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Editorial

For a long time, until the programmes started managing drug resistant TB patients, the National Tuberculosis Control programmes were implemented for achieving good success rates for the patients infected with drug susceptible bacteria so that we are able to minimize the development of drug resistant strains. In addition, the programmes have been implemented on the premise that the drug resistance develops in two ways: Primary Drug Resistance or Acquired Drug Resistance. The main reasons for the acquired drug resistance being patient related issues like compliance or adherence, systemic issues like inadequate drug regimen or monotherapy, issue related to quality or supply of drugs or social issue like myths on the intake of Anti-TB drugs etc. Primary Drug resistance means the patient is infected with bacteria which are already resistant to drugs. The National Programmes adopted DOTS strategy which was expected to address the issues related to compliance, regimen or uninterrupted supply of good quality drugs. However, so far, the programmes have not been acknowledging or addressing issues other than conventionally thought causes for development of drug resistance. Effective first line treatment was expected to prevent emergence of drug resistance as a public health measure. But the data suggest that primary transmission of drug resistant strains is now driving the spread of resistance including the high TB burden countries. It means the traditionally believed causes of development of drug resistance have now taken a back seat and the new emerging data indicates many other causes of development of drug resistance.

The prime way of development of drug resistance in *M. tuberculosis* is through mutations in genes encoding drug targets or enabling enzymes. The effect of drugs treatment is to diminish the population of drug susceptible bacteria which also enables the emergence of a strain able to bypass the treatment. Several studies in individual patients who have developed progressive drug resistance over time have documented the initial acquisition of isoniazid resistance as a result of one or more mutations, followed by acquisition of resistance to rifampicin or ethambutol (or both), pyrazinamide, and finally, the second-line and third-line drugs. When resistance to one or more drugs is acquired in this way, it is referred to as secondary resistance. By contrast, primary resistance occurs when resistant strains are transmitted to a new host in the same manner as a drug-susceptible strain.

Previous exposure to anti-tuberculosis drugs is consistently identified as a strong risk factor for MDR, but other host risk factors can vary in different geographical settings. The studies have proved that population younger than 65 years of age were 2.5 times more likely to have multi drug resistance than those above 65 years. A possible explanation for this is that older patients might have tuberculosis due to activation of a latent infection acquired before the emergence of drug resistance. Studies have not been able to conclusively prove HIV and Diabetes Mellitus as risk factors for drug resistance.

The two deep rooted epidemiological beliefs of the Programme Managers are that resistance has a fitness cost rendering drug-resistant strains less transmissible and resistance is believed to primarily be acquired by patients who were previously exposed to anti-tuberculosis drugs. Consequently, for decades, tuberculosis control policies have targeted prevention of drug resistant tuberculosis through the WHO directly observed treatment, short course (DOTS) strategy and focused on detection of drug-resistant tuberculosis in individuals with a history of prior treatment for active tuberculosis (high-risk group). International policies have largely ignored patients who develop primary resistance. In most regions of the world, drug-resistant tuberculosis is now predominantly caused by transmission rather than acquisition of resistance, with an estimated 95.9% of MDR tuberculosis in new tuberculosis cases and 61.3% in previously treated cases being due to transmission.

Another important cause of acquired drug resistance had been attributed to poor adherence. Thus, acquired drug resistance was dealt with using a programmatic approach, specifically the DOTS strategy, to improve adherence. Emergence of acquired drug resistance eventually became equated with poor adherence, and high rates of acquired drug resistance were considered to be an indicator of poor performance of DOTS programmes. Indeed, careful historical documentation has shown that the problem of *M. tuberculosis* acquired drug resistance arose as soon as drug therapy first became available, and has continued being a problem from the 1950s to the present. In 1970, Hugo David performed fluctuation tests to identify *M. tuberculosis* mutation rates, and identified average mutation rates (as mutation per bacterium per generation) of 2.56×10^{-8} for isoniazid, 2.56×10^{-7} for ethambutol, and 2.25×10^{-10}

for rifampin. The probability of acquired drug resistance to two or more drugs is the product of these mutation rates, so the probability of acquired drug resistance for these three drugs in combination would be $\sim 1 \cdot 0 \times 10^{-25}$. In view of such low probability, it was thought that resistance could develop due to inadvertent monotherapy due to wrong prescription practices, issue related to drug supplies or poor patient adherence. Four scenarios were proposed for inadvertent monotherapy. First, given the high bacillary burden in which mutants probably pre-existed, and that each antibiotic in the combination only works on specific metabolic subpopulations of the bacteria (eg, isoniazid is the only effective drug against rapidly growing bacteria; thus, monotherapy is effectively being given), isoniazid resistant mutants would be selected if patients took the combination treatment for 2 days and then stopped. The second mechanism would arise during the sterilizing effect, given that pyrazinamide would be the only effective drug for semi-dormant M tuberculosis under acidic conditions, and rifampicin for non-replicating persistent bacteria under hypoxia; mathematical models predicted that poor compliance would lead to acquired drug resistance in this situation. The third mechanism involves regrowth during sub-inhibitory concentrations of drugs, especially for drugs (such as isoniazid) that have a high therapeutic margin and a long half-life because they remain present in the body after the clearance of other drugs. This is essentially a version of the pharmacokinetic mismatch hypothesis. The fourth scenario involves differential bacteriopausal mechanisms in which a drug such as rifampicin, whose post-antibiotic effect is shorter than of a companion drug such as isoniazid, selects isoniazid-resistant mutants during regrowth. In the standard anti-tuberculosis regimen, rifampicin and isoniazid both have a short half-life of 2–3 h and pyrazinamide has a half-life of 10 h, while M tuberculosis has a doubling time of 14–96 h and the mutation rates ($2 \cdot 56 \times 10^{-8}$ for isoniazid, $2 \cdot 56 \times 10^{-7}$ for ethambutol, and $2 \cdot 25 \times 10^{-10}$ for rifampin) identified by David, with a total bacterial burden of 10^8 in a cavity. The antibiotics are no longer present because clearance occurs before a single M tuberculosis has replicated, and certainly by the second and third replications. This timing makes the probability of generation of mutants, or even amplifying pre-existing ones, less likely, particularly for non-replicating persistors and semi-dormant bacilli, aptly described as “fat and lazy” by Garton and colleagues. The three meta-analyses conducted, were concordant in showing that supervised therapy was effective in reducing non-adherence and improving treatment completion. The meta-analyses also showed that no benefits were associated with DOTS compared with self-administered therapy when microbiological failure and relapse were examined as clinical endpoints. The incidence of acquired drug resistance was the same whether supervised therapy was given at home, in a health facility, by a family member, or by a community health-care provider.

