culture no growth. Outcome assigned was cured.
- As per the TB In-charge of ERRH, this patient has missed ATT about a month in the 6th month during the Intensive phase and the reasons for disruption of treatment was, the patient has intentionally stopped due to severe CNS side effects such as depression, psychosis, which could be due to Cycloserine CNS toxicity?

- Second patient had smear positive and culture growth at baseline before the initiation of MDR-TB treatment. 1st month was smear negative and culture no growth. 2nd month smear negative and culture was not done. 4th month smear was not done and culture was contaminated. Then, at 11th month, culture no growth. At 14th month smear positive and culture growth and at 15th month smear negative and culture growth. At 18th month culture growth and thereafter patient was loss to follow-up.

As per the TB In-charge of JDWNRH and the treating physician, this patient had issues of non-compliance with treatment, follow-up smear and culture and difficult patient to complete treatment. She was loss to follow –up and treatment stopped. She ultimately succumbed to death, but the outcome remains as loss to follow up.

- Third patient (ND) had smear positive and culture no growth at baseline before initiation of MDR-TB treatment. At 1st and 2nd month, both smear and culture positive and culture remained still positive (growth) at 3rd and 4th month. At 5th month, smear and culture were both negative. At 6th month, smear positive and culture no growth, i.e. culture conversion was attained at 6th month. At 7th month, smear was still positive and culture no growth. At 8th and 9th months, both smear and culture negative. 19th month smear negative and culture not done. At 11th month, both smear and culture were negative. At 12th and 15th month smear negative, culture not done. At 20th, 21st, 22nd and 23rd month, smear and culture were positive. Therefore, culture reversion at 21st month and switched to Pre-XDR treatment.

- There was no significant difference in the median days to initial sputum culture conversion by treatment regimen; longer regimen median was 91 days (IQR 59-184, range 52-212 days) and shorter regimen median was 89 days (IQR 61-184, range 60-184 days). 125 (**87.4%**) of 143 patients on longer regimen and 52 (**91.2%**) of 57 patients on shorter regimen have had initial sputum culture conversion within three to four months after initiation of second line anti-TB treatment. The maximum time to initial sputum culture conversion was less in shorter regimen (184 days) when compared to longer regimen (212 days) (Table 6).

Table 6: Comparison of initial time to sputum culture conversion by MDR-TB treatment regimen among the study participants from 2016-2019, Bhutan.

Month of initial culture conversion	Treatment regimen		Total
	Longer Regimen	Shorter Regimen	
Total	143	57	200
2 Month	64(44.76)	28(49.12)	92(46)
3 Month	42(29.37)	15(26.32)	57(28.5)
4 Month	19(13.29)	9(15.79)	28(14)
5 Month	12(8.39)	4(7.02)	16(8)
6 Month	4(2.8)	1(1.75)	5(2.5)
7 Month	1(0.7)	0(0)	1(0.5)
8 Month	1(0.7)	0(0)	1(0.5)

Longer regimen: Median=91 (IQR 59-184), (Range 52-212) & Mean=93.51 (± 33.48) days.

Shorter regimen: Median=89 (IQR 61-184), (Range 60-184) & Mean=91.26 (± 30.75) days.

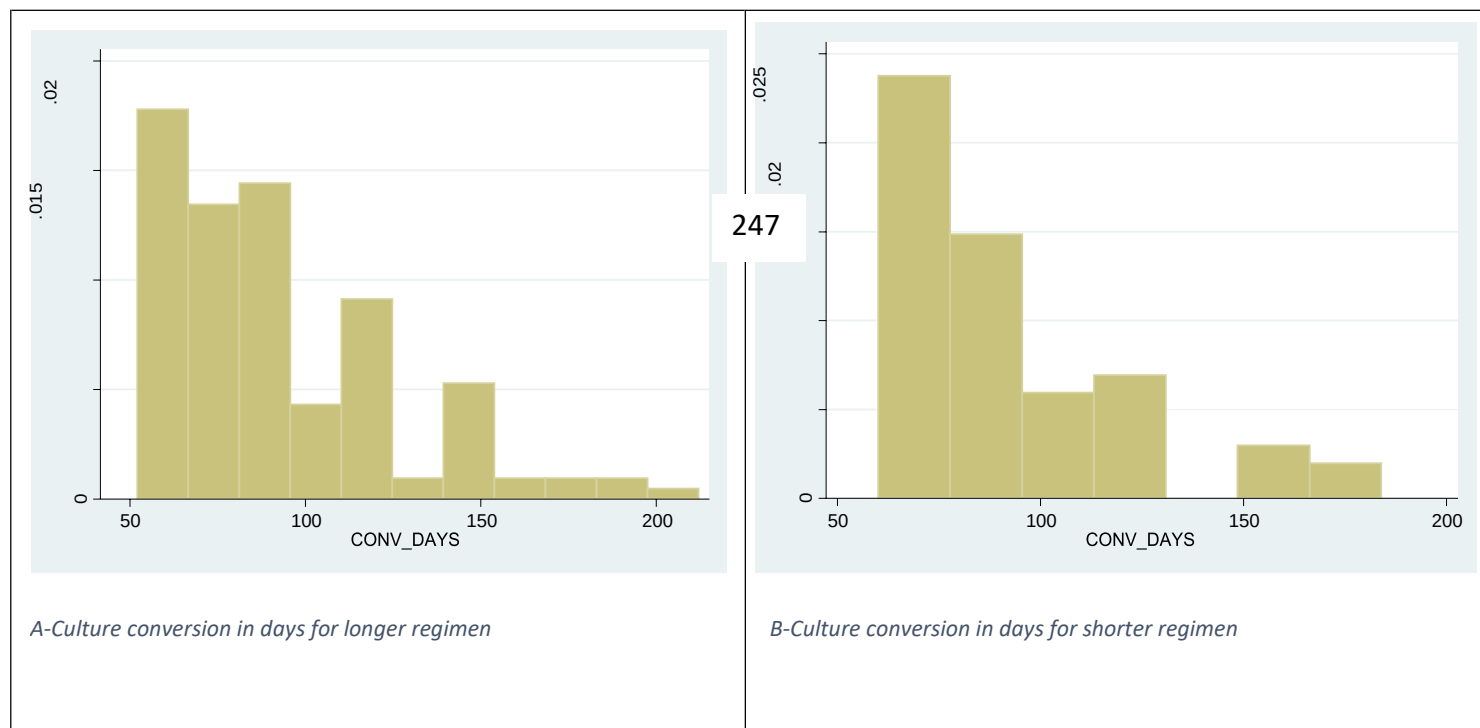


Figure 3 Culture conversion of longer and shorter regimen.

Proportion of both smear 31/67 (46.27%) and culture 28/67 (41.79%) monthly treatment follow-up was comparatively higher in the Shorter MDR-TB treatment regimen group compared to smear 51/182 (28.02%) and culture 46/182 (25.27%) monthly treatment follow-up in the Longer MDR-TB treatment regimen group (Table 7).

Smear and culture treatment follow-up comparison was done for intensive phase (initial 8 months) for the longer regimen and for full 9 months for the shorter regimen as smear and culture follow up is mandated to be done monthly in both the regimens.

About 71.98 % (131/182) smear and 74.73 % (136/182) culture has missed more than two monthly follow up in longer regimen during intensive phase, while 53.73% (36/67) smear and 58.21% (39/67) culture has also missed more than two monthly follow up in the shorter regimen (Table 7).

Table 7: Completeness of smear & culture follow-up for Longer treatment regimen done or not done till 8th month and smear & culture follow-up for Shorter Regimen done or not done till 9th month.

Longer Regimen

	Smear		Culture	
Monthly Follow-up schedule (1-8 months)	<i>n</i>	%	<i>n</i>	%
Done	51	28.02	46	25.27
Not Done	131	71.98	136	74.73
Total	182	100	182	100

Shorter Regimen

	Smear		Culture	
Monthly Follow-up schedule (1-9 months)	<i>n</i>	%	<i>n</i>	%
Done	31	46.27	28	41.79
Not Done	36	53.73	39	58.21
Total	67	100	67	100

Section D: Treatment regimen, adherence and outcome

182 (73.1%) of the study participants have completed Longer treatment regimen and 67(26.9%) have completed Shorter treatment regimen respectively. Shorter regimen was introduced in the country since 2018 onwards in a phase wise scalable manner.

Table 08: MDR-TB treatment course completed (Longer and Shorter Regimen) by the study participants from 2016-2019, Bhutan.

Type of Regimen	2016 n(%)	2017 n(%)	2018 n(%)	2019 n(%)	Total n(%)
Longer Regimen	54 (100)	59 (100)	50 (73.5)	19 (27.9)	182 (73.1)
Shorter Regimen	0(0)	0(0)	18(26.5)	49(72.1)	67(26.9)
Total	54	59	68	68	249

The overall treatment adherence of MDR-TB treatment was about 97.8% (combined adherence of intensive phase and continuation phase) (Table 9).

The treatment adherence of longer MDR-TB regimen was 99.3% in intensive phase and 97.2% in continuation phase, whereas treatment adherence of shorter regimen was 93.3% (Table 10). Treatment adherence was slightly higher in longer regimen compared to shorter regimen.

Table 09. Overall treatment adherence of MDR-TB Longer and Shorter regimen treatment among the study participants from 2016-2019, Bhutan.

Year n(%)	2016 n(%)	2017 n(%)	2018 n(%)	2019 n(%)	Total n(%)
Total	54	59	68	68	249
Treatment adherence_IP					
Yes	40(97.6)	50(100)	54(100)	60(98.4)	204(99)
No	1(2.4)	0(0)	0(0)	1(1.6)	2(1)
<i>Dose missed_IP</i>					
≥ 7 missed dose	1(100)	0(0)	0(0)	1(100)	2(100)
< 7 missed dose					
Treatment adherence_CP					
Yes	39(95.1)	49(98)	53(100)	56(93.3)	197(96.6)
No	2(4.9)	1(2)	0(0)	4(6.7)	7(3.4)
<i>Dose missed_CP</i>					
≥ 7 missed dose	1(50)	1(100)	0(0)	3(75)	5(71.4)
< 7 missed dose	1(50)	0(0)	0(0)	1(25)	2(28.6)

Table 10: Treatment adherence of MDR-TB Longer and Shorter treatment regimen among the study participants from 2016-2019 in Bhutan.

	Longer Regimen				
Year n(%)	2016 n(%)	2017 n(%)	2018 n(%)	2019 n(%)	Total n(%)
Treatment adherence_IP					
Yes	40(97.6)	50(100)	39(100)	16(100)	145(99.3)
No	1(2.4)	0(0)	0(0)	0(0)	1(0.7)
<i>Dose missed_IP</i>					
≥ 7 missed dose	1(100)	0(0)	0(0)	0(0)	1(100)
< 7 missed dose					
Treatment adherence_CP					
Yes	39(95.1)	49(98)	38(100)	15(93.8)	141(97.2)
No	2(4.9)	1(2)	0(0)	1(6.3)	4(2.8)
<i>Dose missed_CP</i>					
≥ 7 missed dose	1(50)	0(0)	0(0)	0(0)	1(25)
< 7 missed dose	1(50)	1(100)	0(0)	1(100)	3(75)

	Shorter regimen				
Year <i>n</i> (%)	2016 <i>n</i> (%)	2017 <i>n</i> (%)	2018 <i>n</i> (%)	2019 <i>n</i> (%)	Total <i>n</i> (%)
Treatment adherence					
Yes			15 (100)	41 (91.1)	56 (93.3)
No			0 (0)	4 (8.9)	4 (8.9)
Dose missed					
≥ 7 missed dose			0 (0)	3 (75)	3 (75)
< 7 missed dose			0 (0)	1 (25)	1 (25)

Treatment Outcome

For assigning correct treatment outcomes, the following treatment outcome definitions as per the national TB guidelines have been followed, which is as per the WHO updated treatment outcome definitions (Table 11).

Table 11: Definitions of treatment outcomes for drug-resistant patients

Treatment Outcome	Definition
Cured	Treatment completed as recommended by the national guideline without evidence of failure AND three or more consecutive cultures taken at least 30 days apart are negative after the intensive phase for conventional regimen and three or more culture negative done monthly for shorter regimen
Treatment completed	Treatment completed as recommended by the national guideline without evidence of failure BUT no record that three or more consecutive cultures taken at least 30 days apart are negative after the intensive phase.
Treatment failed^a	Treatment terminated or need for permanent regimen change of at least two anti-TB drugs because of: Lack of conversion ^a by the end of the intensive phase; or Bacteriological reversion in the continuation phase after conversion to negative; or Evidence of additional acquired resistance to Fluoroquinolones or second-line injectable drugs; or Adverse drug reactions.
Died	A patient who dies for any reason during the course of treatment.
Lost to follow-up	A patient whose treatment was interrupted for two consecutive months or more.
Not Known	A patient whose treatment outcome is not known. (This includes cases “transferred out” to another treatment unit and whose treatment outcome is unknown).

Treatment success	The sum of <i>Cured</i> and <i>Treatment completed</i> .

^aFor *treatment failed*, lack of conversion by the end of the intensive phase implies that the patient does not convert within 6 months (shorter regimen) treatment.

From 2016 to 2019, 182 (73.1%) MDR-TB patients have been assigned and completed longer treatment regimen and 67 (26.9%) have been assigned and completed Shorter treatment regimen. The overall Treatment Success Rate (TSR) was 92.9% for Longer treatment regimen and 94% for Shorter treatment regimen, respectively. These findings are as per the records of Treatment outcomes maintained in the MDR-TB register (Table 12).

The overall TSR was 93.2% as per the records maintained in the MDR-TB register.

Table 12: MDR-TB treatment outcome by treatment regimen and categories of outcome as recorded in the MDR-TB TB Register from 2016-2019, Bhutan.

Outcome	2016 n(%)	2017 n(%)	2018 n(%)	2019 n(%)	Total n(%)
Longer Regimen	54 (100)	59 (100)	50 (73.5)	19 (27.9)	182 (73.1)
Shorter Regimen	0(0)	0(0)	18(26.5)	49(72.1)	67(26.9)
Total	54	59	68	68	249

Longer Regimen					
Outcome	2016 n(%)	2017 n(%)	2018 n(%)	2019 n(%)	Total n(%)
Cured	18(33.3)	31(52.5)	20(40.0)	9(47.4)	78(42.9)
Treatment completed	33(61.1)	25(42.4)	24(48.0)	9(47.4)	91(50.0)
Treatment failed	1(1.9)	0(0)	0(0)	0(0)	1(0.6)
Death	2(3.7)	2(3.4)	3(6.0)	0(0)	7(3.9)
Loss to follow-up	0(0)	0(0)	1(2.0)	0(0)	1(0.6)
Not evaluated	0(0)	0(0)	1(2.0)	0(0)	1(0.6)
Switched to Pre-XDR treatment	0(0)	1(1.7)	1(2.0)	1(5.6)	3(1.7)
Total	54	59	50	19	182
Shorter Regimen					
Outcome			2018 n(%)	2019 n(%)	Total n(%)
Cured			14(77.8)	39(79.6)	53(79.1)
Treatment completed			2(11.1)	8(16.3)	10(14.9)
Death			1(5.6)	0(0)	1(1.5)
Switched to Pre-XDR treatment			1(5.6)	2(4.1)	3(4.5)
Total			18	49	67
Overall treatment outcome of Longer and Shorter Regimen					
Outcome	2016 n(%)	2017 n(%)	2018 n(%)	2019 n(%)	Total n(%)
Cured	18(33.3)	31(52.5)	34(50.0)	48(70.6)	131(52.6)
Treatment completed	33(61.1)	25(42.4)	26(38.2)	17(25.0)	101(40.6)
Treatment failed	1(1.9)	0(0)	0(0)	0(0)	1(0.4)
Death	2(3.7)	2(3.4)	4(5.9)	0(0)	8(3.2)
Loss to follow-up	0(0)	0(0)	1(1.5)	0(0)	1(0.4)
Not evaluated	0(0)	0(0)	1(1.5)	0(0)	1(0.4)
Switched to Pre-XDR treatment	0(0)	1(1.7)	2(2.9)	3(4.4)	6(2.4)
Total	54	59	68	68	249
TSR	94.4	94.9	88.2	95.6	93.3

The overall TSR for treatment assigned by the study was as same as the outcome of the treatment outcome as recorded in the register which was at 93.3%. The TSR recorded and reported by the NTCP was slightly different from the study findings (Table 13).

Table 13. Treatment outcomes assigned as per the smear and culture conversion follow-up and national TB guidelines and study findings for MDR-TB cases from 2016-2019, Bhutan.

Assigned Overall treatment Outcomes	2016 n(%)	2017 n(%)	2018 n(%)	2019 n(%)	Total n(%)
Cured	18 (33.33)	31 (52.54)	34 (50)	48 (70.59)	131 (52.61)
Treatment completed	33 (61.11)	25 (42.37)	26 (38.24)	17 (25)	101 (40.56)
Treatment failed	1 (1.85)	0 (0)	0 (0)	0 (0)	1 (0.4)
Death	2 (3.7)	2 (3.39)	4 (5.88)	0 (0)	8 (3.21)
Loss to follow-up	0 (0)	0 (0)	1 (1.47)	0 (0)	1 (0.4)
Not evaluated	0 (0)	0 (0)	1 (1.47)	0 (0)	1 (0.4)
Switched to Pre-XDR treatment	0 (0)	1 (1.69)	2 (2.94)	3 (4.41)	6 (2.41)
Total	54	59	68	68	249
TSR	94.4	94.9	88.2	95.6	93.3
TB Program Report TSR	91 (50/55)	98 (59/60)	94 (59/63)	97.14(68/70)	95

Comparison of TSR (%)

Sources	2016	2017	2018	2019	Total
TB Register	94	95	88	96	93
Current Study	94	95	88	96	93
NTCP Reported	91	98	94	97	95

When the treatment outcome is grouped into Favourable (Cured and Completed) vs Unfavourable (treatment failed, death, loss to follow-up, not evaluated and switched to pre-XDR treatment), the overall Favourable outcome was at 93.2% (232/249) and Unfavourable outcome at 6.8% (17/249) (Table 14).

Table 14: MDR-TB treatment outcome segregated into Favourable vs Unfavourable from 2016-2019 as per the records maintained in the MDR-TB Register in Bhutan.

Longer Regimen					
Outcome	2016 n(%)	2017 n(%)	2018 n(%)	2019 n(%)	Total n(%)
Favourable	51(94.4)	56(94.9)	44(88.0)	18(94.7)	169(92.9)
Unfavourable	3(5.6)	3(5.1)	6(12.0)	1(5.3)	13(7.1)
Total	54	59	50	19	182

Shorter Regimen					
Outcome		2018 n(%)	2019 n(%)	Total n(%)	
Favourable		16(8.9)	47(95.9)	63(94.0)	
Unfavourable		2(11.1)	2(4.1)	4(6.0)	
Total		18	49	67	
Overall treatment outcome of Longer and Shorter Regimen					
Outcome	2016 n(%)	2017 n(%)	2018 n(%)	2019 n(%)	Total n(%)
Favourable	51(94.4)	56(94.9)	60(88.2)	65(95.6)	232(93.2)
Unfavourable	3(5.6)	3(5.1)	8(11.8)	3(4.4)	17(6.8)
Total	54	59	68	68	249

Comparing Treatment outcomes recorded in the MDR-TB Register and the Actual Treatment outcome assigned as per the National TB Guidelines and the study findings

As per the sputum culture conversion during the intensive phase and continuation phase for the Longer and Shorter treatment regimen respectively, correct treatment outcomes were assigned by the investigators as per the national TB guideline treatment outcomes definitions.

The study found that about 35% (87/249) treatment outcomes were wrongly recorded in the MDR-TB Register. Hence the study has assigned 35% treatment outcome correctly as per the treatment outcomes definitions (Table 14).

Table 14. Correct assignment of the wrongly recorded treatment outcomes in the MDR-TB Register from 2016-2019, Bhutan.

Treatment Outcome as per TB Register record (Register Outcome)	Correctly recorded in TB Register	Wrongly recorded as per Treatment Outcome definition	Total	% Wrongly recorded
Cured	112	58	170	34
Treatment completed	35	6	41	15
Death	8	0	8	0
Loss to follow-up	1	2	3	67
Not assigned	0	21	21	100
Switched to Pre-XDR treatment	6	0	6	0
Total	162	87	249	35

Reassigned Outcome as per smear & Culture (Study Outcome)	Treatment as per smear follow-up	Correctly recorded in TB Register	Correctly Reassigned per Treatment Outcome definition	as	Total	% Correctly Reassigned
Cured		112	19		131	15
Treatment completed		35	66		101	65
Treatment failed		0	1		1	100
Death		8	0		8	0
Loss to follow-up		1	0		1	0
Not evaluated		0	1		1	100
Switched to Pre-XDR treatment		6	0		6	0
Total		162	87		249	35

ANALYTICAL STATISTICS

Univariate analysis

A univariate analysis of socio-demographic, clinical, diagnostic, treatment regimen were performed using the log-rank test. Kaplan-Meier curves for time to culture conversion and time to reversion was explored to test the proportional hazards assumptions across independent variable categories.

In the univariate analysis, ethnicity, place of residence during initial diagnosis, AFB positivity at initial diagnosis, smear positive at zero months and culture positive at zero month had overall significant (p-value) for unfavourable outcome, based on time to culture conversion and culture reversion (Table 15).

Table 15: Log-Rank Test to compare statistical significance difference between groups of variables among the MDR-TB study participants, Bhutan.

Variables		<i>n</i> Unfavourable outcome (%)	<i>p-value</i>	X ²
Sex			0.75	0.11
Female	146	6(4.1)		
Male	103	11(10.7)		
Median age			0.59	0.3
≤ 25/Median Age	134	4(3)		
> 25-Median Age	115	13(11.3)		
BMI Category			0.26	1.26
Underweight	63	2(3.2)		
Normal	90	6(6.7)		
Marital status			0.60	0.28
Married/Living Together	124	8(6.5)		

Single/Separated	106	5(4.7)	
Education Level		0.37	3.18
None/No Formal Education	66	7(10.6)	
Primary/LSS	35	5(14.3)	
HSS/Diploma	96	3(3.1)	
Degree and above	42	1(2.4)	
Ethnicity		0.02	7.58
Lhotshampa	31	4(12.9)	
Ngalop	77	3(3.9)	
Sharchop	141	10(7.1)	
Location of Residence		0.06	3.59
Rural	64	6(9.4)	
Urban	185	11(5.9)	
Initial Registration Type		0.13	2.3
New	205	13(6.3)	
Retreatment	44	4(9.1)	
AFB Positivity		0.03	4.53
Yes	201	15(7.5)	
No	36	1(2.8)	
X-Ray Chest Positivity		0.67	0.18
Yes	96	6(6.3)	
No	120	8(6.7)	
Month 0 Smear		<0.01	13.48
Positive	163	13(8)	
Negative	55	1(1.8)	
Not done	31	3(9.7)	
Month 0 Culture		<0.01	8.72
Growth	179	12(6.7)	
No Growth	30	2(6.7)	
Treatment regimen		0.74	0.11
Longer Regimen	182	13(7.1)	
Shorter Regimen	67	4(6)	
Treatment start Year		0.8742	0.7
2016	54	3(5.6)	
2017	59	3(5.1)	
2018	68	8(11.8)	
2019	68	3(4.4)	

Kaplan-Meier curves for the univariate analysis presented in the (Figure 4) shows that those MDR-TB patients who resided in the urban areas are likely to attain culture conversion slightly earlier than the rural counterparts (A).