Hollow fibre studies in tandem with in-silico clinical trial simulations predicted that, given the xenobiotic metabolism patterns in the Western Cape in South Africa, a proportion of patients would actually be on monotherapy despite being given the full multidrug regimen and being part of a DOTS programme. This is because of the differential rapid elimination of some drugs in the regimen, leading to prolonged monotherapy with the drug that is not rapidly or extensively metabolised over tens to hundreds of rounds of bacterial replication. The in-silico study predicted that 0.68% of patients would develop acquired drug resistance and MDR tuberculosis within 2 months despite 100% adherence, because of such differential pharmacokinetic variability of regimen components. Acquired drug resistance, including MDR tuberculosis, was encountered in 0.7% of patients during the first 2 months, despite adherence to standard doses of isoniazid, rifampicin, pyrazinamide, and ethambutol. All cases of acquired drug resistance, including MDR tuberculosis, were preceded by suboptimal drug concentrations due to pharmacokinetic variability. A meta-analysis of 13 randomised studies with 1631 rapid isoniazid acetylators and 1751 slow acetylators showed that rapid acetylators had 2.0 times higher occurrence of microbiological failure and acquired drug resistance compared with slow acetylators.

Dheda and colleagues have proposed and tested further pharmacokinetic variability at the level of drug penetration into tuberculosis lesions, which is dependent on the architecture of the tuberculosis lung cavity, for more than eight drugs. The lung cavity and surrounding fibrosis, depending on the size, will create a physicochemical barrier to drug entry, leading to anatomical site-based monotherapy. The programmatic level issues with drugs can also lead to pharmacokinetic variability and resulting monotherapy. In addition, health-care workers might prescribe lower doses than those needed to achieve the required optimal drug concentrations because of error or weight-based capping dosing practices (when dosing is capped at a particular maximum for the individual patient weight), which are often used in tuberculosis programmes, especially when fixed-dose formulations are given.

The role of efflux pumps in antibiotic resistance: As part of the bacterial stress reaction to the suboptimal antibiotic concentrations—and to effective monotherapy—efflux pumps in the bacilli are upregulated within hours. This

increase can be demonstrated by quantifying transporter messenger RNA, and is followed within a few days by phenotypically demonstrable low-level resistance that is reversed by efflux pump inhibitors such as verapamil. This efflux pump-dependent low-level resistance process allows the bacteria time to undergo multiple rounds of replication under suboptimal antibiotic pressure or monotherapy, allowing for development of mutations in the canonical drug resistance genes, in efflux pump genes, or in negative regulators of efflux pumps. The mutations in efflux pump regulators lead to high-level resistance, usually to multiple antibiotics. Indeed, mathematical modelling predicted a probability of the emergence of resistance to both isoniazid and rifampicin of 1×10^{-5} to 1×10^{-4} before commencement of therapy, suggesting that prior existence of MDR might be common. These patients would have a mixture of both drug-susceptible M tuberculosis and drug-resistant M tuberculosis that have arisen from a single strain.

Preclinical studies, prospective clinical studies, and meta-analyses have not identified the role of adherence in acquired drug resistance, contrary to common beliefs. Hence, the time is ripe that the Programmes start dealing with the individual drug resistant TB patients and identify those who require specialized care in view of the new emerging causes of drug resistance.

Chief Editor
Director, STAC

Original article

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 receive treatment care - they are “missed” to identify by the 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. Sputum was collected from eligible participants and tested for AFB, Gene-Xpart and Chest X-ray was done. Standard test algorithm and case categorization was done according to WHO recommendations. Descriptive analysis of demographic, clinical and laboratory data was done.

A total of 22,596 participants were included and among them 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 than the actual reports by the Afghanistan National TB program, which signifies the program was missing significant numbers of TB cases. Hence active TB case finding might be an effective approach in terms of case notification in Afghanistan. We recommend extending active community TB surveillance of the country.

Key words: Afghanistan, Tuberculosis, Prevalence survey, Household surveillance

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%

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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⁽⁵⁾. A number of factors, including on going 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. 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 populations 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⁾.

Socio-demographic and other relevant characteristics of the study participants using mean, standard deviation, median and percentages as appropriate. Data was statistically analysed with the significance level setting as two-tailed and at p value <0.05. Descriptive statistics for continuous variables were described as means ± 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 was 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.

RESULTS

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 + 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.

Gender distribution among the presumptive Tuberculosis cases

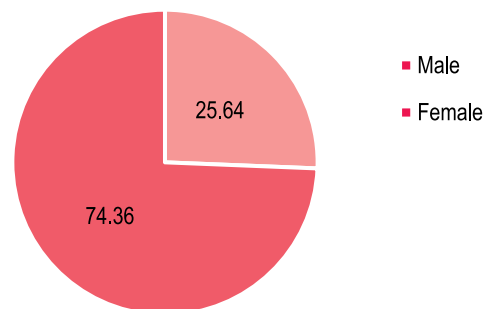


Figure 1: The gender distribution of the presumptive TB cases, Kabul, Afghanistan, 2018

While analysing 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 analysed 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 occupations were diverse and the data is plotted in figure 2.

Occupation of the presumptive Tuberculosis patients

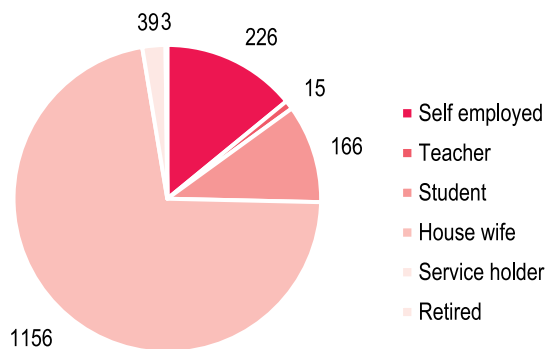


Figure 2: Occupational distribution of the presumptive TB cases, Kabul, Afghanistan

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 lesions 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), 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. The household composition of the study population was 8.4%, although, this rate is 7.2% according to AHS 2018.

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. The prevalence of TB in Kabul city found to be 465 in 100,000 populations which are higher than WHO estimates of 340 in 100,000 populations.

CONCLUSION

The findings of this study shows higher rate of respiratory symptomatic patients in Kabul city. The presumptive TB patient's identification rate was higher than the NTP data for over the past one decade. 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 active surveillance approach to find missed TB cases in cities 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.