Those MDR-TB cases with AFB positivity at the time of initial diagnosis has delayed culture conversion, compared to those cases who were AFB negative at the time of initial diagnosis (Figure 4, B).

Those with baseline smear positive and culture growth before the initiation of MDR-TB treatment were found to convert late compared to baseline line smear negative and culture no growth (Figure 4, C & D).

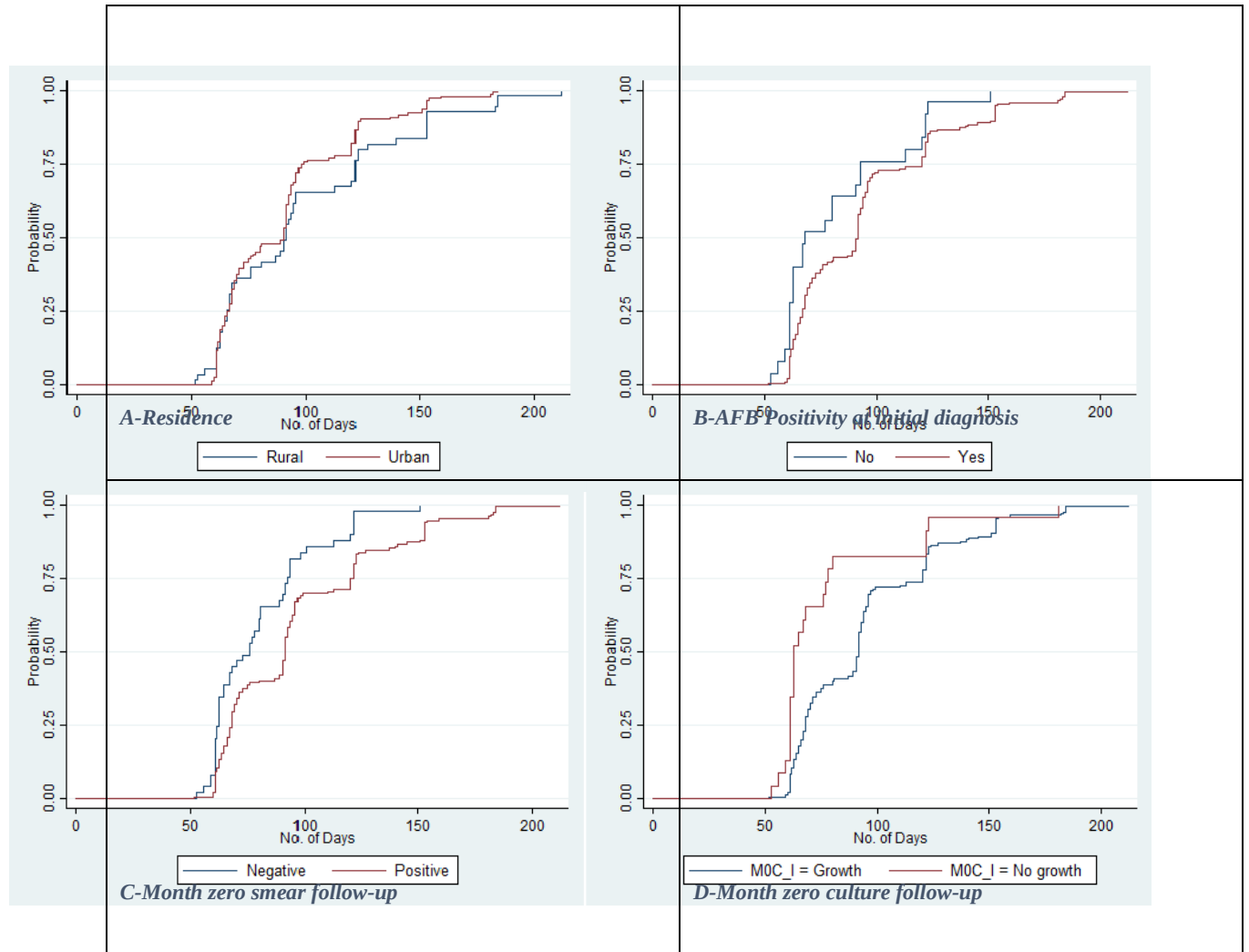


Figure 4. Kaplan-Meier curves for time to culture conversion by residence, AFB positivity, month zero smear and culture follow up.

Multivariate analysis

The assumption of proportion hazard was verified using the Cox-models. Initially, the variables were included in a bivariate Cox-proportional regression model. All variables which were significantly associated and those variables with threshold p -value of ≤ 0.2 were included in the final multivariable logistic regression analysis.

For multivariate logistic regression, treatment failed, death, loss to follow-up, not evaluated and switched to pre-XDR treatment were combined as a single unfavorable outcome (time to culture conversion), while cured and treatment completed were combined as favourable outcome.

Table 15: Cox-proportional model with Hazard Ratios (HR) in the multivariate regression for unfavourable outcome (time to culture conversion) among the MDR-TB patients from 2016- 2019, Bhutan.

Variables	HR	<i>p-value</i>	95% (CI)
BMI Category			
Normal	1.29	0.19	0.881912 - 1.891679
Residence			
Urban	1.56	0.04	1.023269 - 2.364398
Month 0 Smear			
Positive	0.61	0.04	0.389479 - 0.968597
Month 0 Culture			
Growth	0.51	0.04	0.264254 - 0.970641

In the final Cox-model, urban residents had hazard ratio of 1.56 compared to rural residents with significant (p -value 0.04) and 95% CI (1.023269-2.364398) for unfavourable outcome (time to culture conversion). This indicates that urban residence among the MDR-TB patients were 1.56 times more likely to attain culture conversion earlier compared to rural counterparts.

Similarly, hazard ratios of baseline smear and culture before the initiation of MDR-TB treatment was <1 with significant (*p-value* 0.04). This indicates that smear positive and culture growth at baseline were more likely to convert late, compared to baseline smear negative and culture no growth.

Although hazard ratio of normal BMI was 1.29, this finding was not significant in the final model.

DISCUSSIONS

MDR-TB patients treated under the NTCP PMTD Guidelines has continually reported treatment success rate of more than $>90\%$ consistently in the last five years. The latest global RR/MDR-TB treatment success rate was 57% and TSR in the South-East Asia for RR/MDR-TB cases was 52%. Bhutan has consistently reported a high TSR with 98% in 2019⁶. A high treatment success rate notification by the NTCP in the face of increase in MDR-TB cases among the newly diagnosed pulmonary cases led us to examine and assess the treatment adherence and treatment outcomes among MDR-TB cases who have completed the standardized treatment regimen. In general, this retrospective study showed that there was a good treatment adherence level and attained high level of treatment success rate among MDR-TB patients in Bhutan. However, this study explored deeper into treatment non-adherence which was at 2.8% for longer regimen and 6.7% shorter regimen and unfavourable treatment outcome was 6.8% (17/249). Treatment wrongly recorded in MDR-TB Register was 35% (87/249), which requires immediate programmatic interventions.

Socio-demographic characteristics

In our study, we found that participant's age was highest in the age range of 15-34 years (79%) with female sex predominance 58.6% (146/249). This age group is economically productive and most mobile age group.

The education background of people with MDR-TB was maximum among those who went to HSS/Diploma holders at 40.2% (96/249), followed by no formal education/NFE. Most of the people who suffered from MDR-TB were among the students and trainees 34.5% (86/249). This finding indicates that primary transmission of MDR-TB cases are probably occurring in these congregate setting such as schools and training institutes and they are a suitable target population for programmatic interventions. Similar finding was also reported in the Tuberculosis Epidemiological Review in Bhutan, June 2020⁷.

Spatial distribution of the MDR-TB cases showed that most of the cases resided in urban areas at the time of diagnosis, in the western region of the country, namely in Thimphu, Chukha, and

⁶ Global Tuberculosis Report 2020. The World Health Organization.

⁷ Tuberculosis Epidemiological Review in Bhutan, June 2020.

Samtse districts followed by Sarpang districts in central Bhutan. However, the study noted that rural residents 25.7% (64/249) were likely to achieve culture conversion late, compared to urban residents. This calls for programmatic intervention in terms of improving follow-up smear and culture among MDR-TB cases residing in rural areas.

Clinical characteristics

The proportion of PTB 85.1% (212/249) was higher than EPTB 14.9% (37/249) in our study. PTB among newly diagnosed was 82.3% (215/249) compared to retreatment category 17.7% (44/249). We have also noted that AFB positive at initial diagnosis was 84.8% (201/237). A higher proportion of PTB mostly diagnosed as new MDR-TB at first instance fueled with high rate of positive sputum smear increases the risk of transmission of MDR-TB in the community, prior to diagnosis and initiation of effective MDR-TB treatment.

The DRS report of 2017 published by the RCDC showed that 13.4% (50/373) of RR/MDR-TB cases were among new and 16.7% (3/18) were among retreatment group MDR-TB cases⁸.

In 2018, DRS report showed 13.07% (52/398) RR/MDRTB among new and 20% (1/5) among retreatment group⁹.

The DRS report of 2019 showed 14.7% (76/518) RR/MDRTB among new and 9.4% (5/53) among retreatment group¹⁰. This indicates that MDR-TB treatment has potentially transmitted and spread as a primary infection in the population, probably among the target age group population discussed above.

The DRS report of 2020 showed 10.55% (53/502) RR/MDR-TB among new and 26.42% (14/53) among retreatment group¹¹. In 2020, there was a fall in the rate of MDR-TB detected among newly diagnosed group with a concurrent increase in RR/MDR-TB detected among the retreatment group by about three folds, compared to 2019 DRS report. The noticeable drop in MDR-TB among newly diagnosed could probably be forecasting actual fall in TB epidemiology,

⁸ Tuberculosis Drug Surveillance Report, NTRL, RCDC, 2017. ⁹ Tuberculosis Drug Surveillance Report, NTRL, RCDC, 2018. ¹⁰ Tuberculosis Drug Surveillance Report, NTRL, RCDC, 2019. ¹¹ Tuberculosis Drug Surveillance Report, NTRL, RCDC, 2020.

which will be able to be appreciated only if there is continuous fall is observed in the next few years. This shows that those active MDR-TB cases are diagnosed in time and transmission is being interrupted thereby leading to a gradual decline in detection rate. The decrease in primary transmission could also be attributed to positive impact of COVID-19 prevention practices such as wearing face masks, keeping safe distance which could have led to interruption of transmission of TB in general, although not yet proven in our setting. In the meantime, the program data reports a gradual decline in of DS-TB notification over the last 10 years with a small spike in 2016¹².

In 2020, sputum samples were directly subjected to first line LPA, where the proportion of samples tested is higher, whereas till 2019, LPA was done only after culture positives and culture recovery among the retirement group is less. Therefore, this could have actually lead to apparent increase in MDR-TB rate among the retreatment groups without actual increase in the detection rate. The other prospective reason could be a natural shift of actual increase in MDR-TB cases among the retreatment groups due to development of resistance. Most recently, a large, retrospective, population-based cohort study showed that the incidence density of MDR-TB was

22.6 per 1,000 person-years (95% CI 20.9–24.3) for individuals with previous history of TB treatment¹³.

VCT converge was very good at 97.6% and among the HIV tested, only two MDR-TB cases had HIV co-infections. Of the 10 people with co-morbidity, 2.10% (5/238) had diabetes mellitus (DM). In a study by Wangchuk et. al, the prevalence of DM among the PTB patients was 0.87% (95% CI: 0.20-2.50) at the national referral hospital in Bhutan¹⁴. The findings from Bhutan is comparatively lower than the prevalence of DM among TB patients around the world, which varies from 12% to 44% according to different regions¹⁵.

¹² Annual Tuberculosis Report 2020, NTCP, Department of Public Health, Ministry of Health.

¹³ Incidence Density and Predictors of Multidrug-Resistant Tuberculosis among Individuals with Previous Tuberculosis History: A 15-Year Retrospective Cohort Study.

¹⁴ Prevalence and risk factors of pulmonary tuberculosis among people living with diabetes mellitus at the National Referral Hospital, Thimphu, Bhutan

¹⁵ High diabetes mellitus prevalence with increasing trend among newly-diagnosed tuberculosis patients in an Asian population: A nationwide population-based study; Po-Yen Ko et. al.

Diabetes is a recognized a known risk factor for development of TB in general and bi-directional screening is an important approach to catch either of the diseases at early stage and manage them.

DST method and drug resistance pattern

Rapid diagnosis of MDR-TB using molecular techniques are useful in early treatment initiation before phenotypic DST results are available. With the introduction of GeneXpert technology in Bhutan, from 2016 onwards R resistance which is being considered as a surrogate marker for MDR-TB has been used for rapid diagnosis of MDR-TB in the country. Our study findings shows that more than 99% of the registered MDR-TB cases were screened using the GeneXpert technology and more than 80% cases confirmed as MDR-TB by FL-LPA. Additional resistance against S was the most prevalent drug resistance pattern observed among the cases. These finding confirms that the continuous use of S as first line drug in Bhutan until its withdrawal in 2018 (2019 TB Guideline revision has totally removed S)¹⁶ had minimal relevance and ineffective for TB treatment due to high resistance levels and questions the effectiveness of S as second line drugs for the treatment of MDR-TB.

Lower proportion of MDR-TB cases had additional baseline 2nd line LPA results available and with the presence of resistance in both FLQs and 2nd line injectables drugs being observed among MDR-TB cases in this study, it is essential that all MDR-TB cases have baseline 2nd line drugs resistance pattern available prior to initiation of MDR-TB treatment so that correct treatment can be given as per the drug resistance pattern.

Initiation of MDR-TB treatment prerequisites baseline test as per the PMTD guideline which includes sputum smear microscopy and culture for monitoring of treatment. In this study, it was observed that only about 85% of the registered MDR-TB cases had their baseline smear and culture result available. It is essential to note that positive cultures are often detected before positive smears which are critical in monitoring of treatment. These findings should contribute evidence to programmatic decision-making to ensure that all MDR-TB cases receives baseline smear and culture tests as per the PMTD guideline.

¹⁶ Integrated National Guidelines for Management of Tuberculosis in Bhutan 2019, 7th edition.

Overall, 93% of the patients had a favorable treatment outcome (culture converted) within 3 to 4 months after the initiation of MDR-TB treatment and no significant difference was observed in the median days to initial sputum culture conversion by treatment regimen; longer regimen median was 91 days (IQR 59-184, range 52-212 days) and shorter regimen median was 89 days (IQR 61-184, range 60-184 days). The high sputum culture conversion rates (94.4%) were comparable to results from other countries (average of 70%)¹⁷.

Five patients with baseline culture positive had culture conversion at >6 months after the initiation of MDRTB treatment and three patients had culture reversion. Further characterization of these patients with delayed culture conversion is needed to determine the potential causes of persistent culture positivity. Common observation among the non-converters (culture reversion) were treatment interruptions and non-adherence due to drug toxicity. For these patients, additional programmatic interventions may be needed to effect conversion which may include enhanced adherence support, aggressive management of adverse events and treatment monitoring by monthly culture follow ups. One of the patients with culture reversion has been ultimately confirmed as XDR-TB and further case-based investigation is obligatory to understand the causes and prevent such cases in the future.

Monthly culture for patients on MDR-TB treatment is essential for treatment monitoring and in this study, it was observed that about 71.98 % smear and 74.73 % culture has missed more than two monthly follow up in longer regimen during intensive phase, while 53.73% smear and 58.21% culture has also missed more than two monthly follow up in the shorter regimen. These finding reiterates that PMTD guideline needs to be reinforced in order to improve the monthly smear and culture follow up among patients undergoing MDR-TB treatment.

Treatment adherence and outcomes

- Adherence by regimen

The overall treatment adherence of MDR-TB treatment was very high with 97.8% (combined adherence of intensive phase and continuation phase) with overall treatment non-adherence of

¹⁷ Nathanson E, Lambregts-van Weezenbeek C, Rich ML, et al. Multidrug-resistant tuberculosis management in resource-limited settings. *Emerg Infect Dis.* 2006;12(9):1389-1397. doi:10.3201/eid1209.051618

2.2%. This study found that the treatment non-adherence was slightly more for the shorter regimen (6.7%) compared to the longer regimen (2.8%).

In this study, patients were assigned as non-adherence to MDR-TB treatment if the patient has missed $\geq$ seven doses in intensive phase (IP) and continuation phase (CP). Patients were assigned as having adherence to MDR-TB treatment if they have missed $<$ seven doses in intensive phase (IP) and continuation phase (CP). For shorter regimen, non-adherence was considered if the patients have missed $\geq$ seven doses in the entire treatment duration. This comparison was made in reference to the previous similar large sample study in India, which looked at the treatment outcomes and associated factors among India's initial cohorts of MDR-TB patients under the revised national TB control programme (2007-2022)¹⁸.

Shorter MDR-TB regimen (9-12 months) has proved to be very effective in some of the developing nations such as Bangladesh and several African countries. In West Bengal, India, of the total 203 cases, cure rate accounted for 44.3% with the shorter regimen. Lost to follow-up was found to be 13.7%, in which alternative dispute resolution accounted for the major cause. A substantial amount of INH resistance is seen among the follow-up culture positive cases speculating the role of ethionamide in the regimen¹⁹.

This shorter regimen was endorsed by the WHO based on the meta-analysis studies done by the Union, Damien Foundation, Medecins Sans Frontieres and the Antwerp Institute of Tropical Medicine in Belgium. Also called as "Bangladesh regimen" as it was started post initial studies in Bangladesh is intended for Rifampin resistance RR-TB and MDR-TB cases who have not been previously treated with 2nd line drugs and in whom fluoroquinolone resistance and 2nd line injectable resistance is excluded or is considered highly unlikely. This shorter regimen is expected

¹⁸ Unacceptable treatment outcomes and associated factors among India's initial cohorts of multidrug-resistant tuberculosis (MDR-TB) patients under the revised national TB control programme (2007-2011): Evidence leading to policy enhancement: Malik M. Parmar et. al.

¹⁹ Das PK, Ganguly SB. Effectiveness of the shorter MDR regimen in the management of tuberculosis: Shortfall in the outcome of disease a multidimensional approach and evaluation for a better alternative. Biomed Biotechnol Res J [serial online] 2020 [cited 2021 Oct 13];4:143-7. Available from: <https://www.bmbtrj.org/text.asp?2020/4/2/143/286840>

to tide over the issues faced due to longer regimen, improve treatment adherence and outcomes, reduce adverse drug reactions (ADRs) and reduce overall cost of MDR-TB treatment²⁰.

- **Treatment outcome – TB Register record & Reassigned by study**

Treatment outcomes categorized into favourable and unfavourable didn't show noticeable difference in between the records maintained in the TB Register and the one reassigned by the study team. The favourable and unfavourable outcome as recorded in the TB Register was 92.9% and 7.1% respectively. The reassigned TB outcome by the study for favourable and unfavourable outcome was 93.2% and 6.8% respectively.

However, the assessment found that when it comes to the individual treatment outcomes category as per the TB Register record, 34% (58/170) of treatment outcome was wrongly classified as cured and 15% (6/41) wrongly classified as treatment completed. So when the treatment outcomes was reassigned as per the laboratory smear and culture follow up and standard definitions in the TB guidelines, cured was assigned to 15% (19/131) correctly and treatment completed to 65% (66/101) correctly. The highlight is that most of the cases have actually just completed the treatment course without evidence of systematic smear and culture follow up requirement fulfilled, but wrongly classified as cured in the TB Register. Since cured and treatment completed together makes the favourable outcome, they finally complements each other and the overall Treatment Success Rate (TRS) remains almost the same. Six cases were switched over to Pre XDR-TB treatment and eight TB death cases were correctly recorded. Overall, 35% (87/249) treatment outcome were wrongly recorded, which indicates that TB In- charges and treating physician's needs to be sensitized from time to time through refresher courses and trainings. The program must stress on correctly assigning the treatment outcomes so as to project actual treatment outcomes for programmatic intervention and to avert consequences of XDR-TB development in the long run.

²⁰ New short-course regimen in multidrug-resistant tuberculosis control-A need of the hour: Sankalp Yadav, Gautam Rawal, Mohammad Atif et. al.

Comparison of TSR (%)

When annual TSR was compared, the program notified data (95%) was found slightly higher than the TB Register record and study findings (93%), which needs further validation of the TSR.

In the final Cox-model, the study found that urban residents had hazard ratio slightly higher compared to rural residents for unfavourable outcome which indicates that urban residence among the MDR-TB patients were slightly more likely attain culture conversion earlier compared to rural counterparts. This could be attributed to easy access of urban residents to the health care centres for follow up smear and culture, better transportation facilities and higher level of education, awareness, understanding self-care and better economic status compared to rural counterparts. However, the challenge is that MDR-TB cases in the last five were found to be concentrated in Thimphu, Phuentsholing in Chukha, Samtse and Sarpang districts indicating population density, people's movement and mixing and commerce as driver of transmission in the community. These are also towns in the bordering areas where highest economic activities and mixing of people takes place.

Similarly, hazard ratios of baseline smear and culture before the initiation of MDR-TB treatment was less than one which indicates that smear positive and culture growth at baseline were more likely to convert late, compared to baseline smear negative and culture no growth. This could be attributed to high bacilli load that takes time to be killed by the anti-tubercular drugs compared to negative smear and culture at baseline.