Improved and faster case detection was noted by using Xpert MTB/RIF assay than the culture and histopathology tests. We hence recommend the use of the same as the first-line investigation at laboratories for all suspected Spine TB cases and to gradually scale-down the processing by AFB staining and conventional culture. The results of

the study may be used to improve existing Spinal TB diagnostics at medium sized hospitals by assessing the usefulness of the tests in their own set-ups and pick on the most optimal and accurate one. Age-old HPE may no longer be viewed as a reference standard and needs further evaluation.

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CONFLICT OF INTEREST

None

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A SYSTEMATIC REVIEW ON THE DIAGNOSTIC ACCURACY OF LINE PROBE ASSAY IN THE RAPID DIAGNOSIS OF DRUG RESISTANT TUBERCULOSIS IN INDIAN SCENARIO

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ABSTRACT

Owing to the drastic increase in the number of patients with drug resistant TB around the world, it is important to increase the testing for it. Line probe assay (LPA) is the rapid diagnostic tool to detect drug resistant TB and it was endorsed by WHO for testing first line drugs such as Isoniazid (INH) and Rifampicin (RIF). This systematic review evaluated the accuracy of this LPA by analysing its sensitivity and specificity against the phenotypic drug susceptibility testing (DST) methods like LJ and liquid culture DST. A total of 4774 samples were included in this review from 19 articles. The average sensitivity and specificity for the detection of RIF resistance from 17 articles was 95.79% and 96.71% and for INH resistance it was 89.85% and 97.33% respectively when compared to phenotypic DST. Out of 19 articles included, 2 articles have mentioned the sensitivity and specificity for multi-drug resistant TB (MDR TB) and the average was 98.50 % and 97 % respectively. The accuracy for RIF resistance detection through first line LPA was good and the sensitivity detection for INH was less across the studies. This could be improved further in future generation assays. Our finding supports the use of LPA especially on smear positive specimens but use on smear negative specimens still be considered as studies have shown some interpretable results.

Key Words: Line Probe Assay LPA, Drug susceptibility testing (DST), DRTB, Isoniazid, Rifampicin

INTRODUCTION

Tuberculosis (TB) is a major cause of ill health and it's the leading cause of death from a single infectious agent. More than a quarter of the world's population is infected with M. tuberculosis which results in the development of TB disease. Around the world, an estimated 10 million people fell ill with TB in 2018. In India the number of patients

diagnosed newly for TB varies from 1.2 million to 2.0 million between 2013 and 2018⁽¹⁾.

The emergence of multidrug and extensively drug-resistant tuberculosis (MDR & XDR) is a major threat to global tuberculosis control. In 2018, there were about half a million new cases of rifampicin-resistant TB. Out of which 78% had multi drug resistant TB⁽²⁾. Overall, there were an estimated 484,000 incident cases of MDR/RR-TB in 2018. Out of which 27% of MDR-TB cases had been reported from India. In addition to MDR TB cases a total of 13,068 cases of XDR-TB were reported in 2018⁽¹⁾.

Given the global statistics of drug resistant TB, it is important to increase the testing for drug resistance among bacteriologically confirmed TB cases and

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it should be rapid. Due to which the number of patients having TB will be enrolled for treatment as early as possible.

The diagnosis of drug resistance of *M.tuberculosis* is done by performing drug susceptibility tests (DST) on clinical isolates either by using Lowenstein Jensen media or by automated liquid culture method such as Mycobacterium growth Indicator Tube (MGIT) system. These methods are laborious and they have longer turnaround time .Hence molecular methods were accompanied with conventional methods which detects the mutations in the genes responsible for drug resistance in *M.tuberculosis*.

The genotypic methods include line probe assays and nucleic acid amplification method like CBNAAT which reduce the time of detection from several weeks to few days⁽³⁾. Line probe assays such as , MTBDR plus and MTBDR sl (Hain life science) and the Cartridge Based Nucleic Acid Amplification tests (CBNAAT) like Xpert MTB/Rif assay have been endorsed by WHO for rapid and effective detection of *M.tuberculosis* as well as the genetic mutations in *M. tuberculosis* that confer drug resistance.

WHO has also endorsed the use of commercially available molecular line probe assays (LPA) like MTBDR plus and MTBDR sl (Hain life science) for rapid drug susceptibility testing of first-line drugs such as isoniazid and rifampicin as well as selected second-line drugs such as fluoroquinolones and second-line injectable drugs only on smear positive pulmonary TB samples during the year 2008 and 2016 respectively. The turnaround time of this line probe assay is 1 to 2 days. The sensitivity and the specificity of the first line LPA was high with 98% and 99% compared to phenotypic methods with 87.7% and 89.7% respectively⁽⁴⁾.

In India until the use of LPA for DST, conventional LJ and MGIT 960 were in practice. In 2011 first line LPA was included as part of programmatic management of drug resistant TB (PMDT) under National Tuberculosis Control Program (NTCP) by setting up LPA labs in several states of India starting with 30 labs which has extended to 64 labs till 2019. In 2011 the number of DST using LPA was 635 ⁽⁵⁾ which has substantially increased to

3,46,282 tests by 2019 ⁽⁶⁾, of which 10,837 MDR TB ,20,329 Isoniazid (INH) resistance and 2,247 rifampicin (RIF) resistance cases were identified.

In 2016 ,a systematic review was done on first line LPA with 74 publications by WHO from different part of the world including six publications from India⁽⁷⁾. The number of publications from India on LPA increases due to the drastic increase in the number of LPA tests performed in different parts of the country. A systematic review has been performed in order to elucidate the accuracy of LPA in Indian lab settings.

METHODOLOGY

We performed comprehensive search of the data bases like Pub med, Web of science and Google scholar for relevant citations. We have restricted to the time period from 2011 to 2019 as LPA became part of PMDT programme in 2011 in India. Key words used were TB, Drug resistant TB, Mycobacterium tuberculosis and accuracy of Line probe assay. We have included only full text articles and none from conference publications.

Studies published only on Indian data were included. The studies were prospective studies which compared LPA first line DST with a reference tests in a particular point of time. The reference standard tests were phenotypic tests like MGIT DST and LJ DST for first line drugs and their sensitivity and specificity data were included. Publications without sensitivity and specificity to detect both INH and RIF resistance, mono resistance to INH and RIF resistance were excluded. Studies with minimum 40 samples (both pulmonary and extra pulmonary samples) independent of the smear status were included.

The diagnostic accuracy of LPA first line DST with the sensitivity and specificity against the reference tests.

RESULTS

A total of 52 articles were collected which got published from different states of India on LPA and all these articles were full text articles. Of which 19 articles were included in this review as our systematic review focussed on the diagnostic

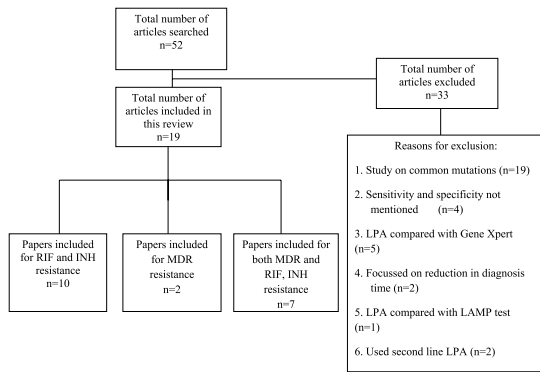


Figure 1. Characteristics of the studies included and excluded

accuracy of drug resistant tuberculosis using LPA compared to phenotypic diagnostic tests like LJ DST and liquid culture DST. Thirty three articles were excluded from this review and the reasons for not including these articles are mentioned in figure 1.

Table 1 demonstrates the characteristics of the 19 articles which provided data on RIF and INH

resistance separately and also MDR TB (RIF and INH resistance together). All these 19 articles were prospective in design. Most of the studies were performed in either a regional or national reference laboratory setting.

Out of 19 articles, 15 evaluated LPA on direct sputum samples where 14 were on smear positive for Acid fast bacilli (AFB) and 1 was on smear negative samples. Four were on indirect samples/ culture isolates which are smear positive for AFB. Only eight studies have mentioned the version of the Hain genotype MTBDR *plus* kit used (version 1 (n= 3), version 2(n=5)).

Seven studies have mentioned the number of invalids in their study results. The reasons of these invalids were mentioned as incomplete amplification of RIF and/or INH genes or absence of TUB while the specimen is culture positive for *M.tuberculosis* in phenotypic test. None of the studies have mentioned indeterminate in their study results. Studies did not report whether repeat testing was done on the invalid results.

Table-1 -Characteristics of data on RIF and INH

S.No	Author	Year of publication	Reference Test	Source of the sample	Sample size	MDR (%)		RIF (Mono resistance) (%)		INH (Mono Resistance) (%)		Direct testing or Indirect testing	Design of the study
						Sens	Spec	Sens	Spec	Sens	Spec		
1	Binit Kumar Singh	2017	MGIT 960	Sputum (Sm -ve)	572	NA	NA	100	99.2	97.6	98.6	Direct testing	Prospective study
2	Richa Kumari	2016	LJ	EPTB	51	92.86	97.3	92.86	97.3	93.33	94.44	<i>M.tuberculosis</i> isolates	Prospective study
3	Raveendran	2012	Bact/Alert	Sputum (Sm+ve)	106	NA	NA	100	97	92	93	<i>M.tuberculosis</i> isolates	Prospective study
4	Shariq Ahamed	2017	LJ	Sputum (Sm+ve)	62	NA	NA	94.4	95.35	92	91.89	Direct testing	Prospective study
5	Raj N Yadhav	2013	LJ	Sputum (Sm+ve)	242	97	100	98	99	92	99	Direct testing	Prospective study
6	Neeraj Raizada	2014	LJ	Sputum (Sm+ve)	248	96	99	93	94	72	97	Direct testing	Prospective study
7	Jai shankar	2016	LJ	Sputum (Sm+ve)	105	100	94	NA	NA	NA	NA	Direct testing	Prospective study
8	Parveen Kumar	2013	MGIT 960	Sputum (Sm+ve)	567	93.1	100	96.8	100	92.8	100	<i>M.tuberculosis</i> isolates	Prospective study
9	Manoj Kumar	2016	LJ	Sputum (Sm+ve)	652	100	96	92	99	97	96	Direct testing	Prospective study
10	Jadhav	2015	LJ and MGIT	Sputum (Sm+ve)	47	95.74	100	NA	NA	NA	NA	Direct testing	Prospective study
11	Sana Nadrat	2017	LJ	Sputum (Sm+ve)	200	NA	NA	89	100	91	100	Direct testing	Prospective study
12	AK Mayura	2013	Bact/Alert	Sputum (Sm+ve)	125	97.7	99.1	95.8	98.5	96.3	98.4	<i>M.tuberculosis</i> isolates	Prospective study
13	Marilyn	2016	LJ/MGIT 960	Sputum (Sm+ve)	91	NA	NA	100	93.8	89.3	100	Direct testing	Prospective study
14	Madhuri	2015	LJ	Sputum (Sm+ve)	100	95	98.3	98.1	97.8	92.1	97.9	Direct testing	Prospective study
15	Pranali Medhaekar	2016	LJ	Sputum (Sm+ve)	510	98.9	97	98.9	96.8	97.5	98.4	Direct testing	Prospective study
16	Ritu Singhal	2012	MGIT 960	Sputum (Sm+ve)	120	NA	NA	97.6	94.4	83.3	93.8	Direct testing	Prospective study
17	Himanshu Vashishta	2017	MGIT 960	Sputum (Sm+ve)	483	NA	NA	87	87	76	97	Direct testing	Prospective study
18	Leimapokpam shivadutta	2014	Bact/Alert	Sputum (Sm+ve)	375	98.3	100	95	100	81.3	99.2	Direct testing	Prospective study
19	A.Jain	2016	MGIT 960	Sputum (Sm+ve)	118	NA	NA	100	95	92	100	Direct testing	Prospective study

Outcome of Interest:

Table 2. Average Sensitivity and Specificity of RIF, INH and MDR TB detection of first line LPA compared to phenotypic tests.		
	Sensitivity	Specificity
RIF resistance detection	95.6% (87-100)	96.25% (87-100)
INH resistance detection	88.70% (72-100)	97.59 % (91.89 -100)
MDR TB detection	98.50% (92.86-100)	97% (94-100)

A total of 4774 samples were included in this review from 19 articles. As shown in table 2 the average sensitivity and specificity for the detection of RIF resistance was 95.79% (ranges from 87% to 100%) and 96.71% (ranges from 87% to 100%) and for INH resistance it was 89.85% (ranges from 72% to 100%) and 97.33% (ranges from 91.89 % to 100%) respectively. Out of 19 articles included, 2 articles have mentioned the sensitivity and specificity of MDR TB detection (RIF and INH resistance together) and the average was 98.50 % (ranges from 92.86 % to 100%) and 97 % (ranges from 94 % to 100%) respectively.