SUMMARY OF MAIN FINDINGS

Socio-demography

- From 2016 to 2018, the proportion of females was 146 (58.63%) slightly higher compared to 103 (41.37%) males among the people who suffered from MDR-TB in Bhutan. About 84% were in the age range of 15-44 years;

- MDR-TB cases were seen maximum among students/trainees 86 (34.5%) and majority of the cases, 185 (74.3%) resided in urban areas, compared to rural 64 (25.7%) residence;
- Spatial distribution of cumulative number of cases from 2016 to 2019 shows highest concentration of the cases in western region followed by central Bhutan. Thimphu 105 (42.2%) had the highest case notification of MDR-TB cases followed by Chukha 32 (12.9%), Samtse 20(8%), Sarpang 15(6%), Samdrupjongkhar 13 (5.2%) and Wangdue 12 (4.8%) districts;

Clinical characteristics

- By type of case, Pulmonary form of MDR-TB 212 (85.1%) dominated over Extrapulmonary form of MDR-TB 37(14.9%) with 205(82.3%) initially registered as New MDR-TB and 44(17.7%) were among Retreatment category;
- About 97.6% (243/249) MDR-TB patients were screened for HIV and only two positive cases, one each in 2016 and 2019 were detected through the Voluntary Counselling and Testing (VCT) method;
- Of the 10 who responded to have comorbid conditions, five had diabetes mellitus, two had hypertension and rest of them had COPD, cancer and autoimmune disorder each;
- 84.8% (201/237) of the MDR-TB cases were AFB positive indicating infectiousness while those cases with chest X-Ray results, 44.4% (96/216) of cases were suggestive of pulmonary TB;

Laboratory diagnosis

- The diagnosis of MDR-TB was established in 247 (99.2%) patients with genotypic method with more than 94% (233/247) cases being diagnosed using GeneXpert technology. Two case was diagnosed using phenotypic method while 129(51.81%) cases had both genotypic and phenotypic results available for at least isoniazid and rifampicin;
- Prior to MDR-TB treatment initiation, baseline sputum smear was done in 87.6% (218/249) and culture in 85.1% (212/249) of MDR-TB patients. About 12.4% (31/249) sputum smear and 14.9% (37/249) culture were not performed as baseline test among MDR-TB patients;
- Of 179 patients who were sputum culture positive at baseline, 94.4% (169/179) converted their culture in a median of 91 days (IQR 59-184; range 52-212 days). About 88.1% (149/169) patients have had initial sputum culture conversion within three to four months after second line anti-TB treatment initiation. Only 5 (3.0%) of 169 patients with sputum culture positive at treatment initiation had culture conversion at ≥ 6 months;
- There were three patients who did not culture convert;
- There was no significant difference in the median days to initial sputum culture conversion by treatment regimen; longer regimen median was 91 days (IQR 59-184, range 52-212 days) and shorter regimen median was 89 days (IQR 61-184, range 60-184 days). 125 (**87.4%**) of 143 patients on longer regimen and 52 (**91.2%**) of 57 patients on shorter regimen have had initial sputum culture conversion within three to four months after initiation of second line anti-TB treatment;

- The maximum time to initial sputum culture conversion was less in shorter regimen (184 days) when compared to longer regimen (212 days);
- Proportion of both smear 46.27% (31/67) and culture 41.79% (28/67) monthly follow up done was comparatively higher in the Shorter MDR-TB treatment regimen group compared to smear 28.02% (51/182) and culture 25.27% (46/182) monthly follow in the Longer MDR-TB treatment regimen group;
- About 71.98 % (131/182) smear and 74.73 % (136/182) culture has missed more than two monthly follow up in longer regimen during intensive phase, while 53.73% (36/67) smear and 58.21% (39/67) culture has also missed more than two monthly follow up in the shorter regimen;
- The treatment adherence of longer MDR-TB regimen as was 99.3% in intensive phase and 97.2% in continuation phase, whereas treatment adherence of shorter regimen was 93.3%;

Treatment outcome and TSR

- The overall Treatment Success Rate (TSR) was 92.9% for longer treatment regimen and 94% for shorter treatment regimen, respectively, as per the records of Treatment outcomes maintained in the MDR-TB register;
- When the treatment outcome was grouped into Favourable (Cured and Completed) vs Unfavourable (treatment failed, death, loss to follow-up, not evaluated and switched to pre-XDR treatment), the overall Favourable outcome was at 93.2% (232/249) and Unfavourable outcome at 6.8% (17/249);
- The study found that about 35% (87/249) treatment outcomes were wrongly recorded in the MDR-TB Register. Hence the study has assigned 35% treatment outcome correctly as per the treatment outcomes definitions and laboratory smear and culture follow up;
- When it comes to individual treatment outcomes category as per the TB Register record, 34% (58/170) of treatment outcome was wrongly classified as cured and 15% (6/41) wrongly classified as treatment completed. So when the treatment outcomes was reassigned as per the laboratory smear and culture follow up and standard definitions in the TB guidelines, cured was assigned to 15% (19/131) correctly and treatment completed to 65% (66/101) correctly;
- When annual TSR was compared, the program notified data (95%) was found slightly higher than the TB Register record and study findings (93%);

Inferential statistics

- Kaplan-Meier curves for the univariate analysis shows that those MDR-TB patients who resided in the urban areas are likely to attain culture conversion slightly earlier than the rural counterparts;
- Those MDR-TB cases with AFB positivity at the time of initial diagnosis has delayed culture conversion, compared to those cases who were AFB negative at the time of initial diagnosis;
- Those with baseline smear positive and culture growth before the initiation of MDR-TB treatment were found to convert late compared to baseline smear negative and culture no growth;
- In the final Cox-model, urban residents had hazard ratio of 1.56 compared to rural residents with significant (*p-value* 0.04) and (95% CI 1.023269-2.364398) for unfavourable outcome (time to culture conversion). This indicates that urban residence among the MDR-TB patients were 1.56 times more likely attain culture conversion earlier compared to rural counterparts;
- Similarly, hazard ratios of baseline smear and culture before the initiation of MDR-TB treatment was <1 with significant (*p-value* 0.04). This indicates that smear positive and culture growth at baseline were more likely to convert late, compared to baseline smear negative and culture no growth.

RECOMMENDATIONS FOR PROGRAMMATIC INTERVENTIONS

Sn	Critical Findings & Gaps	Recommendations: Actions by NTCP & Field Workers
Socio-demography		
1	BMI at the start of treatment (Height and weight) and at completion of treatment not recorded for all MDR-TB cases.	Height and weight to be accurately recorded and BMI calculated by the TB In-charge at the start of and end of treatment.
2	MDR-TB cases were seen maximum among students/trainees (34.5%) and majority of the cases (74.3%) resided in urban areas, compared to rural (25.7%) residence.	- Targeted awareness program should focus schools and training institutes in urban areas. - MDR-TB contact investigations should be done vigorously and intensively in school, institutions & congregate settings.
3	MDR-TB high burden districts are: Thimphu 105 (42.2%) had the highest case notification of MDR-TB cases followed by Chukha 32 (12.9%), Samtse 20(8%), Sarpang 15(6%), Samdrupjongkhar 13 (5.2%) and Wangdue 12 (4.8%) districts.	- Resource allocation, including laboratory capacity (GeneXpert), manpower capacity building, contact tracing, supervision & monitoring should be prioritized in these districts. - Institutional, community and public awareness on MDR-TB transmission, control and prevention should be strengthened in these districts.
Clinical		
4	By case type, Pulmonary form of MDR-TB 212 (85.1%) dominated over Extrapulmonary form of MDR-TB 37(14.9%) with 205(82.3%) initially registered as New MDR-TB and 44(17.7%) were among Retreatment category.	- This indicates that >80% of the cases are primary and highly infectious MDR-TB cases in the community, mostly affecting the productive age group of 15-44 years. - DST screening using Xpert MTB/RIF as first line test should be conducted for all TB cases diagnosed (as per the revised TB diagnostic algorithm), followed by FL-& SL-LPA & culture - Perform vigorous contact investigations and follow up of the close contacts as per the latest contact tracing algorithm/guidelines.

5	- Of the 4.2% (10/249) who responded to have comorbid conditions, five had diabetes mellitus & two had hypertension - A very small percent reported to have comorbidity	- All MDR-TB patients should be provided with random blood glucose testing and depending upon the RBS results, subjected to fasting and post prandial blood sugar estimation. - The treating physician and the TB-ICs should thoroughly take history of any co-morbidity and classify it prior to or during MDR-TB treatment.
6	About 97.6% (243/249) MDR-TB patients were screened for HIV	100% should be screened for HIV through VC method
Laboratory		
7	About 12.4% (31/249) sputum smear and 14.9% (37/249) culture were not performed as baseline test among MDR-TB patients.	All MDR-TB patients should have baseline smear and culture done before starting the treatment.
8	5 (3.0%) of 169 patients with sputum culture-positive at treatment initiation had culture conversion at ≥ 6 months and 3 patients had culture reversion.	Additional interventions is needed to effect conversion which may include enhanced adherence support, aggressive management of adverse events and treatment monitoring by monthly culture follow-ups.
9	About 71.98 % smear and 74.73 % culture has missed more than two monthly follow up in longer regimen during intensive phase, while 53.73% smear and 58.21% culture has also missed more than two monthly follow up in the shorter regimen.	This finding reiterates that PMDT guideline needs to be re-enforced in order to improve the monthly smear and culture follow up among patients undergoing MDR-TB treatment.
10	The study observed lower proportion of MDR-TB cases had additional baseline 2nd line LPA results available and with the presence of resistance in both FLQs and 2nd line injectables drugs being observed among MDR-TB cases	It is essential that all MDR-TB cases have baseline 2nd line drugs resistance pattern available prior to initiation of MDR-TB treatment so that correct treatment can be given as per the resistance pattern.
11	In this study, it was observed that only about 85% of the registered MDR-TB cases had their baseline smear and culture result available.	It is essential to note that positive cultures are often detected before positive smears which are critical in monitoring of treatment. These findings should contribute as evidence to programmatic decision-making to ensure that all MDR-TB cases receive baseline smear and culture tests as per the PMTD guideline.

12	One of the patients with culture reversion has been ultimately confirmed as XDR-TB (First case in Bhutan).	A case-based investigation for every pre-XDR-TB and XDR-TB is obligatory to understand the causes and prevent such cases in the future. Therefore, the NTCP should conduct this task with the technical help of available experts without fail.
Treatment outcomes and TSR		
13	The overall Treatment Success Rate (TSR) was 92.9% for longer treatment regimen and 94% for shorter treatment regimen.	The ongoing, all oral, shorter regimen recommended by the WHO should be spanned over the country to reach maximum MDR-TB patients for better treatment outcome.
14	The overall Favourable outcome was at 93.2% (232/249) and Unfavourable outcome at 6.8% (17/249).	All unfavourable outcomes should be such as treatment failed, death, loss to follow-up, not evaluated and switched to pre-XDR treatment should be examined and audited on timely basis so that proper treatment is assigned.
15	The study found that about 35% (87/249) treatment outcome were wrongly recorded in the MDR-TB Register	The TB In-charge and the treating physician should arrive to the decision of MDR-TB treatment outcomes as per the laboratory smear and culture follow up frequency and outcome definition as per the guidelines.
16	When the overall annual TSR from 2016-2019 was compared, the program notified data (95%) was found slightly higher than the TB Register record and study findings (93%).	Further validation of the TSR required.
17	- When it comes to individual treatment outcomes category as per the TB Register record, 34% (58/170) of treatment outcome was wrongly classified as cured and 15% (6/41) wrongly classified as treatment completed. - So when the treatment outcomes were reassigned as per the laboratory smear and culture follow up and standard definitions in the TB guidelines, cured was assigned to 15% (19/131) correctly and treatment completed to 65% (66/101) correctly.	- The highlight is that most of the cases have actually just completed the treatment course without evidence of systematic smear and culture follow up requirement fulfilled, but wrongly classified as cured in the TB Register. - Treatment outcome to be assigned as per the definition of MDR-TB outcomes in the guidelines. - The TB In-charge and the treating physician should arrive to the decision of MDR-TB treatment outcomes.

18	• The final Cox-model analysis indicated that urban residence among the MDR-TB patients were 1.56 times more likely attain culture conversion earlier compared to rural counterparts.	• Although the magnitude of risk is not so high, the program should keep eye on and track all the MDR-TB patient coming from rural settings for laboratory follow up and adherence to medication on periodic basis.

LIMITATION OF THE STUDY

Although this study has planned to recruit all MDR-TB cases diagnosed from 2015 to 2019 (including those treatment completed at the time of data collection) but we could recruit cases from 2016 to 2019 for this study as records prior to 2016 had several missing data and not well kept. One such study should have been conducted on a large sample size for a robust statistical inference. However, limited and small sample size it may appear, mainly the descriptive findings have led to several recommendations for programmatic management of MDR-TB in Bhutan.

As this retrospective study was based on historical data retrieval from MDR-TB Register, patient treatment cards and TbISS online system and even calling up the patients/relatives where ever feasible, issues of missing data and ambiguous patient's information were verified and validated to the extent possible.

Only a small percent (4.2%) has reported co-morbidity conditions in this study. It's assumed that a slightly more proportion of people with MDR-TB may possibly have suffered from co-morbid conditions and gone unrecorded. Therefore, it is highly recommended to take detailed history of previous illness prior to initiation of MDR-TB treatment and any illness that develops during MDR- TB treatment as some co-infections may increase the risk of developing other concomitant infections and complicate treatment process and hamper the treatment outcomes.

Treatment adherence was assessed purely based on the records maintained in the TB treatment card and also through follow-up calls during the data collection period when in doubt. It was not directly based on DOTs. If the anti-tubercular drugs missed dose was ≥ 7 , then it was considered as non-compliance to treatment. However, the current assessment was not based on direct supervision of medicines intake by the patient. Hence, the investigators are of the opinion that the treatment adherence may possibly have been overstated towards higher side of estimation than the expected practice and authenticity.

CONCLUSIONS

The overall treatment adherence and outcome of MDR-TB achieved by the program is at a very high level, which has fulfilled the main objective of the study more than desired. In addition, few factors such as urban residency was found in favor of earlier culture conversion and thus favourable MDR-TB treatment outcomes while others like baseline smear positive and culture growth were associated with delayed culture conversion.

The NTCP is advised to urgently follow up and address to the issues reflected and suggestion provided in the programmatic recommendations as the main output of this study.

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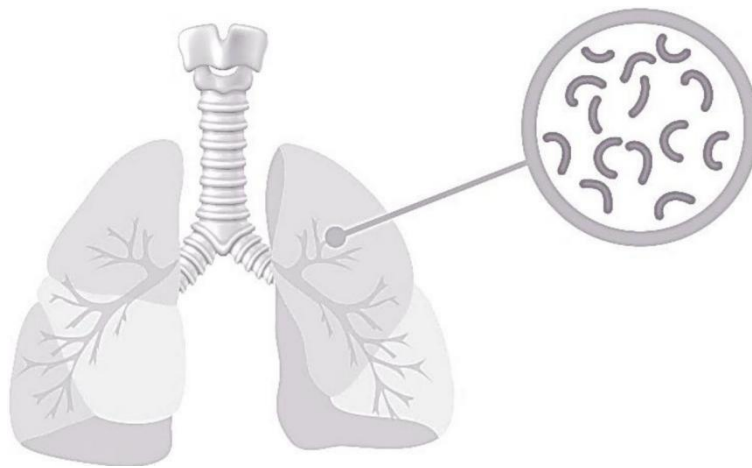
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Prevalence and risk factors of latent tuberculosis infection among healthcare workers of directly observed tuberculosis treatment centre of Kathmandu, Nepal 2019

Prevalence and risk factors of latent tuberculosis infection among healthcare workers of directly observed tuberculosis treatment centers of Kathmandu, Nepal



Report

National Tuberculosis Center
Thimi, Bhaktapur

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Executive summary

Latent TB infection is the state of the persistent immune response to stimulation by *Mycobacterium tuberculosis* antigens. It can progress into active TB disease with the lifetime risk of approximately 10%. Recent estimate has reported that 1.7 billion people worldwide have latent TB infection. Screening and treating people with high risk of latent TB may prevent progression to the active TB disease as well as help decrease the reservoir population. The WHO End TB Strategy calls for targeted screening and treatment of high-risk individuals in its quest for TB elimination by 2035.

Healthcare workers are among the high-risk individuals due to their repeated contacts with the patients with TB. It has been found that healthcare workers have more than two times higher risk of latent TB infection in comparison to the general population. The problem is even higher in resource constrained settings where the TB burden is high and TB infection control practices in the healthcare settings are often inadequate. In a systematic review and meta-analysis, the mean prevalence of latent TB infection among the healthcare workers of low- and middle-income countries was 49% based on TST results, and as 39% based on IGRA results. The objectives of this study were to estimate the prevalence of latent tuberculosis among the healthcare workers at directly observed TB treatment centers of Kathmandu district and to assess the risk factors for latent TB among these populations. The information obtained will provide evidence of the existing occupational risk of infection in health care settings.

This was a cross-sectional study conducted at the 13 directly observed TB treatment (DOT) centers across Kathmandu district. With two healthcare workers per DOT center on average, the total number of study participants was 226. All the non-pregnant healthcare workers at the DOT centers without previous or current active tuberculosis were included in the study. After obtaining written informed consent, trained data collectors conducted face-to-face interview and administered TST. For those with TST reading of 5 mm, whole blood sample was collected for IGRA (QuantiFERON TB Gold). Those with positive IGRA were regarded as having latent TB infection. Data entry was done in Microsoft Excel 2016 and analyzed in R statistical software. Descriptive statistics were used to summarize the sociodemographic and other characteristics of the study participants. Appropriate univariate and multivariate statistical tests were used to assess the association of latent tuberculosis with the potential risk factors. A p-value of less than 0.05 was considered significant. Ethical approval was obtained from the Nepal Health Research Council.

The median age of the study participants was 40 years and most of them (140, 61.9%) were females. Most of them (199, 88%) were from the government centers and were allied health professionals. The median duration of employment as healthcare workers was 10 years and the median number of patients served by the study participants was 7. There were 115 (50.8%) study participants who had TST reading of 5 mm or more. The prevalence of latent TB infection was 17.6% (95% CI 13.08 - 23.44). On univariate analyses, age, duration of employment as a health worker, number of patients

encountered per day and TB in colleagues were found to have a significant association with latent TB infection. In the multivariate regression model, the employment duration as health workers was found to be an independent risk factor for latent TB infection (adjusted OR 1.06, 95% CI 1.01 - 1.11, $p = 0.006$). Because there was multicollinearity between age and work experience, only work experience with lower inflation factor was included in the model.

Using TST as the screening tool and IGRA as the confirmatory test, 17.6% (95% CI 13.08 - 23.44) of the healthcare workers studied had LTBI with the duration of employment as a healthcare worker as an independent risk factor for LTBI (adjusted OR 1.06, 95% CI 1.01 - 1.11, $p = 0.006$) after adjusting for other factors. Further exploration into the validity as well as cost-effectiveness and acceptability of the algorithm used in this study is recommended for informing policy decisions and program interventions.

Abbreviations

AFB	Acid Fast bacilli
BCG	bacille Calmette-Guerin
CFP-10	Culture Filtrate Protein 10
CI	Confidence Interval
DOT	Directly Observed Treatment
ELISA	Enzyme-linked Immunosorbent Assay
ELISPOT	Enzyme-linked Immunospot
ESAT-6	Early Secretory Antigenic Target 6
GENETUP	German-Nepal Tuberculosis Project
HERD	Health Research and Social Development Forum
HIV	Human Immunodeficiency Virus
HP	Health Post
IFN- γ	Interferon Gamma
IGRA	Interferon Gamma Release Assay

IQR	Inter Quartile Range
IU/ml	International Units per milliliter
JANTRA	Japan Nepal Health & Tuberculosis Research Association
LTBI	Latent Tuberculosis Infection
NGO	Non-Government Organization
NTM	Non tubercular Mycobacteria
NTP	National TB Program
OR	Odds Ratio
PHC	Primary Health Care Center
PMN	Peripheral Blood Mononuclear cells
PPD	Purified Protein Derivative
QFT-G	QuantiFERON-TB Gold
QFT-GIT	QuantiFERON-TB Gold In-Tube
SD	Standard Deviation
SHP	Sub Health Post
TB	Tuberculosis
TNF	Tumor Necrosis Factor
TST	Tuberculin Skin test
TU	Tuberculin Units Urban
UHC	Health Center
WHO	World Health Organization

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Introduction

Background

The World Health Organization estimates that in 2017, 10 million people across the globe developed tuberculosis.¹ In Nepal, tuberculosis is the sixth leading cause of death with an estimated 44 000 new TB cases in the year 2016 alone.² Attempts to estimate the reservoir population of tuberculosis - people with latent TB infection have been made. A consensus statement by the WHO almost two decades ago had estimated that around one-third of the world's population at that time had latent TB infection.³ A recent re-estimate has reported that over one-quarter of the world's population (1.7 billion people) have latent TB infection.⁴

Rationale

It is, therefore, important that the people at high risk be screened and treated for latent TB. It confers benefit by preventing the active TB disease and decreasing the reservoir population.^{5,6} The WHO End TB Strategy calls for targeted screening and treatment of high-risk individuals in its quest for TB elimination by 2035.⁷ Healthcare workers, particularly those caring for patients with tuberculosis, are among the high-risk individuals due to their repeated contacts with the patients with TB as well as variable TB infection control practices in the healthcare settings. It has been found that healthcare workers have more than two times higher risk of latent TB infection in comparison to the general population.⁸ Guidelines have recommended screening and treatment of latent TB of healthcare workers based on the background TB epidemiology, available resources and

possible harms of treatment.^{1,9} In Nepal, the majority of the patients with TB receive care from directly observed **TB** treatment centers across the country and the healthcare workers at these centers are at higher risk for tuberculosis.² However, limited data on the burden of **TB** or latent **TB** among the Nepalese healthcare workers have been reported.

In this study, we aim to estimate the prevalence of latent tuberculosis among the healthcare workers at directly observed **TB** treatment centers of Kathmandu district. We also aim to assess the risk factors for latent **TB** among these populations. The information obtained will provide evidence of the existing occupational risk of infection in health care settings.

Objectives

General

- To assess the burden of latent tuberculosis among the healthcare workers at directly observed **TB** treatment centers and describe the risk factors for the same.

Specific

- To determine the prevalence of latent tuberculosis among the healthcare workers at directly observed **TB** treatment centers of Kathmandu District.
- To identify the risk factors of latent tuberculosis among the healthcare workers at directly observed **TB** treatment centers of Kathmandu District.

Literature Review

The WHO defines latent TB infection as the state of the persistent immune response to stimulation by *Mycobacterium tuberculosis* antigens with no evidence of clinically manifest active TB.¹⁰ The intact cell-mediated immunity contains tubercular bacilli within the infected individual and these bacilli remain in the dormant state.¹¹ The infected individual is clinically asymptomatic, the sputum smear for acid-fast bacilli and culture for *Mycobacterium tuberculosis* are negative, the chest X-ray is normal or reveal stable calcified granulomas and the person is non-infectious to others. The only detectable difference from those without TB infection is a positive tuberculin skin test (TST) and Interferon Gamma Release Assay (IGRA) produced as a result of the cell-mediated immune response to *Mycobacterium tuberculosis* antigens.^{12,13} However, if the cell-mediated immune response wanes, the latent TB can progress into active TB disease. The risk of progressing into active TB disease is highest in the first two years after infection and it declines progressively thereafter maintaining at the lifetime risk of approximately 10%.^{13 14} .

A range of risk factors has been identified that play a role in the progression of latent TB into active TB. The ones that confer high risk are human immunodeficiency virus (HIV) infection, transplantation, silicosis, end-stage renal disease, recent exposure to TB (less than two years}, malignancy and patients on tumor necrosis factor- α (TNF- α) inhibitors.¹⁵

Screening and treating latent TB infection

WHO End TB Strategy incorporates, for the first time, the provision of systematic screening of high-risk individuals along with preventive treatment to those who will benefit

from it.^{7,16} The WHO guidelines prioritize systematic screening and treatment of latent TB in people living with HIV, household contacts of bacteriologically confirmed pulmonary TB patients, patients initiating anti-TNF treatment, patients receiving dialysis, patients preparing for an organ or hematological transplant, patients with fibrotic radiologic lesion and patients with silicosis. For these risk groups, clear evidence of benefit from systematic testing and treating of latent TB has been found. For another category of risk-groups, that also includes healthcare workers, there is weak evidence of benefits of screening and treating latent TB, but it has been judged that it may outweigh the harms depending upon the background TB epidemiology and settings.

Diagnosis of latent tuberculosis infection

No gold standard tests are available to diagnose latent TB infection. The evidence of host immune response to *Mycobacterium tuberculosis* antigens and the absence of clinically manifested active TB are required for its diagnosis. Two types of tests are available to demonstrate such evidence of host immune response - Tuberculin Skin Test (TST) and Interferon Gamma Release Assays (IGRA).