Diagnostic accuracy of LPA from Direct testing compared to phenotypic tests:

A total of 3925 samples from 15 studies were tested on LPA directly from specimens which showed the average sensitivity and specificity as 95.61% and 96.25 % for RIF resistance, 88.70% and 97.59% for INH respectively. Two articles have mentioned the sensitivity and specificity of MDR TB detection (RIF and INH resistance together) for which the average sensitivity and specificity was 97.25 % and 97.67% respectively.

Diagnostic accuracy of LPA from Indirect testing compared to phenotypic tests:

A total of 4 articles performed LPA on culture isolates with the sample size of 849. The average sensitivity and specificity of RIF resistance detection was 96.36% and 98.20% .For INH resistance the sensitivity and specificity were 93.60% and 96.46% respectively.

Hain genotype MTBDR plus Version 1 vs. Version 2:

Eight articles have mentioned the version of kit used. The average sensitivity and specificity of RIF resistance detection using Version 1, Version 2 was 94.78%, 98.40% and 94.56%, 99.60% respectively. Similarly the average sensitivity and specificity of INH resistance detection was 83.76%, 95.20% and 98.32%, 99.30% respectively.

DISCUSSION

This literature search identified 17 articles which reported the sensitivity and the specificity of RIF and INH detection. Two articles reported the sensitivity and specificity of MDR TB detection. Higher sensitivity and specificity was observed for RIF and INH detection such as 95.61% and 96.25 % for RIF resistance, 88.70% and 97.59% for INH respectively. Raizada et al from India has also reported high sensitivity and specificity for RIF and INH detection which similar to our findings⁽⁸⁾.

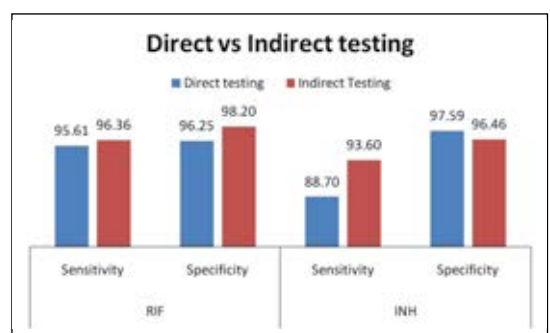


Figure 2 Sensitivity of LPA for indirect testing

On the other hand when sensitivity of RIF resistance detection was compared with the INH found that sensitivity of INH detection was less. The overall sensitivity for RIF detection was 95.79% and for INH resistance detection it was 89.85 % only. Similarly Singhal et al⁽⁹⁾, Ninan et al from India⁽¹⁰⁾ and Maschmann et al from Brazil⁽¹¹⁾ have reported less sensitivity and secificity for INH detection in their study.

According to Barnard et al in 2008 the low sensitivity range in the detection of INH resistance is due to the mutations being detected in a wide range of genetic loci compared to RIF⁽¹²⁾. In addition Meaza et al in 2017 have stated that nearly 10-25 % of INH resistant strains have mutations outside kat G and inh A regions⁽¹³⁾.

As shown in figure 2 sensitivity of LPA for indirect testing was higher for both RIF and INH detection when compared to direct testing and no studies performed LPA testing on specimens and culture isolates from the same patients. This might be due to the increased bacillary load when using culture isolates for LPA compared to direct samples in the studies included in this review. This study finding was in contrast with the finding from Nathavitharana et al in 2016 where they have reported less sensitivity and high specificity in indirect testing compared to direct testing⁽¹⁴⁾.

There was no significant observation on smear data in this review. However our review demonstrated that assay performed well in smear positive samples as invalids from the included studies were mainly smear negative specimens which was similar to the findings of Meaza et al in 2017⁽¹³⁾, Yadhav et al in 2013⁽¹⁵⁾ and Ahmed et al in 2017⁽¹⁶⁾. Binit Kumar Singh et al in 2017 has reported high sensitivity and specificity for RIF and INH resistance detection in LPA on sputum smear negative pulmonary TB cases in his study⁽¹⁷⁾. Further studies are needed which compares the accuracy of LPA on smear negative with smear positive samples.

We performed a comprehensive search of articles through different databases. Review of the articles was done independently. The quality review of the studies and disagreements were sorted out with discussions. This review was limited by small numbers of available studies on first line LPA published only in India. Most of the studies from India focussed on the mutations associated with the RIF and INH drug resistant TB. We can foresee more publications towards the clinical impact of LPA like patient management and treatment outcomes and how much LPA has been contributed in the rapid diagnosis over conventional tests.

CONCLUSION

We have observed excellent accuracy for RIF resistance detection through first line LPA. As RIF resistance is the surrogate marker for MDR TB, LPA can serve as a good diagnostic tool for MDR TB detection in high TB burden countries like India. As the sensitivity detection for INH was less across the studies, it could be improved further in future

generation assays. Our finding supports the use of LPA especially on smear positive specimens but use on smear negative specimens still be considered as studies have shown some interpretable results. With good microbiological laboratory practices there is a high chance of improving the quality of testing with minimal invalids or indeterminate results as they are the mainly due to the mistakes during setup or performance of the amplification reaction or presence of amplification inhibitors.

CONFLICT OF INTEREST

None

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COMPARISON OF CD4 AND CD8 COUNTS IN HIV NEGATIVE PULMONARY TUBERCULOSIS PATIENTS WITH NORMAL HEALTHY CONTROLS IN AND AROUND PRAYAGRAJ

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ABSTRACT

Anti-tuberculosis immunity involves a cellular immune response for their control. A critical marker of immunologic integrity is the CD4 and CD8 cell counts. Tuberculosis may be a cause of non-HIV associated CD4 and CD8-T cell lymphopenia. This study compares mean CD4, CD8 cell count and CD4:CD8 Ratio in pulmonary tuberculosis patients never had treatment for tuberculosis, pulmonary tuberculosis patients had received anti-tuberculosis treatment for more than one month and normal healthy controls. A case control study done in Prayagraj from October 2019 to October 2020 includes HIV negative, sputum positive pulmonary tuberculosis patients never had treatment for tuberculosis (n=25), pulmonary tuberculosis patients had received anti-tuberculosis treatment for more than one month (n=24), and normal healthy controls (n=36). We collected details including age, sex, symptoms of pulmonary tuberculosis, anti-tuberculosis treatment and investigated for HIV testing by ELISA, Sputum for AFB, Sputum for CBNAAT, CD4 and CD8 cell count determined by flow cytometrically.

The mean CD4 and CD8 cell count was significantly lower in HIV negative pulmonary tuberculosis patients never had treatment for tuberculosis than in normal healthy controls (p value<0.001) and CD4:CD8 ratio also lower (p value=0.013). The mean CD4 and CD8 cell count higher in pulmonary tuberculosis patients who have received anti-tuberculosis treatment for more than one month than in pulmonary tuberculosis patients never had treatment for tuberculosis (p value<0.001) and CD4:CD8 ratio also higher (p value=0.013). CD4 and CD8 lymphopenia is an acceptable phenomenon in HIV negative pulmonary tuberculosis patients and such lymphopenia improves with anti-tuberculosis drug regimens as per protocol. This study highlights the importance of CD4, CD8 cellular immune response conducted by T- lymphocytes in outcome of pulmonary tuberculosis.

Key Words: Pulmonary tuberculosis, CD4 count, ELISA, CBNAAT

INTRODUCTION

Tuberculosis has been a major cause of suffering and deaths since ancient times. The history of

tuberculosis is old as the mankind.¹ It is one of the top 10 causes of death and the leading cause from a single infectious agent (Mycobacterium tuberculosis), ranking above HIV/AIDS. The disease can affect anyone, anywhere, but most people who develop tuberculosis (about 90%) are adults, the male: female ratio is 2:1. Almost 90 % of cases each year are in 30 high tuberculosis burden countries.² An estimated 10.0 million (range 9.0-11.1 million) people fell ill with tuberculosis in 2018,

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a number that has been relatively stable in recent years.² In 2018, India was able to achieve a total notification of 21.5 lakh tuberculosis cases of which 25% was from the private sector.³ Uttar Pradesh, with 17% of population of the country, is the largest contributor to the tuberculosis cases in with 20% of the total notifications, accounting to about 4.2 lakh cases (187 cases per lakh population).³

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* (MTB). Rising trend in HIV infection in some countries together with the emergence of multi-drug resistant (MDR) strains of tuberculosis pose an additional threat.⁴

Anti-tuberculosis immunity involves innate as well as adaptive immunity at various levels following *Mycobacterium tuberculosis* infection. Anti-tuberculosis immunity involves a cellular immune response for their control. A critical marker of immunologic integrity is the CD4 and CD8 cell counts. CD4+T helper lymphocytes play a central role in regulation of immune response.⁵ They have capacity to help B cells for generating antibodies, to recruit and activate macrophages, to recruit neutrophils, eosinophils and basophils to sites of infection and inflammation.⁶ Patients with tuberculosis manifest significant immunologic abnormalities, including anergy and failure of T lymphocytes to proliferate and produce INF- γ in response to mycobacterial antigens.⁷

A critical marker of immunologic integrity is the CD4 cell counts. It has been shown that CD4+ T-lymphocytes are most important in protective response against mycobacterium tuberculosis. In murine studies, T-cell deficiency was associated with increased susceptibility to disease.⁽⁸⁻¹⁰⁾ CD8+ T-lymphocytes are also important for effective T-cell immune response against *Mycobacterium tuberculosis* infection, forming a cuff at the periphery of epithelioid cell granulomas.¹¹ They are capable of secreting cytokines such as INF- γ and IL-4 and thus may play a role in regulating the balance of Th-1 and Th-2 cells in the lungs of patients with pulmonary tuberculosis.⁸

The present study was carried out in department of pulmonary medicine Swaroop Rani Nehru Hospital Prayagraj to look at the occurrence of CD4 and CD8 cell lymphopenia in HIV negative pulmonary tuberculosis patients and compared to normal healthy controls.

METHODOLOGY

The present study was conducted in Department of Pulmonary Medicine Swaroop Rani Nehru Hospital Prayagraj Uttar Pradesh India from October 2019 to October 2020. It was a case control study. Institutional ethics committee MLN Medical College Prayagraj grant the permission prior to the start of the study, meeting held on 05-10-2019.

The following groups of subjects were included in the study after a written informed consent.

Case-Previous ATT-: A total of 25 patients with negative serology for HIV and sputum positive pulmonary tuberculosis who never had treatment for tuberculosis were registered in our study.

Case-Previous ATT+: A total of 24 patients with negative serology for HIV and sputum positive pulmonary tuberculosis had received anti-tuberculosis treatment for more than one month.

Control: A total of 36 normal healthy controls of ethnically matched age, sex who had never been treated for any form of tuberculosis and negative serology for HIV.

All subjects were enrolled in this study as per inclusion and exclusion criteria and detailed history was recorded and routine investigations were done.

All subjects were investigated for following parameters:

1. HIV testing ELISA
2. Sputum smear for AFB
3. Sputum for CBNAAT
4. CD4 cell count by flow cytometer
5. CD8 cell count by flow cytometer

And ratio of CD4 and CD8 also estimated and recorded accordingly.

Inclusion Criteria:

- Subject with negative serology for HIV.
- Pulmonary tuberculosis case confirmed by sputum smear microscopy positive for Acid Fast Bacillus (AFB) or pulmonary tuberculosis case confirmed by sputum for CBNAAT (Cartridge Based Nucleic Acid Amplification test).
- Apparently healthy control with sputum for AFB

negative.

- Age Greater than 18 year.
- Patient / guardian giving informed consent.

Exclusion Criteria:

Patients having any of the following conditions were excluded from the study:

- Consent not given by patient / guardian.
- Seropositive for HIV.
- Chronic illness like diabetes, chronic liver disease, chronic kidney disease or any other comorbid condition.
- History of alcohol intake.
- History of smoking, tobacco or other addiction.
- Patients on steroid or cytotoxic drugs.
- Concurrent use of immunosuppressant.
- Other immunocompromised patients.
- Age less than 18 years.

HIV testing by ELISA:

2-3 ml blood collected aseptically in a clean sterile tube for HIV testing after counselling and obtaining due informed consent. In MLN Medical College the collected sample was tested for HIV antibody (HIV1/HIV2) using rapid diagnostic kit following the NACO, India guidelines.