Tuberculin Skin Test (TST)

The basis for TST is the delayed-type hypersensitivity reaction to the antigens derived from *Mycobacterium tuberculosis*. It entails intradermal injection of 0.1 ml (equivalent to 5 tuberculin units (TU)) of purified protein derivative (PPD) into the volar aspect of the forearm by a 26- to a 27-gauge needle with its bevel faced upward. A visible wheal 6 to 10 mm in diameter should result during the injection. The presence of induration (not the

erythema) as determined by touch is measured transversely after 48 to 72 hours. The cutoff point for a positive reaction depends on the characteristics of the population being tested, the risk for TB infection, and the prevalence of environmental mycobacteria. For the healthcare workers, the induration of 10 mm or more is typically regarded as positive for TST.^{17,18} In a systematic review, it was shown that it had a pooled sensitivity of 77% (CI, 71% to 82%) and specificity of 97% (CI, 95% to 99%) in non-BCG-vaccinated

populations with low specificity in BCG-vaccinated.¹⁹ Because the PPD contains the antigens that are common to non-tuberculous mycobacteria (NTM) and the *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG) sub-strains, TST can be falsely positive in the individuals with NTM infections as well as with prior BCG vaccinations. On the other hand, negative TST does not rule out TB infection. Conditions causing a decrease in delayed-type hypersensitivity and cutaneous anergy like HIV infection, viral infections such as measles or varicella-zoster virus, live virus vaccination, use of immunosuppressive drugs, sarcoidosis, bacterial infections (including fulminant TB), malignancies (particularly lymphoreticular forms}, and malnutrition have also been implicated in false-negative TST results.¹⁷

Interferon Gamma Release Assays (IGRA)

The IGRA is based on the release of interferon- γ (IFN- γ) by antigen-specific memory T cells, which has encountered mycobacterial antigens previously, in response to the repeated exposure by the antigens. These mycobacterial antigens are antigenic target 6 (ESAT-6), culture filtrate protein 10 (CFP-10), and TB7.7. These proteins are absent from

all strains of *Mycobacterium bovis* BCG and the majority of NTM and therefore offer more advantages in comparison to TST in this regard. Two different systems for IGRA are commercially available currently - the first system, called QuantiFERON-TB Gold (QFT-G) and its variant QuantiFERON-TB Gold In-Tube (QFT-GIT) was developed by Cellestis, Victoria, Australia. In both these tests, whole-blood specimens are inoculated in the tubes prefilled with antigens and concentration of IFN- γ in plasma released by antigen-activated T lymphocytes is measured by enzyme-linked immunosorbent assay (ELISA). The other system, called T-SPOT.TB was developed by Oxford Immunotec, Oxford, England and uses the enzyme-linked immunospot (ELISPOT) method. In this, the number of peripheral blood mononuclear cells (PMN) in the assay is quantified to measure counts of IFN- γ -secreting T cells ("spots") on stimulation by *Mycobacterium tuberculosis*-specific antigens in microplate wells.^{17,20} In a systematic review, it was reported that the pooled sensitivity was 78% (95% CI, 73% to 82%) for QFT-G, 70% (CI, 63% to 78%) for QFT-GIT, and 90% (CI, 86% to 93%) for T-SPOT.TB. The pooled specificity for both QuantiFERON tests was 99% among non-BCG-vaccinated participants (CI, 98% to 100%) and 96% (CI, 94% to 98%) among BCG-vaccinated participants. The pooled specificity of T-SPOT.TB was 93% (CI, 86% to 100%).¹⁹

Burden of latent TB infection among healthcare workers

The healthcare workers are repeatedly exposed to the patients with infectious forms of pulmonary tuberculosis and are therefore at increased risk of getting infected with TB. Furthermore, due to often poor TB infection control practices, particularly in high TB

burden and resource-constrained settings, the risk is even higher. A systematic review and meta-analysis have shown a higher risk of latent TB infection among healthcare workers as compared to the general population (Odds Ratio [OR], 2.27; 95% confidence interval [CI], 1.61-3.20).⁸ The burden of TB infection among healthcare workers is, therefore high - the estimated pooled prevalence has been reported as 37% (95% CI, 28%-47%).⁸ A systematic review of the studies reporting latent TB burden among healthcare workers of low- and middle-income countries reported the average prevalence of latent TB infection of 54%.²¹ An updated systematic review has reported the mean prevalence of latent TB infection among the healthcare workers of low- and middle- income countries as 49% (95% CI 43 - 55) based on TST results, and as 39% (95% CI 30 - 47) based on IGRA results.²² Another systematic review and meta-analysis of studies limited to high-burden countries have reported even higher latent TB burden among healthcare workers - 57% (95% CI 44%-70%).²³

Risk factors for latent TB infection

A myriad of factors has been implicated for latent TB infection among healthcare workers with varying factors shown to be significantly associated based on the underlying TB epidemiology, healthcare settings, TB infection control practices as well as individual factors. Among the healthcare workers, the occupational factors found to have a significant association with latent TB infection were more years of work, work location, job category, type of facility, contact with a TB patient or with a co-worker with TB, and average daily time with direct patient contact in the preceding year. The non-occupational

factors among healthcare workers revealed to have significant association were male sex, age, BCG scar/history of BCG vaccination, education level, household contact with a TB case, smoking status, chronic disease, immunosuppression, and diabetes mellitus.

Methodology

Research design

This was an analytical cross-sectional study.

Study site

The directly observed TB treatment (DOT) centers under the National TB Program (NTP) are responsible for a large proportion of TB care in Nepal. Due to resource constraints, this study has been confined to the DOT centers from Kathmandu District - the country capital. There are 13 DOT centers across Kathmandu district which range from the health posts to the tertiary level central hospitals and are spread across one metropolitan city and 10 municipalities of Kathmandu District. All these DOT centers were included in the study.

Study Population

Healthcare workers at the DOT Centers of Kathmandu District

Sampling unit

Healthcare workers of the DOT centers of Kathmandu District

Sample size

The sample size was calculated by the formula $n = Z^2P(1-P)/d^2$ where Z is level of confidence taken as 1.96, P is expected the prevalence of latent TB among the healthcare workers taken as 41% (the pooled prevalence value corresponding to the TB incidence of Nepal) and d is the degree of precision taken as 10%. The sample size thus derived was

93. Because the cluster sampling technique was applied, it was multiplied by the design effect of 2. An additional 20% non-response rate was estimated, and the final sample size was 223. With two healthcare workers per DOT center on average, the total number of study participants was 226.

Inclusion criteria

- Healthcare workers at the DOT centers who encounter the patients with TB.
- Healthcare workers without the prior or current history of TB.

Exclusion criteria

- Pregnant females
- Previous or current active tuberculosis

Data Collection Technique

A team of healthcare workers received a training session for data collection which involved the explanation of the study details, questionnaires to be administered, the techniques of administering Tuberculin Skin Test and reading the results as well as standard operating procedures for whom to collect the whole blood sample for **IGRA** and how to transport the blood samples to the laboratory. These data collectors then visited each of the study centers (DOT centers of Kathmandu District} and identified the healthcare workers as the study participants. After obtaining informed written consent, the data collectors interviewed healthcare workers with a predesigned questionnaire. Those with no symptoms of TB were administered TST by the Mantoux testing method. The result was read in 72 hours and recorded in millimeters. To increase the sensitivity,

the TST reading of 5 mm was regarded as positive TST and samples were collected for IGRA. Those who had one or more symptoms of active TB underwent further evaluation for active TB by X-Ray Chest and sputum smear examination for AFB. Those study participants in whom active TB was ruled out underwent the interview and TST administration as other study participants. Those healthcare workers who had positive TST underwent **IGRA** (QuantiFERON TB Gold). The data collector obtained a whole blood sample from these healthcare workers and subjected to **IGRA** at the outsourced laboratory. Those with positive **IGRA** were regarded as having latent TB infection. These study participants were offered a three-month course of Isoniazid and Rifampin therapy. Those with negative TST were regarded as having no latent TB infection and no further testing was done.

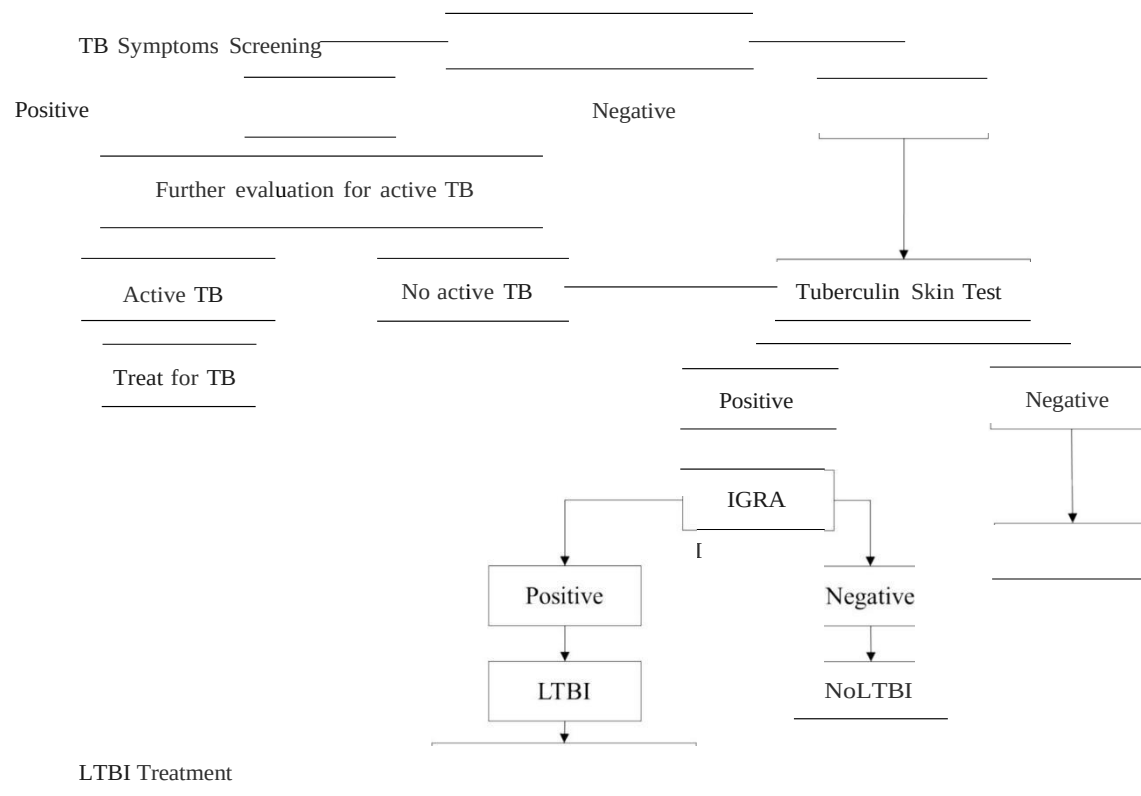


Figure 1: Algorithm for the diagnosis of latent tuberculosis infection.

Data collection tool

Appendix A

Data management and analysis

Data entry was done in Microsoft Excel 2016 and analyzed in R (R Core Team (2019). R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics were used to summarize the sociodemographic and other characteristics of the study participants using mean ($\pm$ Standard Deviation [SD]) or median (Inter-Quartile Range **IQRI**) for continuous variables and proportions for categorical variables. Appropriate univariate statistical tests were used to assess the association of latent tuberculosis with the potential risk factors

from earlier studies using the odds ratio as a measure of association. The factors found significantly associated with latent TB in the univariate analyses were analyzed further by binary logistic regression and the adjusted odds ratio was calculated. The goodness of fit was tested, and the accuracy of the model was determined along with its 95% CI. A p-value of less than 0.05 was considered significant.

Ethical considerations

The participants were well explained about the details of the study and the data collecting team ensured that the respondents understood the study. Their identification, as well as the details on their responses, have been kept confidential. After data entry, cleaning, coding, and validation, the data collection tools with the collected data were destroyed. Ethical approval was obtained from the Nepal Health Research Council (Proposal registration number 618/2019: approval letter number 390 dated 8 August 2019).

Results

Missing values

Among the 226 healthcare workers, two had symptoms (cough for more than two weeks) suggestive of active tuberculosis. The X-ray chest and sputum smear of these participants tested negative for active pulmonary tuberculosis. Therefore, all the 226 study participants were evaluated for latent TB infection and the data analyzed. There were 53 (0.6%) data points missing (Figure 2 and Table I). There were no systematic interactions between the missing data points (Figure 3).

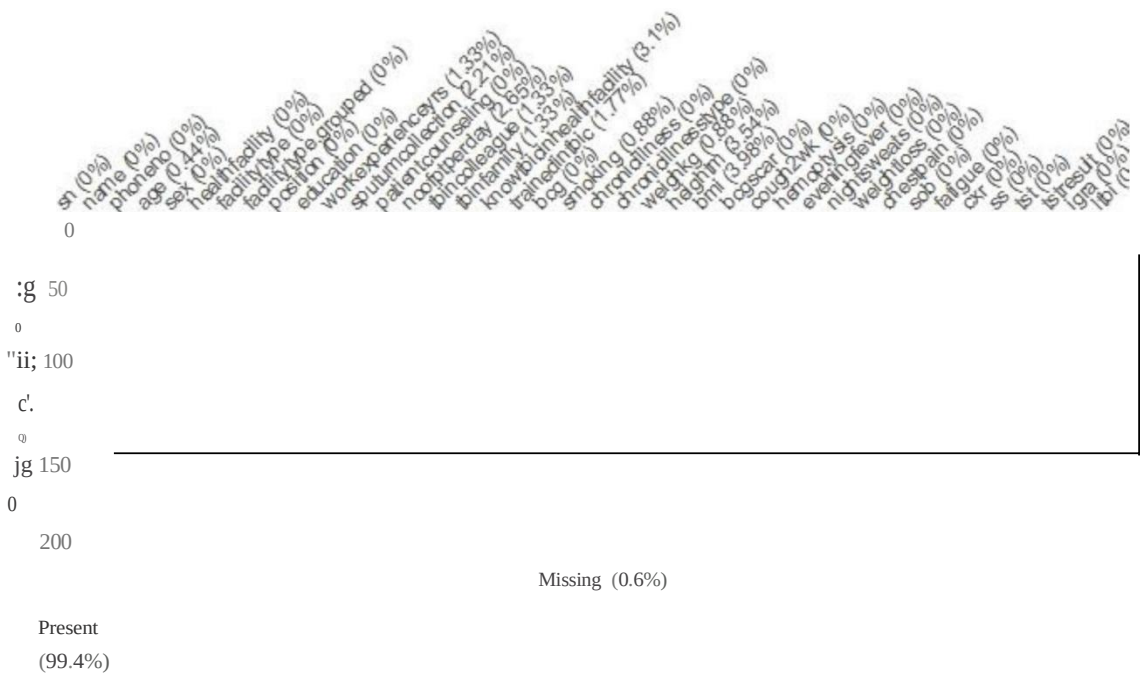


Figure 2: Plot for missing values.

Table I: Number and percentage of missing values in the variables.

Variable	Number	Percentage
Body Mass Index	9	3.98
Height	8	3.54
Knowledge of TB infection control in the health facility	7	3.10
Number of patients encountered per day	6	2.65
Sputum collection	5	2.21
Trained in TB infection control	4	1.77
TB in colleague	3	1.33
TB in family	3	1.33
Work experience (years)	3	1.33
Smoking	2	0.88
Weight (Kg)	2	0.88
Age		0.44

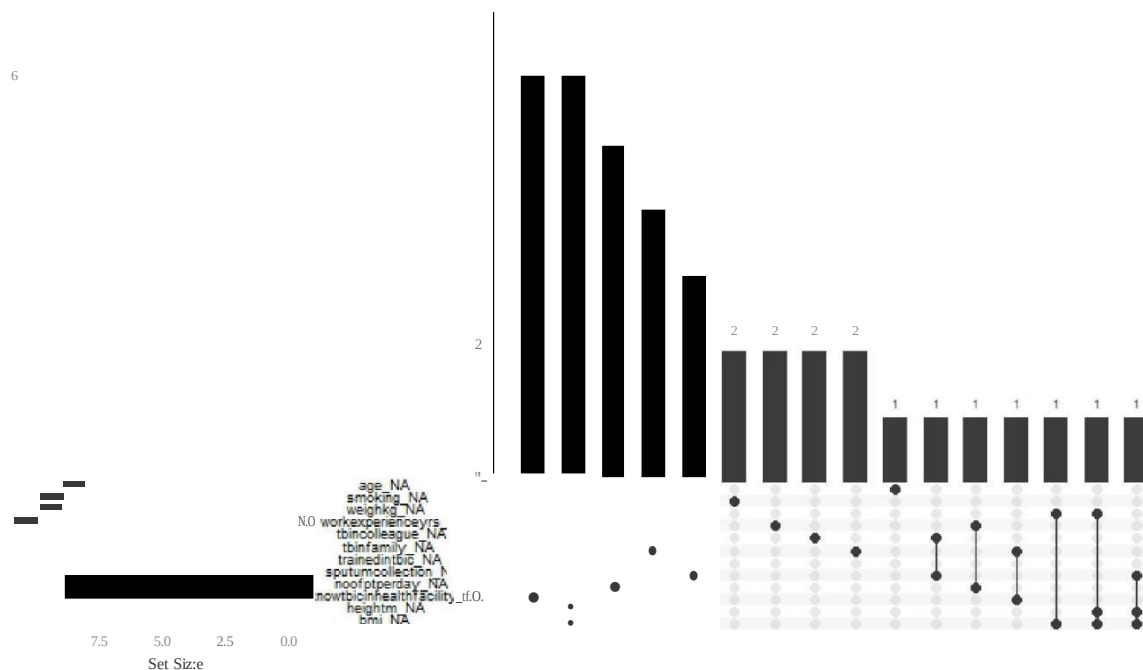


Figure 3: Plot for intersections between the missing data points.

Descriptive analyses

The median age of the study participants was 40 years (IQR 33 - 46) with a minimum age of 20 years and the maximum age of 60 years. Most of them belonged to the 40 to 50 years category (Figure 4). A majority of them (140, 61.9%) were females and the rest were males (Figure 5).

Age distribution

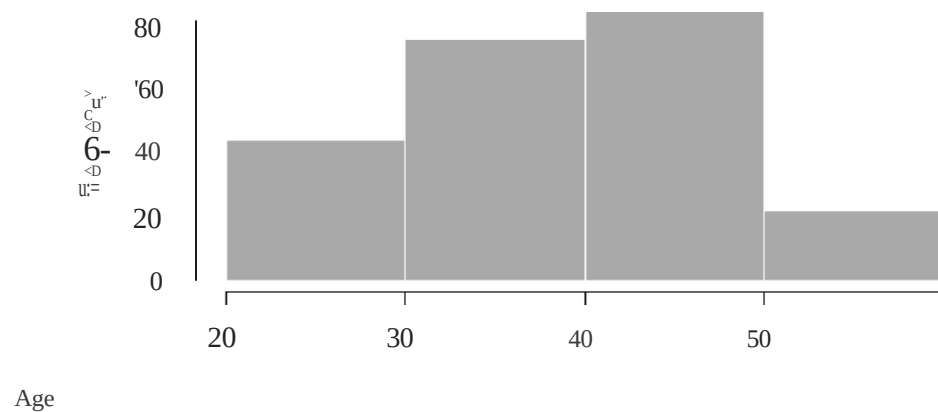


Figure 4: Age distribution of the study participants.



Figure 5: Sex distribution of the study participants.

Most of them (199, 88%) were from the government centers (Figure 6) and were allied health professionals (Figure 7). Senior Auxiliary Health Worker was the most common position (53, 23.4%) followed by Health Assistant (45, 19.9%).

Type of health facility

199

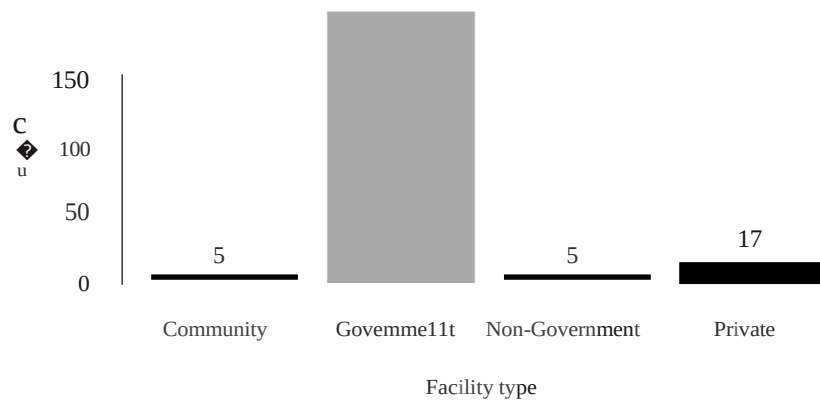


Figure 6: Distribution Of the study participants according to the type Of facility.

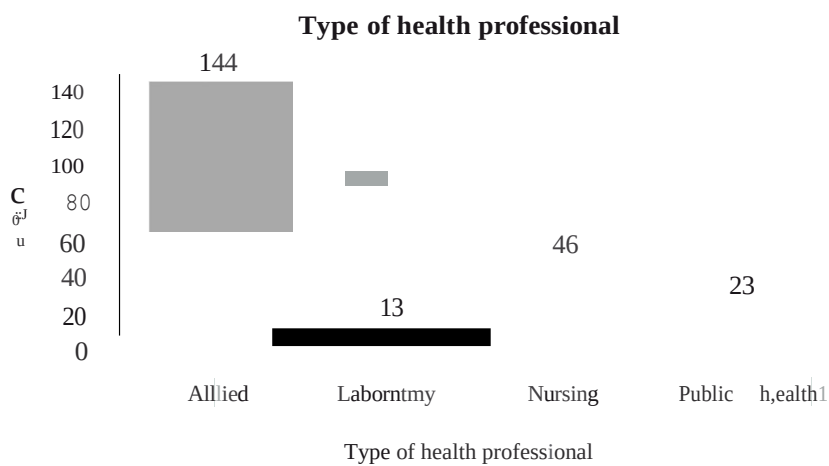


Figure 7: Type Of health professional.

The median duration of employment as healthcare workers was 10 years (IQR 4.75 - 20) with most of the study participants having work experience of 0 - 10 years (Figure 8).

Emplyoment as healthcare worker (Years)



Figure 8: Duration Of employment as healthcare workers.

Most of the study participants (159, 71.9%) reported that they were involved in sputum collection (Figure 9). Almost all the study participants (221, 97.7%) reported that they were involved in patient counseling (Figure 10).

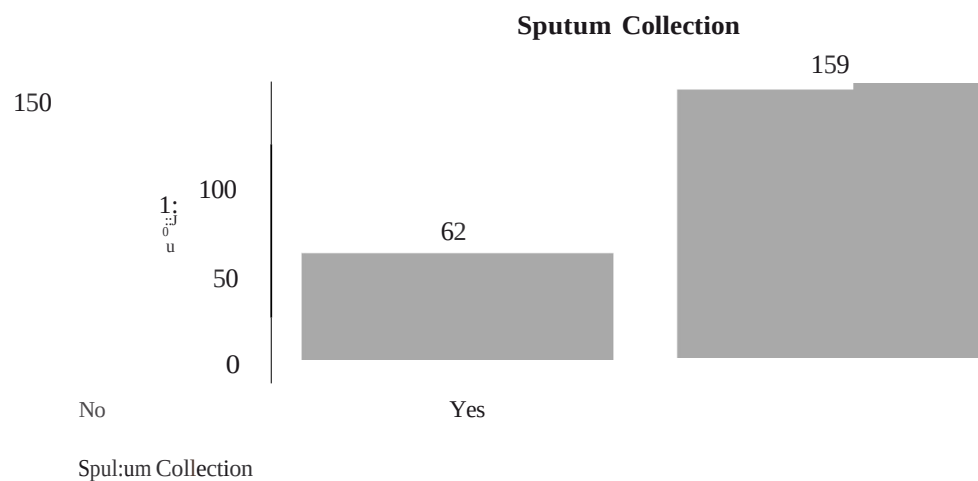


Figure 9: Study participants involved in sputum collection.

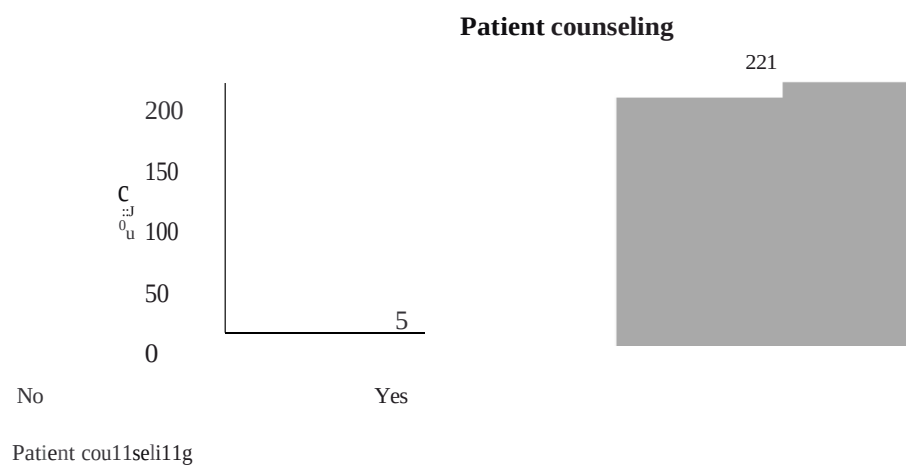


Figure 10: Study participants involved in patient counseling.

The median number of patients served by the study participants was 7 (IQR 3-15) with most of the study participants serving 0 - 20 patients per day (Figure 11).

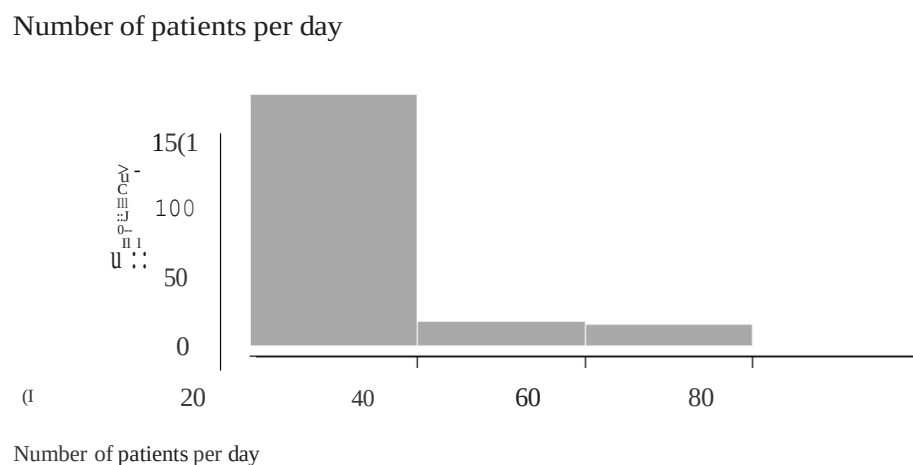


Figure 11: Number of patients served by the study participants per day.

Only 16 (7.1%) study participants reported knowing about tuberculosis disease in colleagues (Figure 12) and 25 (1.2%) study participants reported to have tuberculosis disease in family members (Figure 13).

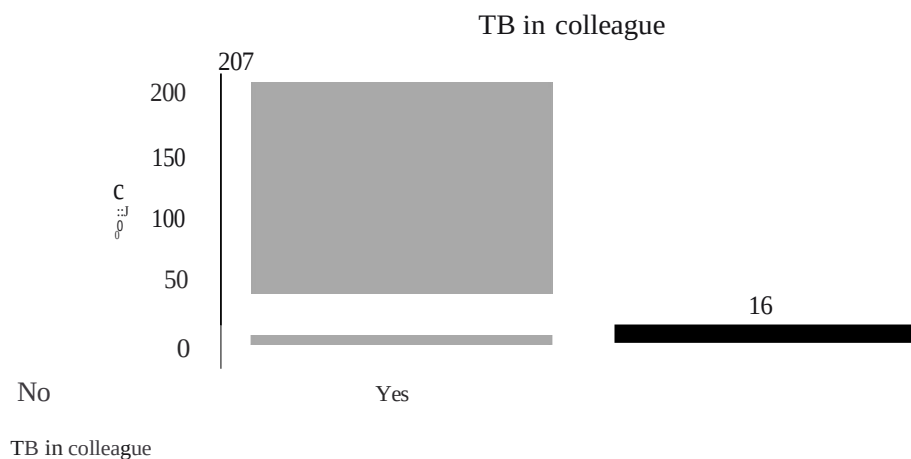


Figure 12: Number Of study participants who know about tuberculosis in colleagues.

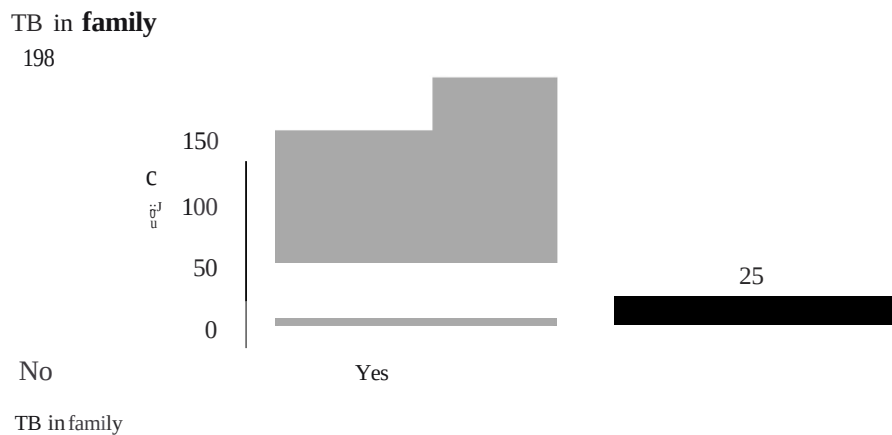


Figure 13: Number Of study participants who reported to have family members with tuberculosis.

More than half of the study participants (121, 54.5%) had received training in TB infection control (Figure 14) and (132, 60.2%) reported knowing that there was TB infection control policy in their healthcare facility (Figure 15).

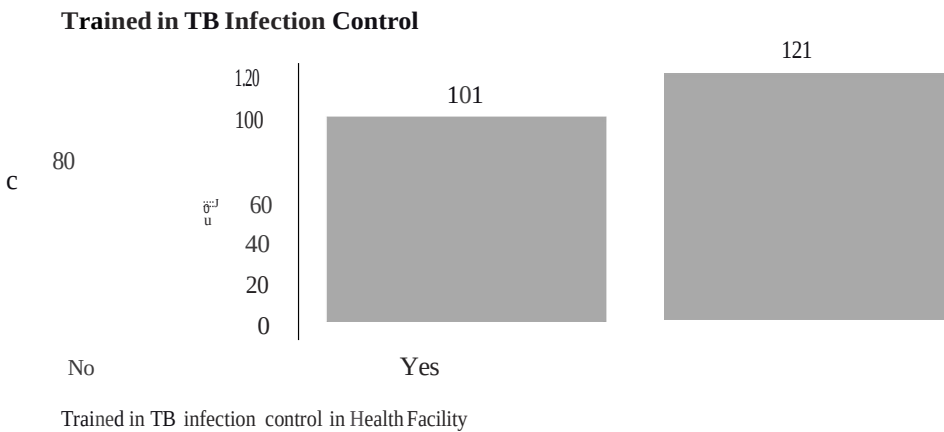


Figure 14: Number of study participants trained in TB infection control.

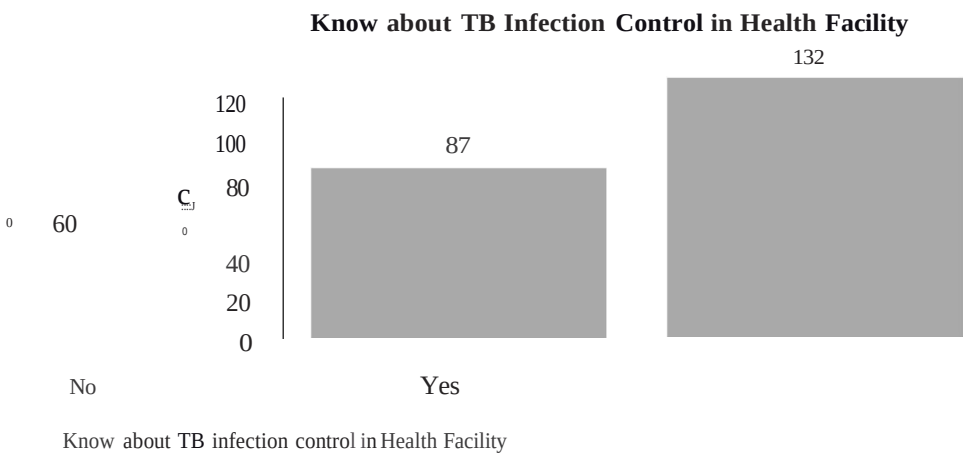


Figure 15: Know about TB infection control in the health facility.

There were 169 (74.7%) study participants who reported to have been vaccinated with BCG (Figure 16) and all these participants had a BCG vaccination scar (Figure 17).

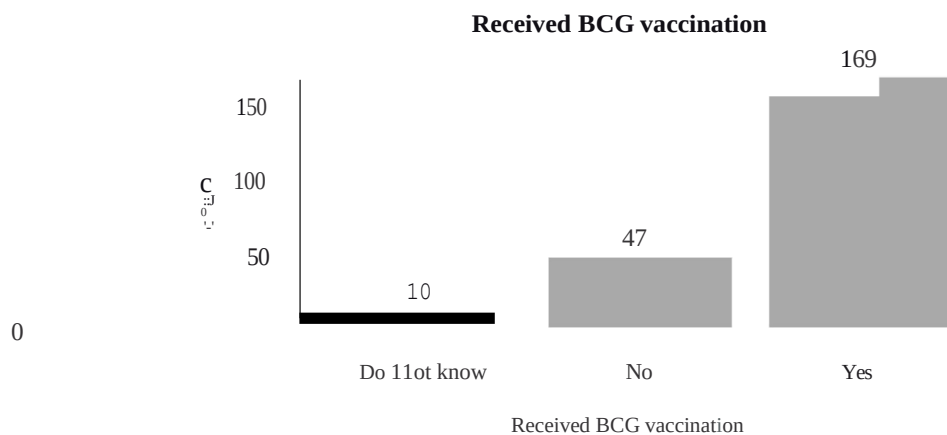


Figure 16: BCG vaccination status of study participants.

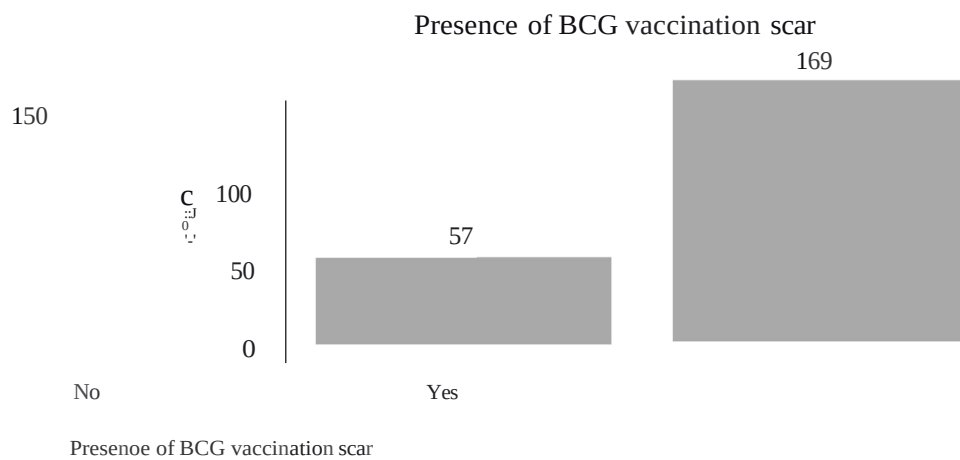


Figure 17: Presence of BCG vaccination scar.

A majority of the study participants (195, 87%) were non-smokers (Figure 18).

Smoking

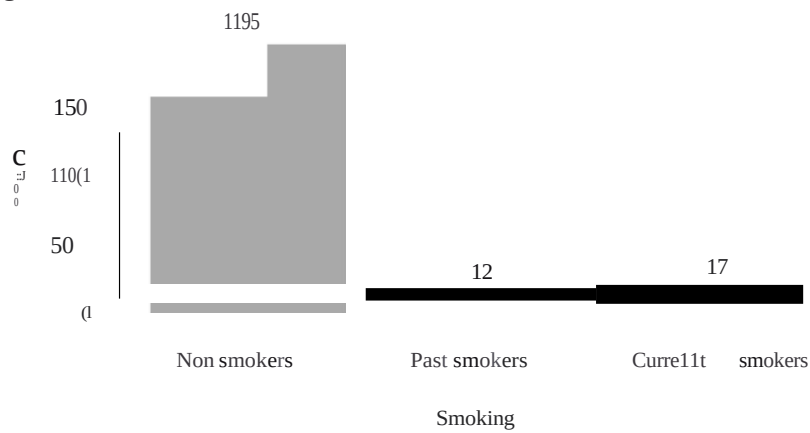


Figure 18: Smoking status of the study participants.

There were 52 (23%), study participants, with one or more of the chronic illnesses (Figure 19) with systemic hypertension being the most common chronic illness, seen in 28 study participants.

Chronic illness

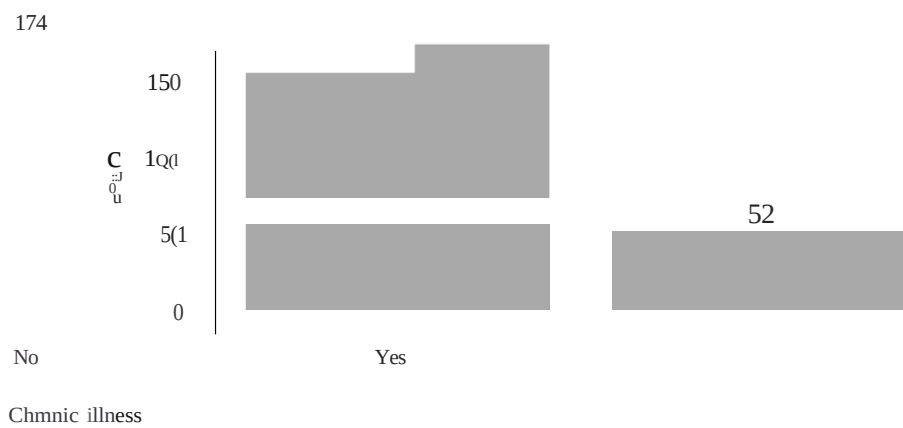


Figure 19: Presence of chronic illness in study participants.

A majority of the study participants had BMI in the range of 20 - 25 (Figure 20) with a median BMI of 24.9 (IQR 22.9 - 27.5).

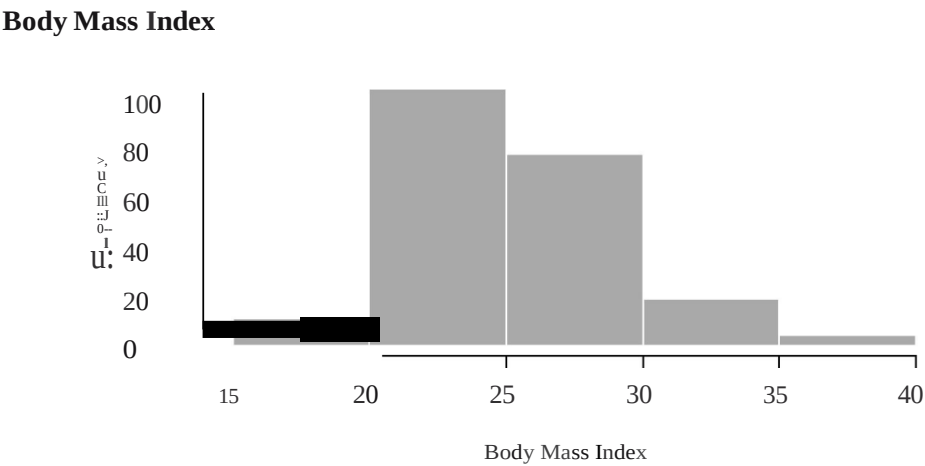


Figure 20: Distribution Of body mass index Of the study participants.

Prevalence of latent TB infection

The median TST reading was 5 mm (IQR 0-10) with most of the study participants had < 5 mm reading (Figure 21 and Table 2).

Tuberculin Skin Test reading (mm)

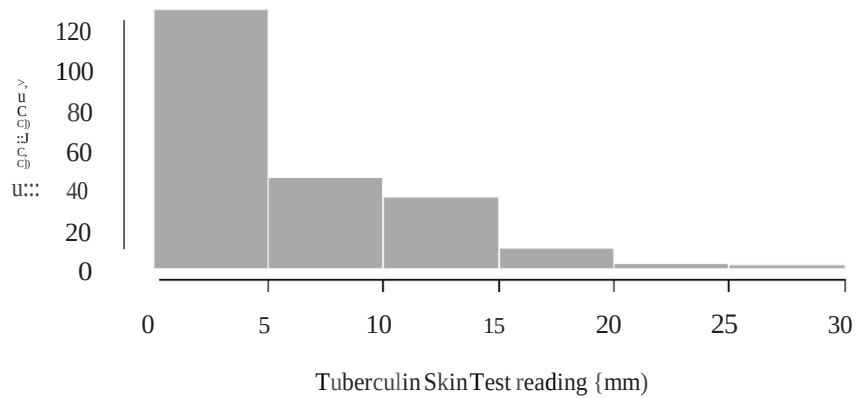


Figure 21: Tuberculin Skin Test reading (in millimeters).

Table 2: Number of participants with different TST cut-offs.

Cut-off in TST	Number	Percentage
5 mm	115	50.88%
10 mm	68	30.09%
15 mm	28	12.39%

With the TST reading of 5 mm or more as a cut-off for positive TST results, 115 (50.8%) participants had positive TST results (Figure 22).

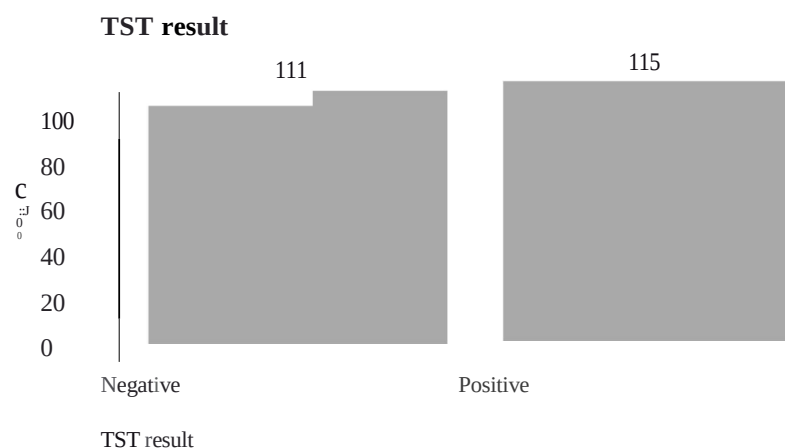


Figure 22: TST results of the study participants.

Of the 115 study participants with positive TST results, a whole blood sample for all these participants was obtained for IGRA, 40 samples tested positive (Figure 23). The prevalence of latent TB infection was 17.6% (95% CI 13.08 - 23.44).

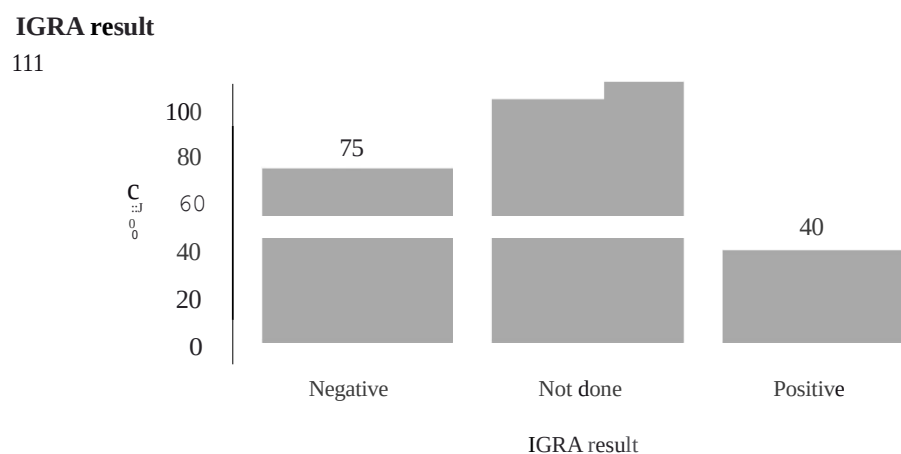


Figure 23: IGRA test results.

Risk factors for latent **TB** infection

On univariate analyses between potential risk factors and latent **TB** infection, age, duration of employment as a health worker, number of patients encountered per day and **TB in** colleagues were found to have a significant association with latent **TB** infection (Table 3). A multivariate logistic regression model was fitted with these variables as independent variables. Because there was multicollinearity between age and work experience, only

work experience with lower inflation factor was included in the model (Figure 24). The final model, thus obtained, was found to be a better fit to the data than the intercept only

model ($\chi^2 = 12.96$, $df = 3$, $p = 0.004$) and had an accuracy of 81.08% (95% CI 64.84, 92.04).

The employment duration as health workers was found to be an independent risk factor for latent **TB** infection (adjusted OR 1.06, 95% CI 1.01 - 1.11, $p = 0.006$) (Figure 25).



Figure 24: Scatter plot between age and work experience.

Relationship of duration of employment and latent TB infection

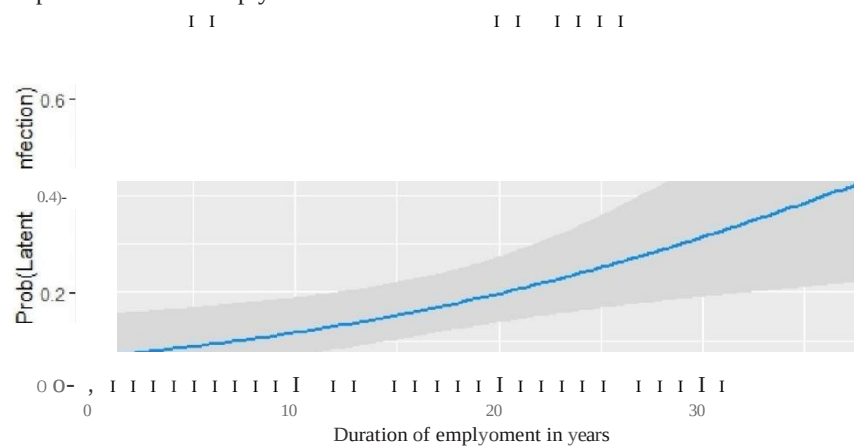


Figure 25: Relationship Of the duration Of employment and latent TB infection.