Sputum smear for Acid Fast Bacillus (AFB):

Two sputum samples were collected in sterile leak proof, disposable, appropriately labeled containers without any fixative as per RNTCP guidelines. In MLN Medical College the collected specimens were subjected for demonstration for Acid Fast Bacilli (AFB) using fluorescent microscopy employing Auramine O stain and grading was done accordingly. The sputum smear microscopy has a sensitivity of 64% and specificity of 98%.

Sputum for CBNAAT:

In MLN Medical College sputum for CBNAAT is a new diagnostic test cartridge based nucleic acid amplification test was done. CBNAAT was rapid, fully automated based on polymerase chain reaction (PCR) that detects deoxyribonucleic acid (DNA) directly from the clinical specimen and also detects rifampicin resistance. This diagnostic test was designed to purify, concentrate, amplify and identify targeted *rpoB* nucleic acid sequences and delivered the result in about 2 hours.

Measurement of CD4 and CD8 T-cell count determination by flow cytometer:

CD4 and CD8 level were measured by taking blood sample in EDTA vacutainers and processed on a flow cytometer using true count tubes with beads and tri test (CD3, CD4, CD8 Cocktail) antibody following a lyse-no-wash protocol. CD4 cell count of 381-1170 cells/ μ l, CD8 cell count of 108-845 cells/ μ l, and CD4: CD8 Ratio of 0.55 to 3.03 were considered to be normal range as per manufacturer's instructions.

Analysis of Data:

Data were coded and recorded in Microsoft excel spreadsheet program. SPSS v23 (IBM Corp.) was used for data analysis. Descriptive statistics were elaborated in the form of means/standard deviations and medians/ IQRs for continuous variables and frequencies and percentages for categorical variables. Data were presented in a graphical manner wherever appropriate for data visualization using histograms/box and whisker plots/column charts for continuous data and bar charts/pie charts for categorical data. Group comparisons for continuously distributed data were made using independent sample 't' test when comparing two groups. If data were found to be non-normally distributed, appropriate non-parametric test in the form of Wilcoxon test were used. Chi-squared test was used for group comparisons for categorical data. In case the expected frequency in the contingency tables was found to be <5 or >25% of the cells, Fisher's Exact test was used instead. Linear correlation between two continuous variables was explored using Pearson's correlation (if the data were normally distributed) and Spearman's correlation (for non-normally distributed data). Statistical significance was kept at $p < 0.05$.

RESULTS

The mean age of Case Previous ATT- group was 37.48 (± 14.54) years and mean age of Case Previous ATT+ group was 29.08 (± 12.18) years and mean age of Control group was 32.86 (± 9.73) years.

18 males (72%) and 7 females (28%) in Case Previous ATT- group, 15 males (62.5%) and 9 females (37.5%) in Case Previous ATT+ group, 23 males (63.9%) and 13 females (36.1%) in Control

CD4 Count (cells/ μ l)	Subgroup			Kruskal Wallis Test	
	Case-Previous ATT-	Case-Previous ATT+	Control	χ^2	p value
Mean (SD)	375.20 (\pm 115.84)	567.71 (\pm 191.63)	748.89 (\pm 176.61)	43.148	<0.001
Median (IQR)	380 (304-437)	538.5 (434.75-612.25)	767.5 (646.5-873)		
Range	80 – 595	298 – 983	364 - 1200		
Pairwise Comparison of Subcategories of Subgroup				Adjusted P Value	
Case-Previous ATT- - Case-Previous ATT+				0.003	
Case-Previous ATT- - Control				<0.001	
Case-Previous ATT+ - Control				0.009	

group.

Case- Previous ATT-: Never had treatment for tuberculosis.

Case- Previous ATT+: Received anti-tuberculosis treatment for more than one month.

Control: Normal healthy controls.

The variable CD4 Count was not normally distributed in the 3 subgroups of the variable Subgroup. Thus, non-parametric test (Kruskal Wallis Test) was used to make group comparisons.

The mean (SD) of CD4 Count in the Case-Previous ATT- group was lower than the mean (SD) of CD4 Count in the Case-Previous ATT+ group. The mean (SD) of CD4 Count in the Case-Previous ATT+ group was lower than the mean (SD) of CD4 Count in the Control group. The median (IQR) of CD4 Count in the Case-Previous ATT- group was lower than the median (IQR) of CD4 Count in the Case-Previous ATT+ group. The median (IQR) of CD4 Count in the Case-Previous ATT+ group was lower than the median (IQR) of CD4 Count in the Control group. The ranged CD4 Count in the Case-Previous ATT- group was lower than the ranged

CD4 Count in the Case-Previous ATT+ group. The ranged CD4 Count in the Case-Previous ATT+ group was lower than the ranged CD4 Count in the Control group.

There was a significant difference between the 3 groups in terms of CD4 Count ($\chi^2 = 43.148$, $p = <0.001$), with the median CD4 Count being highest in the Control group. **Case-Previous ATT-: Never had treatment for tuberculosis.**

Case-Previous ATT+: Received anti-tuberculosis treatment for more than one month.

Control: Normal healthy controls.

The variable CD8 Count was not normally distributed in the 3 subgroups of the variable Subgroup. Thus, non-parametric test (Kruskal Wallis Test) was used to make group comparisons.

The mean (SD) of CD8 Count in the Case-Previous ATT- group was lower than the mean (SD) of CD8 Count in the Case-Previous ATT+ group. The mean (SD) of CD8 Count in the Case-Previous ATT+ group was lower than the mean (SD) of CD8 Count in the Control group. The median (IQR) of

CD8 Count (cells/ μ l)	Subgroup			Kruskal Wallis Test	
	Case-Previous ATT-	Case-Previous ATT+	Control	χ^2	p value
Mean (SD)	337.96 (\pm 119.61)	407.08 (\pm 217.53)	555.22 (\pm 157.57)	26.003	<0.001
Median (IQR)	320 (279-392)	335.5 (267.25-469.5)	558.5 (426.75-630.75)		
Range	141 - 637	176 - 1170	209 - 891		
Pairwise Comparison of Subcategories of Subgroup				Adjusted P Value	
Case-Previous ATT- - Case-Previous ATT+				0.661	
Case-Previous ATT- - Control				<0.001	
Case-Previous ATT+ - Control				<0.001	

CD4:CD8 Ratio	Subgroup			Kruskal Wallis Test	
	Case-Previous ATT-	Case-Previous ATT+	Control	χ^2	p value
Mean (SD)	1.17 (\pm 0.39)	1.62 (\pm 0.68)	1.43 (\pm 0.52)	8.756	0.013
Median (IQR)	1.09 (0.94-1.46)	1.71 (1.09-1.99)	1.24 (1.2-1.54)		
Range	0.57 - 2.06	0.61 - 3.26	0.98 - 3.37		
Pairwise Comparison of Subcategories of Subgroup				Adjusted P Value	
Case-Previous ATT- - Case-Previous ATT+				0.011	
Case-Previous ATT- - Control				0.111	
Case-Previous ATT+ - Control				0.617	

CD8 Count in the Case-Previous ATT- group was lower than the median (IQR) of CD8 Count in the Case-Previous ATT+ group. The median (IQR) of CD8 Count in the Case-Previous ATT+ group was lower than the median (IQR) of CD8 Count in the Control group. The ranged CD8 Count in the Case-Previous ATT- group was lower than the ranged CD8 Count in the Case-Previous ATT+ group. The ranged CD8 Count in the Case-Previous ATT+ group was nearly same the ranged CD8 Count in the Control group.