Table 3: Risk factors for latent tuberculosis infection.

Risk Factors	n %		p value	Odds Ratio (95% Confidence Interval)	Adjusted Odds Ratio (95% Confidence Interval)	p value
	Latent TB Infection (n=40)	No Latent TB Infection (n=186)				
Age in years (Mean± SD)	42.64 (±7.4)	38.86 (±9)	0.014	1.05 (1.01 - 1.09)		
Sex						
Female		27	113	0.425	0.75 (0.36 - 1.54)	
Male		13	73			
Type of health facility						
Government		33	166	0.237	1.76 (0.64- 4.33)	
Non-Government		7	20			
Position						
Allied health professional	21	123				
Nursing professional		12	0.501	0.48 (0.02 - 2.68)		
Public health professional	11	35	0.144	1.84(0.78-4.12)		
Laboratory professional	7	16	0.065	2.56 (0.89 - 6.81)		
Sputum collection						
No		9	53	0.447	1.36 (0.62 - 3.23)	
Yes		30	129			
Patient counseling						
No			4	0.892	0.85 (0.12 - 17.01)	
Yes		39	182			
Work experience in years (Mean ± SD)	19.46 (8)	15.12 (8.8)	0.006	1.06(1.01-1.10)	1.06 (1.01 -1.11)	0.006
No. of patients encountered per day (Mean± SD)	16.6 (15.2)	11.22 (14.4)	0.040	1.02 (1.00 - 1.04)	1.01 (0.98 - 1.03)	0.280
TB in colleague						
No		34	173	0.042	3.05 (0.98 - 8.80)	2.81 (0.72 - 10.29)
Yes		6	10			
TB in family						
No		34	164	0.404	1.52 (0.52 - 3.91)	
Yes		6	19			
Trained in TB infection control						
No		20	81	0.528	0.80 (0.40 - 1.59)	
Yes		20	101			
BCG Vaccination						

Do not know	2	8			
No	7	40	0.688	0.70 (0.13 - 5.27)	
Yes	31	138	0.895	0.89 (0.21 - 6.14)	
Smoking					
No	34	161			
Past smoker	2	10	0.946	0.94 (0.14 - 3.80)	
Current smoker	4	13	0.532	1.45 (0.39 - 4.41)	
Chronic illness					
No		30	144	0.742	1.14
Yes		10	42	(0.49 - 2.46)	
Body Mass Index in Kg/m² (Mean+ SD)					
(0.91 -1.10)		25.44(3.3)	25.3 (3.7)	0.828	1.01

Discussion

The prevalence of LTBI in this study was found to be lower than the pooled estimates of 39% (based on IGRA results) or 49% (based on TST results) reported in the updated systematic review and meta-analysis of the studies among healthcare workers from low- and middle-income countries.²² Several possible explanations could be offered to account for this observed difference.

First, the algorithm for diagnosis of LTBI in this study uses TST as the screening tool to select a subset of the study population and **IGRA** as the confirmatory test to diagnose LTBI in this population. The implications are twofold. While this strategy strives for higher specificity in LTBI diagnosis, the cost is the lower sensitivity in LTBI diagnosis due to the lower sensitivity of IGRA (done by QuantiFERON TB Gold in this study). In addition, it does not consider the individuals with TST negative results for IGRA testing and thus does not accommodate the possibility of TST-/QFT+ discordance. Studies estimate that

8.6% to 15.3% of the individuals with negative TST could test positive in IGRA.^{24,25} If TST had been used alone for diagnosis of LTBI, with the standard cut-off of 10 mm or more, 30.09% of the study participants would have been regarded to have LTBI, which is comparable to the prevalence of 36% (95% CI 21-51) pooled from nine studies from low- and middle-income countries with TB incidence comparable to that of Nepal.²² Furthermore, the IGRA positivity of 34.7% would have reflected the prevalence of LTBI had it been used as the LTBI diagnosing tool. This too is comparable to the prevalence of 41% (95% CI 28-50) pooled from seven studies from similar settings.²²

Second, though the TST cut-off of ≥ 5 mm was used to increase its sensitivity, only one- step TST was used in this study which may have failed to elicit the optimal response of remote TB infection in some individuals due to waned cell-mediated immunity.²⁶ A second TST done 1 - 3 weeks of the first TST (called two-step TST) has been shown to find additional individuals with positive TST.²⁷⁻²⁹ This phenomenon, known as boosting, increases in the settings with high TB burden and high rate of BCG vaccinations.²⁶ Use of one-step TST in this study may have missed a subset of study participants who would have tested positive in two-step TST and thus would have been tested for IGRA subsequently, contributing to the LTBI burden estimation.

Third, there are important differences in the study populations in this study and the studies which contributed to the pooled prevalence estimates.²² Most of the participants (63.7%) in this study were allied health professionals and none were physicians or general services staff (Figure 7). In addition, there were laboratory and public health professionals included as well in this study. The pooled estimates in the systematic-review have been obtained from the studies that had representations from physicians as well as general services staff. In the stratified analysis reported, the LTBI prevalence was lower in allied health professionals (45% versus 49% in overall healthcare workers, based on TST or 31% versus 39% in overall healthcare workers, based on IGRA). In nursing professionals, the prevalence estimates are higher (54% versus 49% in overall healthcare workers) based on TST and lower (34% versus 39% in overall healthcare workers) based on IGRA. Estimates from laboratory or public health professionals were not available for comparison.

Fourth, this study was limited to the screening the healthcare workers at DOT centers for LTBI. These centers are responsible for evaluating the patients with presumptive TB and provide anti-tubercular drugs to the patients diagnosed with TB as per the National DOTS policy. These are typically outpatient centers or outpatient units of hospitals. Because health workers at the DOT centers come in frequent contact with the patient with TB or presumptive TB, they could be more aware of the risks of TB transmission and therefore, likely to adhere to TB infection control practices. Among the study participants in this study, 121 (53.5%) participants reported to have received training in TB infection control and 132 (58.4%) reported having knowledge of TB infection control policy in their health facility (Figures 14 and 15). Furthermore, only 16 (7.1%) of the participants reported having known about TB disease in colleagues and 25 (11.1%) in family members indicating the low transmission of TB and/or progression to active TB disease (Figures 12 and 13). A survey of 190 health workers from randomly selected DOT centers in Kathmandu Valley showed that 66% of the clinical staff and 68.4% of lab or X-ray staff had a good knowledge of TB infection control. Most of these health workers were reported to have a positive attitude and a less satisfactory TB infection control practices. But almost one-third of the study population were administrative and support staff and data on TB infection control attitude and practices were not stratified according to the health worker type.³⁰

Fifth, the prevalence of LTBI also depends on the extent of contact between the health workers and the patients with TB. Though patients with TB receive treatment under the

direct supervision of health workers thus exposing them to TB, in many DOT centers, this system has been deemed suboptimal and inadequate. This could have also contributed in the lower LTBI prevalence among the health workers, particularly among such centers. Sixth, there are possibilities of preanalytical and analytical variabilities that might have impacted the IGRA results. Though the data collectors had been double trained (by the investigators and by the laboratory professionals from the outsourced lab) on optimal amount and methods of obtaining, incubating and transporting the whole blood sample for IGRA and the process verified by random unannounced visits by the investigators, attrition due to these factors could not be ruled out completely. Studies investigating these issues have reported a wide range of possible preanalytical variabilities and their significant impact on the IGRA results. Some of these factors are time of blood collection, inadequate skin disinfection prior to phlebotomy, blood volume collected, tube shaking, and delay in sample processing from collection to incubation. On the other hand, analytical sources of variabilities include imprecision in pipetting, errors during centrifugation, decantation, and washing and imprecise measurements of the final signal by the operator or the instrument.^{31,32}

The duration of employment as a health worker, found to be a statistically significant risk factor in this study, has been reported as an important occupational risk factor for LTBI in earlier studies as well. In the systematic review and meta-analysis of the studies from low- and middle-income countries, eight studies reported the duration of the employment as one of the risk factors for LTBI, either by TST or IGRA.²² This suggests that longer the

duration of employment as a health worker, longer the duration of exposure with the patients with TB and therefore, higher the risk of getting LTBI. This highlights nosocomial TB transmission as an important occupational problem for health workers. Effective implementation of TB infection control policy in DOT centers, and by extension, all healthcare centers, is a priority.

Limitations

In this study, the results of both TST and **IGRA** were dichotomized into positive and negative outcomes. However, the actual outputs of both these tests are continuous data (induration measured in millimeters in TST and T-cell IFN- γ response measured in IU/ml in **IGRA**) and dichotomizing the results leads to underutilization of the data and thus less accurate estimates of LTBI, including in this study. Attempts to improve in the LTBI estimates have been suggested using statistical modeling approaches, for example using Bayesian mixture model analyses.³³ Because there is no gold standard test for LTBI diagnosis, the accuracy of LTBI prevalence estimation by the algorithm in this study or any other strategy could not be assured. This study was restricted to the health workers at DOT centers of Kathmandu district. This implies that the conclusions derived may not be generalizable to the health workers in other types of facilities or the rest of Nepal. This study does not investigate the implementation aspects of LTBI screening like cost considerations, feasibility, and acceptability of the LTBI screening tools and the impact of LTBI screening of health workers.

Conclusion

This study provides an important glimpse of the burden of LTBI among the health workers at the DOT centers in Nepal. Using TST as the screening tool and IGRA as the confirmatory test, 17.6% (95% CI 13.08 - 23.44) of the healthcare workers studied had LTBI with the duration of employment as a healthcare worker as an independent risk factor for LTBI (adjusted OR 1.06, 95% CI 1.01 - 1.11, $p = 0.006$) after adjusting for other factors.

Recommendations

- Further exploration into the validity as well as cost-effectiveness and acceptability of the algorithm used in this study is recommended for informing policy decisions and program interventions.
- Serial testing of the same study participants is recommended to determine the incidence of LTBI as well as the incidence of active TB in this population. This information will help assess the ability of the study algorithm to predict active TB and determine the ongoing TB transmission in these facilities.
- Studies with a nationwide scope could better represent the study population and help obtain a more accurate estimation of LTBI burden and are therefore recommended.

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Appendix

Appendix A: Data collection Tool

- I. SN
2. Name
3. Age ____ _
4. Sex
5. Health Facility
6. Position
7. Qualification
8. Risk factors for **TB** infection
 - a. Number of patients attended per day
 - b. Work period
 - c. Recent close contact a patient with infectious **TB**
9. Injection drug user: Yes/No
- I 0. On immunosuppressive medication: Yes/No I I.
Chronic medical conditions: Yes/No
 - a. If yes, please specify
10. TB Symptoms:
 - a. Cough for 2 or more weeks: Yes/No
 - b. Weight loss: Yes/No
 - c. Blood in sputum: Yes/No
 - d. Decreased appetite: Yes/No
 - e. Night sweats: Yes/No
11. Mantoux Test: Positive/Negative
12. **IGRA**: Positive/Negative



HIV Bio-Behavioral Survey among People Who Injects Drugs in Afghanistan 2019

HIV Bio-Behavioral Survey among People Who Injects Drugs in Afghanistan



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Country Context

The Islamic Republic of Afghanistan, a landlocked country, borders six Central and South Asia countries: China, Iran, Pakistan, Tajikistan, Uzbekistan and Turkmenistan and has an estimated total (settled) population of 27.5 million people with more than 60% below 25 year of age. Afghanistan is administratively divided into 34 provinces and the provinces are further divided into 409 districts.

During the past decade, more than 5.7 million Afghan refugees have returned to Afghanistan and many of these are experiencing difficulty in rebuilding their lives. This is nearly one quarter of the Afghan population. The sexually active population (15-49) is about 43%. Urban areas like Kabul attract many returnees, but face low absorption rate.

The majority of morbidity and mortality is caused by preventable infectious diseases, malnutrition, complications associated with pregnancy and delivery, war related injuries and domestic violence. For every 100,000 live births, 327 women died from pregnancy related causes. Infant mortality rate remains high at 77 per 1,000 live births and the under-five mortality rate is substantial at 97 per 1,000 live births².

The country has taken a devastating toll during almost four decades of war, with human and socio- economic indicators hovering near the bottom of international indices and by all measures, the people of Afghanistan fare far worse in terms of their health than any other country of the region. Poverty, loss of access to productive assets, food insecurity and limited access to basic health and social services are key factors, compounded by traditional practices, the low status of women and the impact of war. The low literacy rate negatively impacts potential for economic growth and well informed formative behavior.

Adults' literacy rate is 31.4% .Low investments in primary education and cultural barriers to education for females have been contributing factors to the country's widespread poverty. The Gender Inequality Index value (GII), reflecting gender-based inequalities in three dimensions – reproductive health, empowerment, and economic activity - was 0.705, ranking Afghanistan 150 out of 152 countries in the 2013 index.

Over one-third of the country's population lives below the national poverty line of less than one USD per day. The economic situation remains precarious. Private investment falls short of expectations and an increase in tax revenue is counteracted by an overall increase in recurrent budgets. At present, poverty reduction strategies, especially targeting the most vulnerable, have not yet been fully developed. Efforts are made to strengthen civil society development; structures in civil society and local decision making

will need to develop further its skills and knowledge on how to support and co-develop services and local structures that include the most vulnerable.

Afghanistan ratified most of the core international human rights treaties, including the International Covenant on Civil and Political Rights, the International Covenant on Economic, Social and Cultural Rights, the Convention on the Elimination of Racial Discrimination, Convention against Torture and Other Cruel, Inhuman or Degrading Treatment or Punishment, the Convention on Elimination of All Forms of Discrimination Against Women and the Convention of the Rights of the Child. These instruments contain a set of standards that form the basis of the UN human rights system and are a part of international law. Afghanistan made a legal commitment to abide by the international human rights treaties, setting forth in Article seven of the Constitution of Afghanistan that “the State shall abide by the UN charter, international treaties, international conventions that Afghanistan has signed, and the Universal Declaration of Human Rights”.

This pledge is further demonstrated by the Government’s commitments to the Millennium Development Goals (MDG) and the Afghanistan Compact and further elaborated in the Afghanistan National Development Strategy (ANDS) and in its Second Pillar - Governance, Rule of Law and Human Rights, which provides a comprehensive and integrated strategy, reflecting recent experience and current conditions. In addition, the Government of Islamic Republic of Afghanistan (GoIRA) has stated its commitment to strengthening health services, establishing national guidelines, promoting evidence-based decision making, and increasing capacity and skills through formal and informal training. It maintains the right to health that embraces a wide range of socio-economic factors that promote conditions in which people can lead a healthy life and extends to the underlying determinants of health, such as food and nutrition, housing, access to safe and potable water and adequate sanitation, and safe and healthy working conditions.

Drug Use in Afghanistan

Afghanistan, the world's leading producer of opium and Heroin, is also facing a major and even growing problem with drug abuse, the United Nations Office for Drug and Crimes (UNODC) Drug Use Survey in Afghanistan reveals.; easy access to cheap drugs, and limited access to drug treatment, combined with over three decades of war related trauma, have resulted in problem drug-use among almost one million Afghans, roughly 8% of the population between 15 and 64 years old. This rate is twice the global average. There is an increase of 53% in the number of opium users, from 150,000 to 230,000, and a leap of 140% in the number of Heroin users, from 50,000 to 140,000, compared to a similar survey conducted in 2005. One of the shocking statistics in the report is the number of parents who give opium to their children; as high as 50% of drug users in the north and south of the country.

Drug use prevalence was highest in the Northern and Southern regions; the latter is where most of the drug cultivation takes place. In terms of number of drug use, the Central region where Kabul is located scores highest in the country. Returning refugees, the majority of whom lived in countries with high drug addiction like Iran and Pakistan, are potentially more likely to be problem drug users. The highest rate of opium use amongst women was in the Northern region. Up to 40% of all opium and Heroin users initiated their habit in Iran, nevertheless there is also an indication of a new generation of PWID who have started injecting drugs in Afghanistan. The more recent Afghanistan National Drug Use Survey (ANDUS), conducted in 24 provinces, reveals that drug use is prevalent throughout Afghanistan with the highest drug use occurring in rural villages, approximately 2.9 to 3.6 million Afghans could test positive for one or more drugs and approximately one to 1.2 million of them are children.

Continued insecurity and the withdrawal of international forces pose a serious risk regarding future political stability. Ethnic and political rivalries continue to limit public sector program implementation in many areas of the country.

Current State of the Epidemic

Afghanistan is experiencing a low and concentrated HIV epidemic and among the countries in Central and South Asia that are confronted with a growing risk of HIV and AIDS, mainly due to the high incidence of injecting drug use that partially intersects with sex work. In Afghanistan, reliable data on HIV prevalence is sparse; at the early stage, the National AIDS Control Program (NACP) collected data from the National Blood Bank and a project of the International Committee of the Red Cross (ICRC).

Then the data from the health management information system (HMIS) were also collected and by 2007 the officially reported number to NACP was 892 HIV cases until end 2015. Since 2008, after implementing several harm reduction interventions with the financial support of the World Bank, Global Fund, UNODC and other partners, case detection improved and so far in 2015, 2033 cases, out of that 133 death cases, were reported to NACP .

The first case of HIV and AIDS in Afghanistan was identified in 1989. In the first years of the epidemic, the majority of cases were HIV infected foreigners, returning Afghan nationals and blood transfusion cases; and the government was slow to respond to the increasing numbers of HIV transmission.

Following this silent rise, it was only in 2000 that the government made its first attempt to form a national body to fight against the epidemic. The NACP) was established in 2003 to coordinate national response, chaired by the Ministry of Public Health (MoPH) involving a wide range of public sector institutions and Civil Society Institutions (CSIs).

Epidemic level and trend

A cumulative total of 2733 HIV infections have been diagnosed in Afghanistan, by the end of June 2018. This officially reported number considerably understates the true figure because not all HIV infections have been diagnosed or reported, partly because many people do not know that they are infected. Hence, these figures are considerably low compared to many regional countries.

Although Afghanistan is among countries with low level HIV prevalence rates, it is worth to evaluate the national trends in new HIV cases. Figure .01 summarizes the trend from 2003 to 2018; and indicates a slow upsurge.

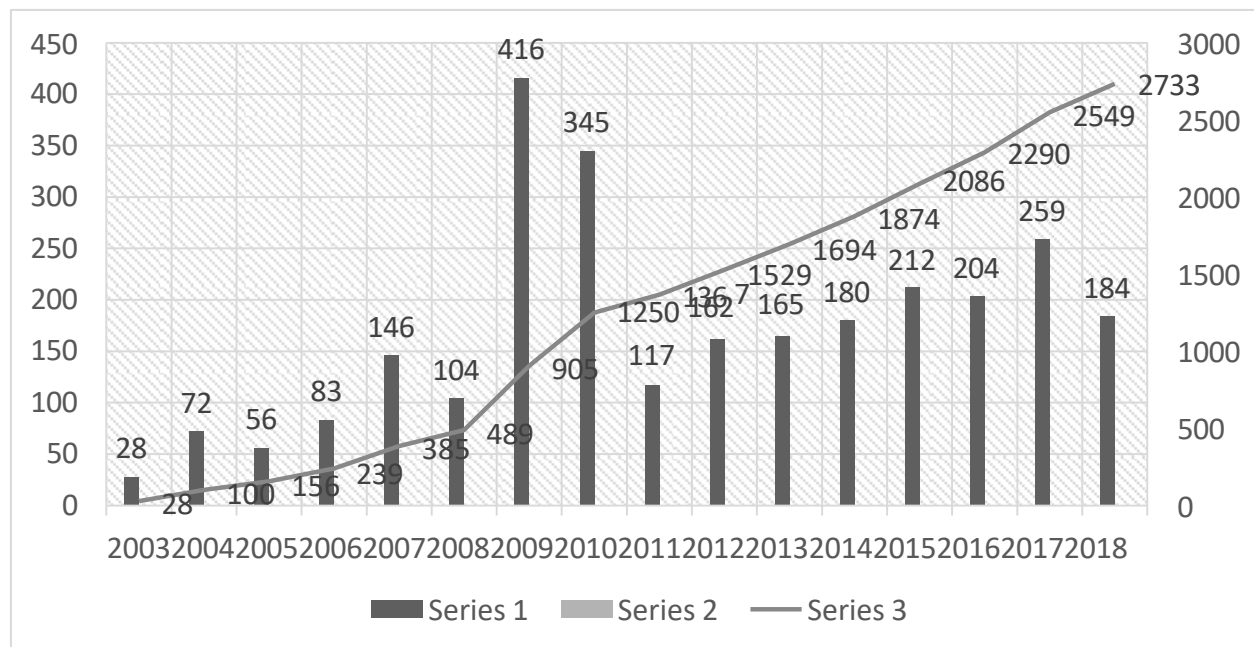


Figure 1 Cumulative cases trend from 2003 to 2018

Majority of the registered cases is from urban areas where the Voluntary Testing and Counselling (VCT) and Anti-Retro Viral Therapy (ART) centers are located. However, UNAIDS and WHO estimate the number of people living with HIV (PLHIV) in 2018 around 7500. Based on the estimation the prevalence in the general population 15-49 year of age is 0.01. High HIV rates are reported among key affected populations (KAPs), particularly, PWIDs. Figure .2 summarizes the annual new cases and cases per

million population per year. In the recent decade (from 1999 to 2009) the cumulative cases increased from 28 to 2733, in other words almost 20 times. These figures also stand for an increase from 1.01 new cases per million population to 6.02 in 10 years.

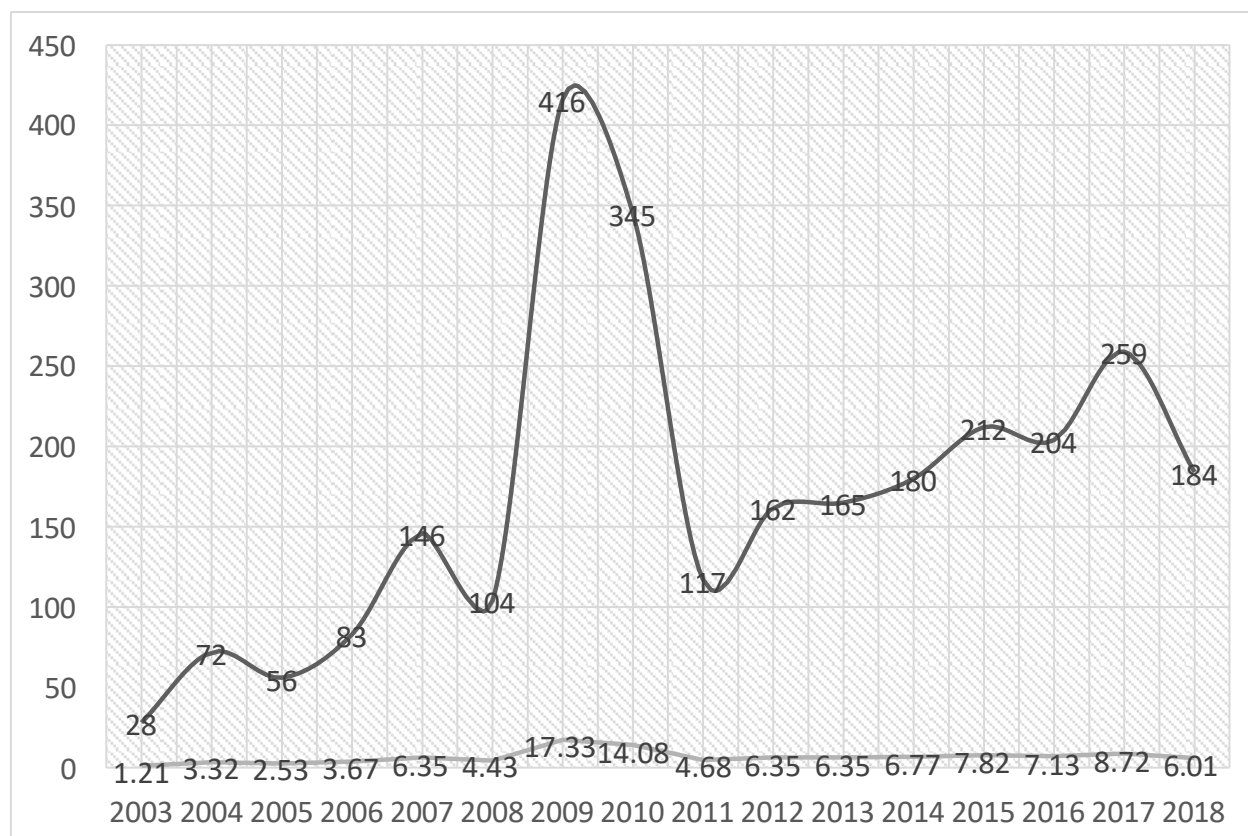


Figure 2 Annual new cases and cases per million

According to the Integrated Bio-Behavioral Survey (IBBS), the average of HIV prevalence among PWID in five cities of Afghanistan is 4.4% (95% CI: 3.3-5.8) with a range from 0.3% in Mazar-i-Sharif to 13.3% in Herat province. Among Men with High Risk Behaviors (MHRB) and Women with High Risk Behaviors (WHRB) the IBBS reported a prevalence of 0.4% and 0.3% respectively. In the cities of Kabul and Herat, HIV prevalence among prisoners was reported as 0.7% (95% CI: 0.2-1.6).

Based on the IBBS, the following intersecting behaviors among HIV key populations are found. More than 52% of PWID (74% in Herat) have purchased sex from a female sex worker, while 8.6% (14.2% in Herat) of FSW has sold sex to PWIDs. More than 16% of the PWID have paid for sex from man or boy and around 30% PWID have been in prison due to any reason. More than 50% of MSM bought sex from a woman and more that 22% of the prisoners had paid for sex for a female sex worker.

The data indicate that young adults, PWIDs, MHRB and WHRB and the partners of all these groups are particularly vulnerable to HIV infection. As injecting drug use is the main driver of the epidemic in the country, it has remained the focus of all efforts to stem the spread of HIV and AIDS. Unsafe injecting drug use includes sharing of needles and other injecting equipment.

Despite the low HIV prevalence among key affected populations, behavioral data suggest alarming signs for an emerging HIV epidemic. For example, ever bought sex from a female was reported among truck drivers (29.1%), prisoners (28.5% in Herat and 21.6% in Kabul), MSM (49.5% in Kabul), PWID (29.3% in Kabul, 37.4% in Herat, 44.5% in Mazar-e-Sharif, 55.8% in Jalalabad, 35.4% in Charikar). Furthermore, data show risk behavior interactions among key populations at high risk where more than 87% of FSWs in Kabul, Herat, and Mazar-e-Sharif reported one or more PWID clients in the last year.

Other than poverty, drug cultivation, trade and use, and low literacy level, underlying factors of vulnerability to HIV include poor HIV-related knowledge and limited access to sexual and reproductive education, high stigma and discrimination against PLHIV and Gender Based Violence (GBV), limited blood screening and high level of TB and STIs. In addition to the poor security and humanitarian environment, HIV prevention is challenged by poor infrastructure and a chronic lack of capacity in data collection, including absence of a comprehensive surveillance system on HIV and STIs and current reliance on data which are only available for some of the most at-risk groups. Injecting drug use as the main driver of the HIV epidemic is partially intersecting with sex work, which creates the potential of the epidemic to grow quickly from a small base of PWID to their sexual partners and heterosexual men an

Mode of transmission and gender differences

Gender distribution indicates a male dominance. Figure 3 clearly shows the difference between male and female.

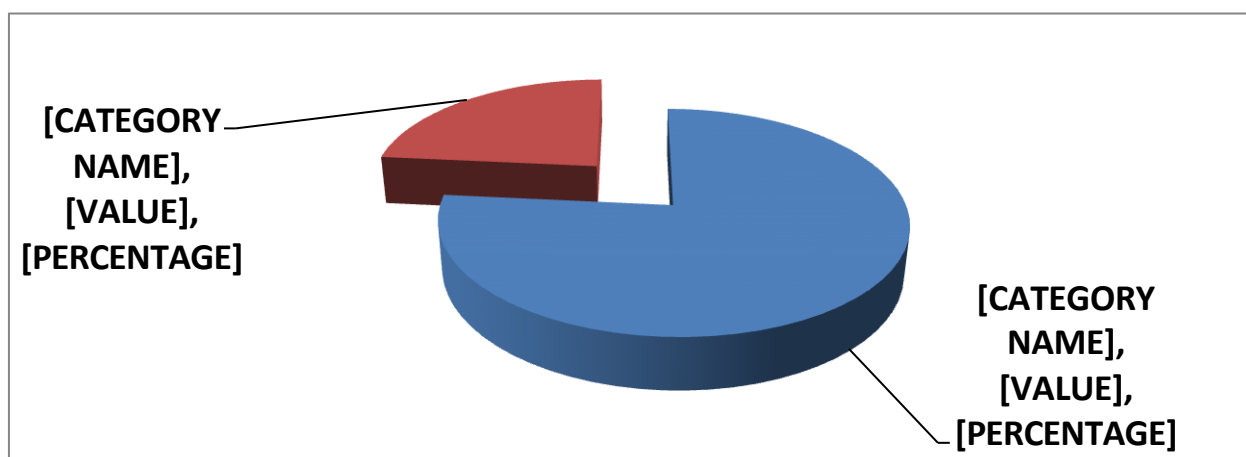
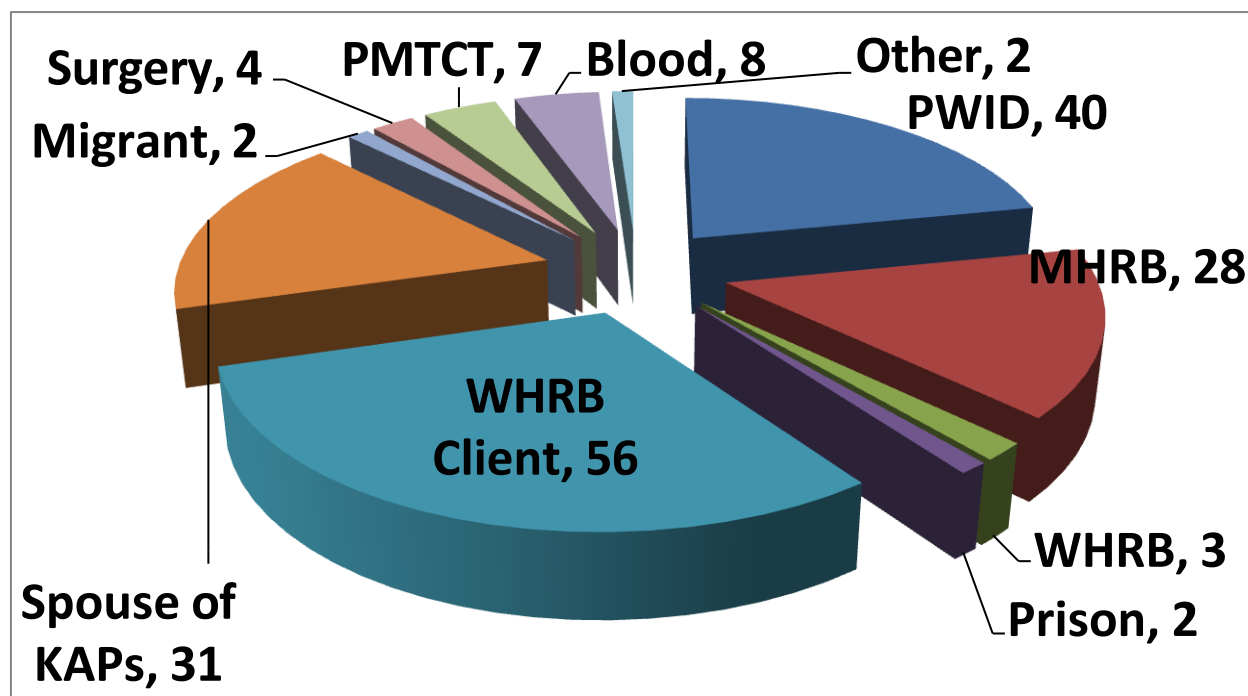


Figure 3 Cases in men and women in 2018

Heterosexual contact is the main mode of HIV transmission in Afghanistan; and constitutes 56 percent of the cases with a reported



mode, followed PWID (40 percent). On the other hand, the mode of transmission is 'unknown' for more than (2 percent) of the cumulative cases (Figure .4).

Figure 4 Cases in different risk groups in 2018

For adolescents and young people (age group 15-24), constitutes 19 percent of cases and (25-49 age group), constitutes 70 percent of cases. (Figure .05).

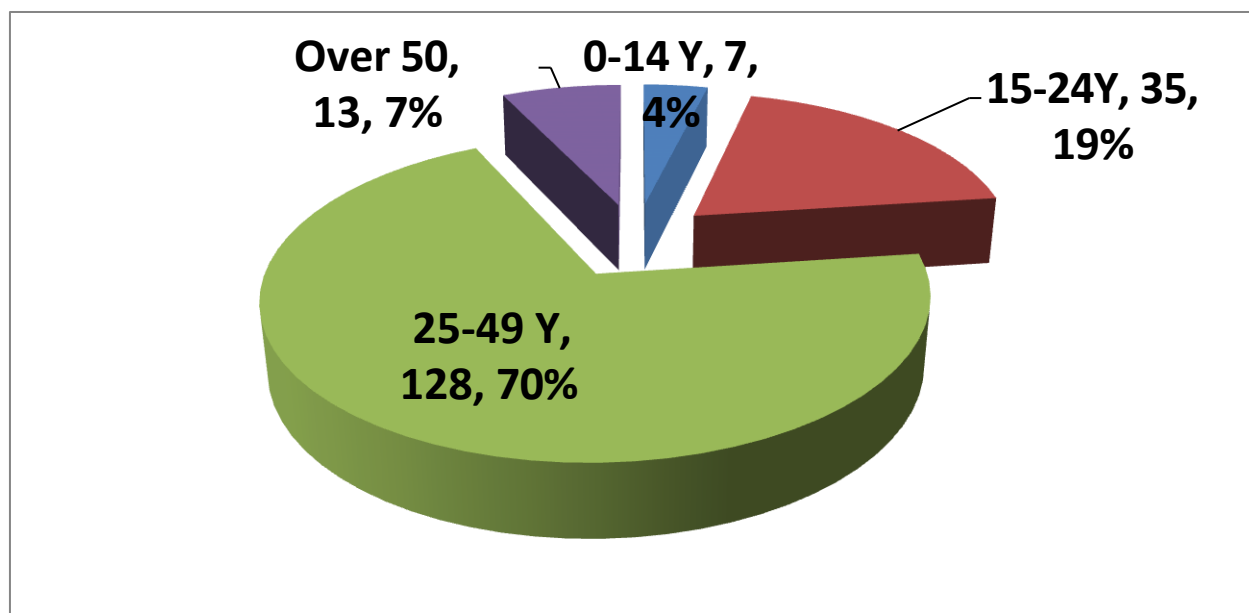


Figure 5 Cases by age groups in 2018

People Who Inject Drugs

Injecting drug-use in Afghanistan is associated with Heroin, opium, tranquillizers and pain killers. According to UNODC the majority of PWIDs are male. Available information suggest a profile of a drug user (as being from any ethnical group) a poor male under 30 years of age, unemployed, with little or no education, married and living with the extended family. If employed, it is usually as a farmer or unskilled worker, supplementing his income to meet the costs of his drug use via selling family assets, borrowing money, stealing, begging or committing other petty crimes. Women injecting drugs are more likely to be widowed or divorced with less education and more than twice as likely not to have a job. It was estimated that 4,000 females were injecting drugs, which is about 20% of PWIDs (UNODC 2009). Among children, opium use was most wide-spread in the North and North Eastern regions.

In a mapping assessment in 2008, the University of Manitoba estimated 1251,159 and 55 PWID in three cities: Kabul, Mazar-i-Sharif and Jalalabad respectively. The study revealed that injecting drug was largely street based and clustered within urban districts, with Kabul having the largest number of PWID per cluster (half of all 177 IDU clusters with at least five PWID).

According to the UNODC estimation in 2009, there were up to 23,000 PWIDs in Afghanistan. The recent size estimation of PWID through IBBS 2012 and rapid assessment in the western provinces of Afghanistan revealed 12,541 PWIDs in Kabul, 1,211 PWID in Herat, 1,496 in Mazar, 1,471 in Jalalabad

and 484 and 257 PWIDs in Nimroz and Farah respectively. While size estimation of rural PWID is yet to be done, some sources point at the existence of injecting drug use outside of urban areas.

In 2015, NACP based on the need to target setting of the NSP III extrapolate the IBBS and rapid assessment data to get provincial and national data. As a result, the national size of PWID extracted from extrapolation process is 40,900 (13,500-80,000) (NACP, 2015).

The IBBS 2009 found the average sample estimation of HIV prevalence among PWIDs was 7.1% ranging between 1% in Mazar-i-Sharif, 3.2% percent in Kabul to 18.2% in Herat, zero prevalence among FSW and no information on other KAP. The IBBS 2012 with a bigger scope found an estimation of 4.4% HIV prevalence among PWIDs with 0.3% in Mazar, 0.9% in Charikar, 1.0% in Jalal Abad, 2.4% in Kabul and 13.3% in Herat. These two figures cannot be compared as the first is only from a sample while the second is estimated for the overall PWID population.

Both 2005 and 2009 UNODC drug surveys point to alarming rates of high risk behavior among PWIDs, including sharing of needles and syringes, use of other substances, low condom use and exchange of sex for money and drugs. According to IBBS 2009, almost one third (27.4%) of all interviewed PWIDs shared needles or syringes which decreased to 11% in 2012. Improvement was reported in the use of non-sterilized syringes and injection equipment, from 31% in 2009 to 6.2% in 2012. Both rounds of IBBS reported a very low condom use among PWIDs at last sex, 26.8% in 2009 and 23.8% in 2012.

The majority of sexually active PWID surveyed in both IBBS rounds (58.4% in round-1 and 52.9% in round-2) bought sex from a woman in their life time and 20.3% of respondents in round-1 and 16.8% in round-2 reported they ever had sex with a boy. Based on the studies

the variation of sexual activity, geographical and periodical, was calculated. Buying sex from a woman varied from 47.1% in Mazar, 55.6% in Herat and 64% in Kabul in 2009 but it increased to 50.8% in Mazar and to 74.7% in Herat and declined in Kabul 34.3% in 2012, . The decline in Kabul may be due to severity of the drug addiction which caused impotence, lack of money and other unknown causes.

The size estimate exercise conducted in 2019 by university of California, estimated about 6,061 (95%CI 4,257 to 8,225) PWID are living in Kabul, which corresponds to 0.27% (95%CI 0.19 to 0.37) of the adult population (15 – 64y) **Table 1.** The population size and prevalence of male and female PWID in Kabul and seven other study sites in 2019.

City	KP	Group	Number of persons			Urban population 15- 64y	Prevalence (%)		
			Point Estimate	Lower Bound	Upper Bound		Point Estimate	Lower Bound	Upper Bound
Kabul	PWID*	Total	6,061	4,257	8,255	2,225,687	0.27%	0.19%	0.37%
Herat	PWID*	Total	690	556	843	291,336	0.24%	0.19%	0.29%
Mazar	PWID*	Total	1,157	906	1,449	245,720	0.47%	0.37%	0.59%
Jalalabad	PWID*	Total	677	528	842	137,868	0.49%	0.38%	0.61%
Kunduz	PWID*	Total	999	717	1,320	96,000	1.04%	0.75%	1.38%
Faizabad	PWID*	Total	392	295	496	19,910	1.97%	1.48%	2.49%
Kandahar	PWID*	Total	1,140	870	1,439	265,355	0.43%	0.33%	0.54%
Zaranj	PWID*	Total	920	712	1,140	15,656	5.88%	4.55%	7.28%

Kabul	PWID*	Male	5,819	4,087	7,924	1,125,624	0.52%	0.36%	0.70%
Herat	PWID*	Male	677	545	826	150,110	0.45%	0.36%	0.55%
Mazar	PWID*	Male	1,145	897	1,434	125,377	0.91%	0.72%	1.14%
Jalalabad	PWID*	Male	663	517	825	70,099	0.95%	0.74%	1.18%
Kunduz	PWID*	Male	989	710	1,307	48,871	2.02%	1.45%	2.67%
Faizabad	PWID*	Male	329	248	417	10,189	3.23%	2.43%	4.09%
Kandahar	PWID*	Male	1,129	861	1,425	134,936	0.84%	0.64%	1.06%
Zaranj	PWID*	Male	755	584	935	8,039	9.39%	7.27%	11.63%
Kabul	PWID*	Female	242	170	330	1,100,063	0.02%	0.02%	0.03%
Herat	PWID*	Female	14	11	17	141,226	0.01%	0.01%	0.01%
Mazar	PWID*	Female	12	9	14	120,343	0.01%	0.01%	0.01%
Jalalabad	PWID*	Female	14	11	17	67,769	0.02%	0.02%	0.02%
Kunduz	PWID*	Female	10	7	13	47,129	0.02%	0.02%	0.03%
Faizabad	PWID*	Female	63	47	79	9,721	0.64%	0.48%	0.82%
Kandahar	PWID*	Female	11	9	14	130,419	0.01%	0.01%	0.01%
Zaranj	PWID*	Female	166	128	205	7,617	2.17%	1.68%	2.69%

*Population size were adjusted for seasonality (winter) effect with a correction factor of 10% (i.e. increased by 10%).

Table 1 Size of PWIDS in different cities

Objectives of the Study

This study measures the prevalence of HIV and related risk behavioral factors in male PWIDs, and their main female sexual partners (If possible their wives)..

Rational for the Study

PWIDs constitute a considerable number of people living with HIV/AIDS worldwide. Due to the common parenteral mode of transmission, they are also at risk of acquiring hepatitis B virus and hepatitis

C. The sexual partners of IDUs are, in turn, at risk of HIV infection through different modes of transmission, and can pass the infection on to other sexual partners or to their offspring through mother to child transmission. In several Asian countries, the majority of females diagnosed with HIV were either partners of men who injected drugs or were sex workers. In Afghanistan such data is not available.

There are an estimated 25736 IDUs in Afghanistan. The majority of whom are men. Of all notified HIV cases in Afghanistan, 35% are among women, most of whom were infected by their sexual partners with whom they may or may not have shared injected drugs. However, to our best knowledge, direct measures of HIV prevalence and related factors among the partners of IDUs are rare globally, and particularly in south Asian region. Establishing the prevalence of HIV and related risk behaviors among PWIDs, as well as their sexual partners, is critical for planning of effective prevention and intervention programs.

Study Design, Methodology and Concepts

The bio-behavioral study included a behavioral component to determine the current situation and to estimate the distribution of characteristics in regard to knowledge and behavior on HIV/AIDS, and a biological surveillance which aims to determine HIV incidence among three target populations. In addition,

Behavioral component

The project team drafted survey questionnaires for PWIDs in close collaboration with partner organizations during the preparatory phase. The questionnaires were intended to cover all data pertaining

to the demographics, history of alcohol and drug use, knowledge on HIV/AIDS, and behaviors related to HIV testing practices of the target audience. During the development process the team accepted the ground rules below in constructing survey questionnaires:

1. Each question should relate directly to the survey questionnaire objectives
2. Every respondent should be able to answer every question
3. Each question should be phrased in such a way that all respondents interpret it the same way
4. Each question should provide answers to what we need to know \

In addition to being brief, using a plain language and limiting the number of questions while constructing the questionnaires, the staff made efforts to collect satisfactory and meaningful data in order to inform programming and to assure consistency with the international surveys by covering related UNGASS indicators (standard indicators adopted by United Nations General Assembly Special Session on HIV/AIDS) to an extent possible. The development process also involved pretesting draft versions of survey questionnaires with minimum 10 respondents from each target population and making necessary revisions.

The survey questionnaires consist of nine sections and the total number of questions were equal to 40 while three to six questions are related to the socio demographic characteristics of the respondents, the remaining questions concern the knowledge, health history and recent behaviors in regard to HIV-AIDS and related health topics. **Table .2** represents the sections and numbers of survey questionnaire.

Type of questions	# of Questions
Alcohol and drug use	12
Sexual history and condom use	6
HIV & AIDES knowledge	4
Socio demographic characteristics	6
Voluntary testing and counselling	4
Exposure to services	5
Other vulnerabilities	3

Table 2 Domains and questions

Biological component

All HIV tests of respondents were performed based on national algorithm guidance.

Sample size and study location

PWIDs will be recruited using simple random sampling (service-based sampling) on four drop-in centers registers (DIC) serving PWIDs. The whole registers will serve as a sample frame.

Place of study: Data was collected from four large cities in Afghanistan i.e., Kabul, Heart, Mazar I Sharif, and Nangarhar. Almost 44 percent of PWIDs live in these cities and harm reduction services are also provided there (Figure.06).



Figure 6 Location study conducted

Data collection technique: The participants were asked to participate in a bio-behavioral survey which included bringing in their main female sexual partners (more often spouses). DICs are supported by the Afghanistan government, and serve subgroups with high risk behaviors who inject drugs and who have high risk sexual behaviors. We used these centers as channels to assess injecting drug users. After the MIDUs were randomly selected. The participants were only enrolled in study if s/he was ≥ 18 years old, had a history of at least one drug injection in one year and married or had at least one female partner within the past 6 months (more often we refer to spouse). The participants were asked if their partners will also consent to participate, and a time was set for them to come together with their main female

partner for interview. The PWIDs were tested for HIV and Syphilis and they were asked question related to high risk behaviors.

An a priori sample size of 230 MIDUs was calculated based on the detection of a minimum of 5% prevalence of HIV infection in their partners within a $\pm 3\%$ margin of error, with 95% CI.¹ The data were weighted and analyzed using survey analysis commands in Stata to take into account clustering at the level of the sites.

Inclusion criteria

1. Male PWIDs and their wives
2. >18 years of age
3. Lives in of the four cities to be surveyed
4. Gives written informed consent
5. Having a history of at least one drug injection in last 12 months.

Data Management and Statistical Analysis

The surveillance and interview data were handled with high confidentiality. Each participant was assigned a specific unique identifier so their personal data couldn't be disclosed to anyone. The standard interview questionnaire and lab test results sheet had coding for computer analysis, monitoring and verification. Measure of association of HIV status and risk behaviors were completed. The results were presented in cross tables. The interview questionnaire is attached in annex. For lab tests standard lab registers were used.

Project Management

The prime investigator was based in MoPH. He ensured all phases of study are completed with high standards of quality and lead overall work on ground. The co-investigators were based in STAC TB and HIV centers in SAARC who provided backstop support on data analysis and report writing. 10 data collectors from both genders were recruited and trained.

Ethical Consideration

After obtaining verbal consent, participants will be interviewed by a trained professional using a standard survey questionnaire covering demographics, drug use and injection practices, and sexual risk behaviors. The partners will be interviewed separately by a same gender interviewer.

¹ IBBS, 2012, MoPH

Rapid tests will be carried out on each participant after obtaining separate consent for serological testing for HIV. If someone was found positive for HIV, other needed tests based on algorithm were completed. The study approval will be obtained from ethical review board at Afghanistan Public Health Institute-APHI.

Duration of study

The study was completed in 16 weeks. The phases of this study included drafting protocol and tools, consultation with partners, program taskforce approval, obtaining ethical approval, recruiting and training data collectors, collecting data, managing and processing data, writing reports and dissemination.

Dissemination of results

Beside publication in scientific media and peer reviewed journal, the result will be shared with all stakeholders, community members and civil society members by mean of a one day workshop.

Results

Age

The age distribution of the achieved sample is summarized in Table .3 as below. 09 % of PWIDs in Kabul and 39 % in Herat aged between 18 to 24 years. 36 % of PWIDs in Kabul, 27 % in Herat, 39 % in Balkh and 53% in Nangarhar aged from 25 to 34 accordingly. 37 % in Kabul, 21 % in Herat, 29 % in Balkh and 26 % in Nangarhar aged between 35 to 44 years. The number of younger PWIDs (18-24) is higher in Herat city comparing to three others.

	Kabul		Herat		Balkh		Nangarhar	
Age	n	%	n	%	n	%	n	%
18-24	9	9	20	38.69	1	2.70	6	16.33
25-34	37	36	14	27.14	19	48.65	20	52.38
35-44	38	37	11	21.11	11	29.05	10	26.53
45-54	14	14	6	11.56	7	17.57	1	3.40
55+	4	4	1	1.51	1	2.03	1	19.68
	102	100	51	100.00	38	100.00	38	100.00

Table 3 Age Group

The mean age of the total sample was 37.5 years (median 30; range 21.0-60.0, STDEV 8.79). Although the total numbers of each subgroup were not even, when they are compared according to their mean ages, non-IV Heroin users represented the youngest.

Education

The majority (35.6%) of the sample was secondary school graduate. This figure shows that our sample has a higher educational level when compared with the general population 15 years of age and over in Kabul.

Marital Status

The vast majority (84.8%) of the sample population was married. 11 % didn't disclose their status and 06

% were single. (Figure-07).

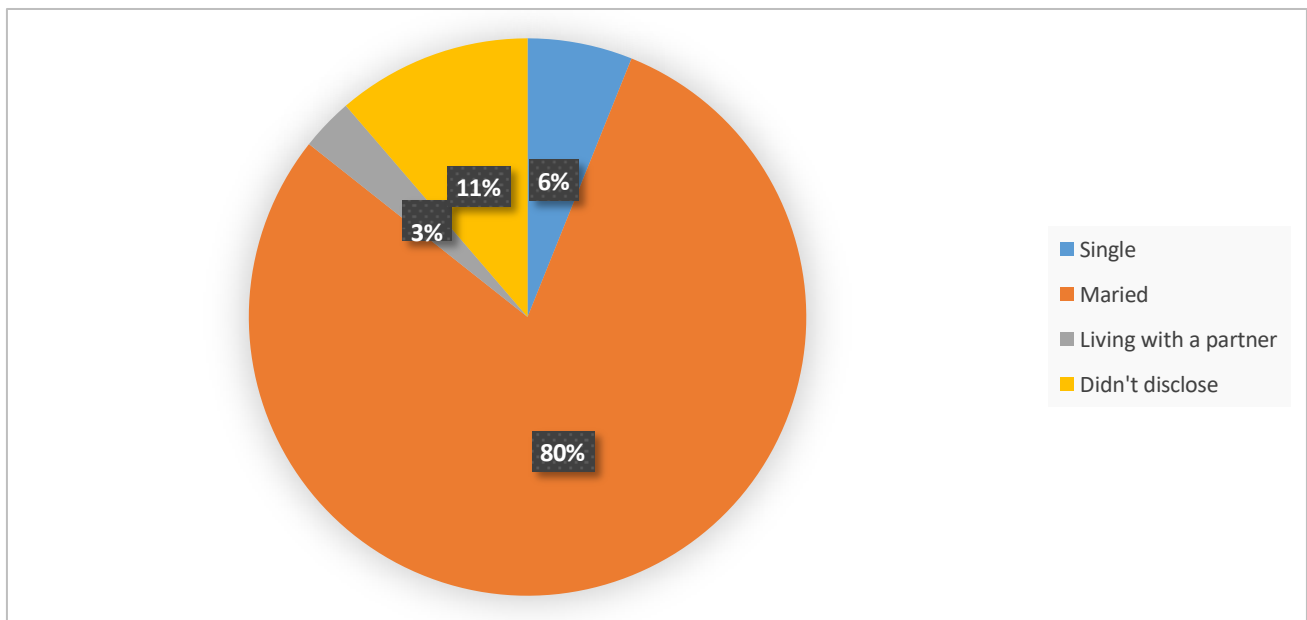


Figure 7 Marital Status

Employment

The vast majority (72.4%) of drug users was unemployed (**Table .04**). Unemployment rate was even higher in IDU sub-group as only less than one fifth (18.2%) of them was currently employed.

Current employment status of Heroin and amphetamine users						
	Heroin users		Amphetamine users		Total	
	n	%	n	%	N	%
Employed	12	18.2	19	40.4	31	27.4
Unemployed	54	81.8	28	59.6	82	72.6
Total	66	100.0	47	100.0	113	100.0

Table 4 Employment

Monthly Income

The vast majority (63.7%) of drug user respondents stated that they had no income or their income was not constant (**Table .8**). When the 'not constant' income group was excluded more than half (52.3%) of drug users appeared to have no income.

	Heroin users		Amphetamine	
	N	%	N	%
Non	29	43.90	16	34.00
Not constant	15	22.70	12	25.50
Less than 250 AFN in 24 Hours	3	4.50	1	2.10
250 -500 AFN in 24 Hours	16	24.20	12	25.50
More 600 AFN	3	4.50	6	12.80

Table 5 Income

Drug Use

Since drug use, particularly the intravenous (IV) route, is relatively rare in Afghanistan, the question was formulated so as to determine the participants' lifetime use. The majority (56.5%) stated ever use of Heroin in Kabul, 94 % in Herat and 78 % in Herat and Nangarhar. (Table .06). For Amphetamine users the rate of ever users slightly decreased to 41.18 % in Kabul, 3.92 % in Herat, 18.42 % in Balkh and

5.26 % in Nangarhar.

Drugs injected frequently	Kabul		Herat		Balkh		Nangarhar	
Heroin	58	56.86	48	94.12	30	78.95	30	78.95
Cocaine	0	0.00	0	0.00	0	0.00	1	2.63
Opium	1	0.98	0	0.00	0	0.00	0	0.00
Amphetamines	42	41.18	2	3.92	7	18.42	2	5.26
Prescription Drugs	0	0.00	0	0.00	1	2.63	1	2.63
Others	0	0.00	1	1.96	0	0.00	4	10.53
	102	100.00	51	100.00	38	100.00	38	100.00

Table 6 Types of different drugs used

Syringe and Needle Sharing

The respondents were also asked whether or not they have shared needle/ syringe (hereafter 'syringe') with someone else during the last time they had injected drugs. Among a total of 89 intravenous drug users in our sample, 83 of the respondents answered the question and more than one third (36.1%) stated that they had shared syringes during their last injection. Although the numbers are very small, we should note that almost half of the IV drug using (44.4%) shared syringes with other users. The respondents were also asked about what they do with the syringes after the injection. 75.49 % in Kabul, 80.77 % in three other cities through the syringes away and 19 to 24 % kept the syringes for reuse. None mentioned about syringes re collection program. (Figure .08)

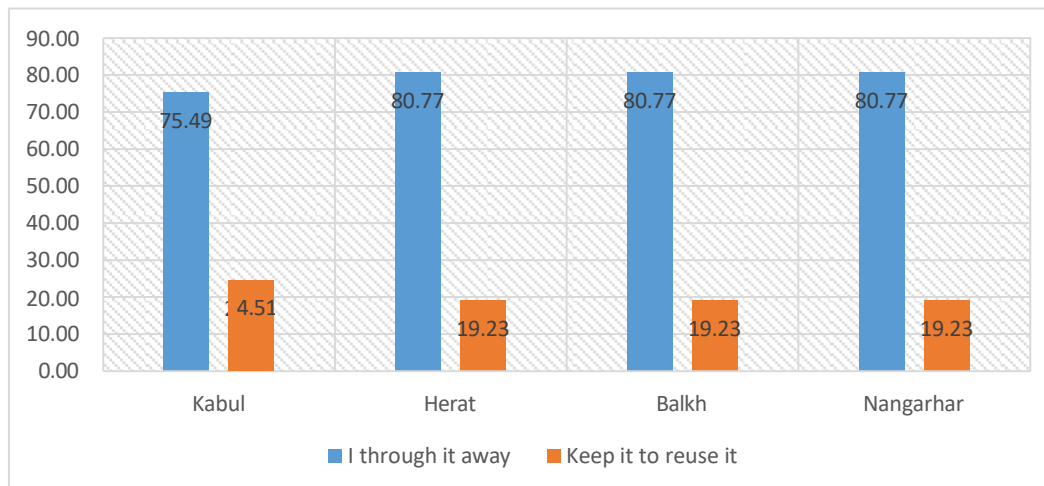


Figure 8 Syringes after usage

In the past one month before the interview, the majority of respondents 98.04 % in Kabul, 71.05 % in Balkh and 92.11 % in Nangarhar and only 22.00 % in Herat in injected drugs. More than half of the (54.00 %) respondents in Herat injected drugs in last 4 to 12 months. The injections in 4 to 12 previous months ranged from 0.98 to 13.00 % in Kabul and Mazar I Sharif. (Table.07)

Last injection	Kabul		Herat		Mazar I Sharif		Jalalabad	
	N	%	N	%	N		N	%
Within the last 1 m	100	98.04	11	22.00	27	71.05	35	92.11
2-3 months	1	0.98	12	24.00	6	15.79	2	5.26
4 to 12 months	1	0.98	27	54.00	5	13.16	1	2.63
	102	100.00	50	100.00	38	100.00	38	100.00

Table 7 Time of last injection

Basic Knowledge on HIV/AIDS

Table below shows the results of the distribution of answers to all questions, including correct/ incorrect and 'don't know' for each city.

The average rate of correct answers was 69.6% for the entire sample. When the percentages for correct answers were counted separately for all cities, PWIDs in Herat had the highest rates for all questions; while PWIDs had the lowest for all questions except “mosquito doesn’t transmit HIV. (Table .08)

	Kabul		Herat		Balkh		Nangarhar	
A healthy looking person can have HIV	15	14.71	16	30.77	2	5.26	5	13.16
Mosquitoes do not transmit HIV	11	10.78	15	28.85	5	13.16	3	7.89
Sharing food does not transmit HIV	14	13.73	7	13.46	4	10.53	4	10.53
A healthy looking person can have HIV	20	10.78	3	5.77	4	10.53	4	10.53
Mosquitoes do not transmit HIV	11	10.78	12	23.08	11	28.95	4	10.53
Sharing food does not transmit HIV	20	19.61	4	7.69	4	10.53	11	28.95
A healthy looking person can have HIV	5	4.90	1	1.92	1	2.63	4	10.53
Mosquitoes do not transmit HIV	3	2.94	6	11.54	4	10.53	1	2.63

Sharing food does not transmit HIV	3	2.94	5	9.62	3	7.89	3	7.89
	102	100.00	52	100.00	38	100.00	38	100.00

Table 8 Knowledge on HIV transmission

Voluntary counseling and HIV testing is a cornerstone of the fight against HIV/AIDS and actually an entry point to prevention and care services. However, the coverage of VCT services has always been inadequate in most parts of the world, in both high-income and resource-constrained settings. On the other hand, use of VCT services has been hampered by many factors including stigma and discrimination, limited access to treatment, care and health services in general, as well as gender issues.

It was found that all participants are aware of their HIV status in Balkh, 96 % in Nangarhar, 77 % in Herat and 57 % in Kabul. The result of low coverage and limited use of VCT services and low levels of knowledge of HIV status in Kabul city is that the majority of people living with HIV access HIV testing and counseling only when they already have advanced clinical disease.

As a way of expanding the supply of HIV diagnosis to populations, Afghanistan has established 11 VCT Centers in four provinces. Those VCT Centers were planned to be easily accessible sites to offer serological testing for the entire population, particularly to vulnerable groups. Since the services were limited to counseling in conjunction with diagnosis, with no community involvement, the majority of the VCT Centers faced low service utilization rates. Despite the huge need in the field, the lack of active prevention activities targeting special populations played a major role in low utilization of services provided by these centers, among many other aspects. (Table .09)

Know HIV	Kabul		Herat		Balkh		Nangarhar	
Status								
Yes	59	57.00	39	77.00	38	100.00	36	95.92
No	43	43.00	12	23.00	0	0.00	2	4.08
	102	100.00	51	100.00	38	100.00	38	100.00

Table 9 Knowledge of HIV status

HIV Testing Practices

HIV testing history of the study sample was determined including their lifetime experiences and practices within the most recent 12 months and beyond, and whether they know the results or not. All the results of HIV testing are shown in Table .13

Time of last HIV Test	Kabul		Herat		Mazar I Sharif		Jalalabad	
In last 12 months	68	67.09	37	72.86	38	99.32	37	98.64
1-2 years ago	5	4.81	7	14.07	0	0.68	0	0.68
More than 2 years ago	4	3.54	0	0.00	0	0.00	0	0.00
Never tested	25	24.56	7	13.07	0	0.00	0	0.68
	102	100.00	51	100.00	38	100.00	38	100.00

Table 10 Time of HIV Testing

First, the results revealed that almost 84 % of the study population have had been tested for HIV in the last 12 months. On the contrary, a quarter of PWIDs in Kabul stated that they had never been tested.

In the antiretroviral therapy era, asking respondents in bio-behavioral surveys to self-report their HIV status is essential for measuring and improving access to – and coverage of – services for the care, treatment and prevention of HIV infection. It is also important for estimating the true size of the unmet

needs in addressing the HIV epidemic and for interpreting the behaviors associated with the acquisition and transmission of HIV infection correctly. The data available indicated the self-reported HIV positives were high in Kabul (36 %) followed by Nangarhar (25 %). 43 % in Kabul and 23 % in Herat were never tested. (Table .14)

Self-reported HIV status	Kabul		Herat		Mazar I Sharif		Nangarhar	
HIV Negative	22	21.00	38	75.00	37	97.00	27	70.07
HIV Positive	37	36.00	1	3.00	1	1.00	10	25.85
Not comfortable to say	0	0.00	0	0.00	1	2.00	0	0.00
Never tested	43	43.00	12	23.00	0	0.00	2	4.08
	102	100.00	51	100.00	38	100.00	38	100.00

Table 11 Self-reported HIV status

Sexual risk behaviors of PWIDs

2.28 % in Kabul and 6.53 % in Herat had exchanged sex for drugs or money. (Table .15)

(For male ever anal or oral sex with another man)	Kabul		Herat		Mazar I Sharif		Nangarhar	
Yes	2	2.28	3	6.53	0	0.00	0	0
No	100	97.72	48	93.47	38	100.00	38	100
	102	100.00	51	100.00	38	100.00	38	100

Table 12 Male ever anal or oral sex with another man

26 % of respondents identified that they engaged in sex (oral or anal) in exchange for drug before 12 months only Herat. There were no reports of such engagement in other cities. (Table .16)

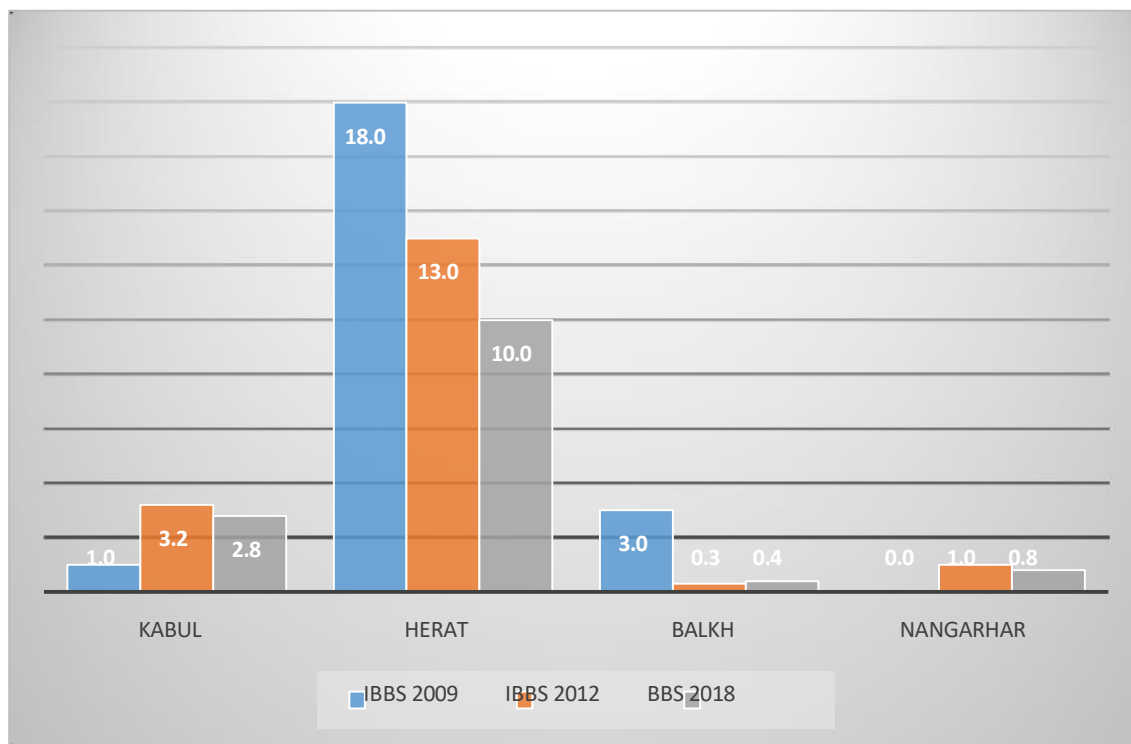
(males) engaged in sex (anal or oral) in exchange for drugs	Kabul		Herat		Mazar I Sharif		Nangarhar	
In last 12 months	2	1.53	1	3	0	0.00	0	0
Before last 12 months	0	0.00	10	26.00	0	0.00	0	0
Never engaged in sex	0	0.00	0	0.00	0	0.00	0	0
Never sex with men	100	98.47	38	78.00	38	100.00	38	100
	101	100	49	100.00	38	100.00	38	100

Table 13 (males) engaged in sex (anal or oral) in exchange for drugs

Prevalence

The overall prevalence of HIV among PWIDs decreased from 18.00 % to 10% in Herat, from 3.2 to 2.8

% in Kabul from 1.00 to 0.8 % in Nangarhar in course of previous decade.



Discussion

The results of our study depict that PWIDs by virtue of their harmful behavior are vulnerable for acquiring blood-borne infections such as HIV. In the current study, we found that one third of the study participants shared needles/syringes with others in the past one month. Similar to this finding, a study conducted by Solomon et al. in South India reported that 37% IDUs used a previously used syringe at least once. As et al. studied risk behaviors of IDUs in Chandigarh and observed that 17.4% shared needles/syringes during last month. Other studies conducted worldwide have also documented a high proportion of unsafe injecting drug sharing practices. Shariful Islam et al. in Bangladesh observed that around three-fourth of IDUs shared needles sometimes. Chikovani et al. in his study among IDUs in Georgia reported that unsafe injecting behavior at the last injection was 51.9%.

The present study found that IDUs who were injecting drugs on daily basis had higher injection sharing risk behavior. Moraros et al. in a study among Canadian IDUs inferred that higher frequency of injection was associated with high-risk behavior. Further, we found that those preferring to avail needles/syringes from NSP had comparatively low-risk behavior.

Our study also showed a high rate of re use of syringes and lack of syringe re collection program. This might have led to transmission of HIV and viral hepatitis. Implementation of Needle Exchange Program (NDP) is highly successful in controlling the HIV epidemic among PWIDs as demonstrated in a recent

publication from Australia. However, drug use remains illegal in Afghanistan and subsequent law enforcement is conflicting with implementation and support for NSP. Indeed, a quarter of IDUs in our survey reported to have been imprisoned because of drug use. Although most received needles from NSP, the main reason reported for not always carrying a needle was the fear of police arrest. The distribution of needles and syringes has shifted from local NGOs to community health centers (run by government), which affected the uptake of services as some proportions of respondents have not been tested for HIV in Kabul. Injecting in a park was another risk factor for HIV infection. It is well established that the size and density of social and injecting networks are key factors in the transmission of HIV among PWIDs.

Indeed, a recent study in Pakistan pointed out large sharing networks as a key factor for high transmission. In the current study, age of the study participants, education, living status, and age of initiation was not related to injection sharing practices.

Heroin was the most frequently used drug among PWIDS in this sample but many reported use of Amphetamines as well, this is in line with reports from Pakistan, China, Thailand, Ukraine, Lithuania, and Poland. This finding is in contrast with current perception that Heroin is changing its place to Amphetamine among PWIDs. This study suffers from the limitations of a cross-sectional study in a population which is difficult to reach, and the question is therefore how representative.

It is important to note that our results demonstrated declining HIV prevalence among PWIDs in all provinces. However, how program data shows the level of hetero sexual transfer is on rise. This highlights that effectiveness of harm reduction programs for PWIDs and necessitates the expansion of services for women with high risk behaviors.

Study Limitations

1. A cross sectional design didn't prove to be appropriate to reach hard to reach population
2. A strict culture caused Male PWIDs don't bring about their female partners for interviews.
3. Lack of approved algorithm for Hep C and Hep B didn't allow estimation of Hep C and Hep B infection in PWIDs
4. This was a cross sectional study. The respondents were selected from services sites not from community. It doesn't reflect behavior and infection rate among PWIDs which does not seek services. The data on biologic component should be used with caution and may not reflect the real picture as only the PWIDS in DICs were tested. The real figures at community level may be found higher. It needs to be verified for large scale IBBs.

Conclusion

It is important to note that our results demonstrated declining HIV prevalence among PWIDs in all studied cities, however, how program data shows the level of hetero sexual transfer is on rise.

Our study provides some evidence of the effectiveness of harm reduction program. For instance reduced risk behavior of exchange of sex for drugs, increased knowledge on how HIV transmit, increased timelines of HIV testing, increased knowledge of HIV status and self-reporting. This comparison was obtained from previous IBBS.

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