There was a significant difference between the 3 groups in terms of CD8 Count ($\chi^2 = 26.003$, $p = <0.001$), with the median CD8 Count being highest in the Control group.

Case-Previous ATT-: Never had treatment for tuberculosis.

Case-Previous ATT+: Received anti-tuberculosis treatment for more than one month.

Control: Normal healthy controls.

The variable CD4:CD8 Ratio was not normally distributed in the 3 subgroups of the variable Subgroup. Thus, non-parametric test (Kruskal Wallis Test) was used to make group comparisons.

The mean (SD) of CD4:CD8 Ratio in the Case-Previous ATT- group was lower than the mean (SD) of CD4:CD8 Ratio in the Case-Previous ATT+ group. The mean (SD) of CD4:CD8 Ratio in the Control group was lower than the mean (SD) of CD4:CD8 Ratio in the Case-Previous ATT+ group. The median (IQR) of CD4:CD8 Ratio in the Case-Previous ATT- group was lower than the median (IQR) of CD4:CD8 Ratio in the Case-Previous ATT+ group. The median (IQR) of CD4:CD8 Ratio in the Control group was lower than the median (IQR) of CD4:CD8 Ratio in the Case-Previous ATT+ group.

The ranged CD4:CD8 Ratio in the Case-Previous ATT- group was lower than the ranged CD4:CD8 Ratio in the Case-Previous ATT+ group. The ranged CD4:CD8 Ratio in the Case-Previous ATT+ group was lower than the ranged CD4:CD8 Ratio in the Control group.

There was a significant difference between the 3 groups in terms of CD4:CD8 Ratio ($\chi^2 = 8.756$, $p = 0.013$), with the median CD4:CD8 Ratio being highest in the Case-Previous ATT+ group.

DISCUSSION

The present study was conducted in Department of Pulmonary Medicine, Swaroop Rani Nehru Hospital Prayagraj from October 2019 to October 2020. A total of 85 candidates were selected for the study who fulfilled the inclusion and exclusion criteria and gave consent for the study. Among 85 candidates, 49 were HIV negative pulmonary tuberculosis patients (sputum smear microscopy positive for AFB or sputum for CBNAAT) and 36 were normal healthy controls. Of these 49 HIV negative pulmonary tuberculosis patients, 25 pulmonary tuberculosis patients never had treatment for tuberculosis and 24 pulmonary tuberculosis patients had received anti-tuberculosis treatment for more than one month. There were no significant difference between mean age (p value=0.637) and sex distribution (p value=0.74) in HIV negative pulmonary tuberculosis patients and in normal healthy controls. The CD4 cell count and CD8 cell count of the respective sample were recorded in all the candidates. The ratio of CD4 and CD8 also estimated and recorded accordingly.

Comparison of the Variable subgroups in terms of CD4 count (n=85)

In our study we found CD4 cell count was lower in HIV negative pulmonary tuberculosis patients than in normal healthy controls. Similarly, **Uppal**

et al ⁽¹²⁾, **Sabhapandit et al** ⁽¹³⁾ and **Siraj et al** ⁽¹⁴⁾ also found CD4 cell count was lower in patients of pulmonary tuberculosis compared to healthy controls. In our study we also found that mean CD4 cell count was lower in pulmonary tuberculosis patients who never had treatment for tuberculosis than in pulmonary tuberculosis patients who have received anti-tuberculosis treatment for more than one month.

From our study and previous studies this can be predicted that pulmonary tuberculosis is responsible for CD4-T Cell lymphopenia.

Comparison of the Variable subgroups in terms of CD8 count (n=85)

In our study we found CD8 cell count was lower in HIV negative pulmonary tuberculosis patients than in normal healthy controls. Similarly, **Sabhapandit et al** ⁽¹³⁾ and **Siraj et al** ⁽¹⁴⁾ also found CD8 cell count was lower in patients of pulmonary tuberculosis compared to healthy controls. In our study we also found that mean CD8 cell count was lower in pulmonary tuberculosis patients who never had treatment for tuberculosis than in pulmonary tuberculosis patients who have received anti-tuberculosis treatment for more than one month.

From our study and previous studies this can be predicted that pulmonary tuberculosis is responsible for CD8-T Cell lymphopenia.

Comparison of the Variable subgroups in terms of CD4: CD8 Ratio (n=85)

In our study we found CD4:CD8 Ratio was lower in HIV negative pulmonary tuberculosis patients than in normal healthy controls. Similarly, **Uppal et al** ⁽¹²⁾ and **Siraj et al** ⁽¹⁴⁾ also found CD4:CD8 Ratio was lower in patients of pulmonary tuberculosis compared to healthy controls. This finding is contrary to that observed by **Sabhapandit et al** ⁽¹³⁾ In our study we also found that mean CD4:CD8 Ratio was lower in pulmonary tuberculosis patients who never had treatment for tuberculosis than in pulmonary tuberculosis patients who have received anti-tuberculosis treatment for more than one month. From our study and previous studies this can be predicted that pulmonary tuberculosis may be responsible for change in CD4:CD8 Ratio.

CONCLUSION

In conclusion from our study found significantly lower mean CD4 and CD8 cell count and CD4:CD8 ratio among HIV negative pulmonary tuberculosis patients compared with normal healthy controls. We also found lower mean CD4 and CD8 cell count and CD4:CD8 ratio in pulmonary tuberculosis patients who never had treatment for tuberculosis than in pulmonary tuberculosis patients who have received anti-tuberculosis treatment for more than one month. According to our study results patients of pulmonary tuberculosis had lower number of both CD4 and CD8 cell count than normal healthy controls which can be a sign of suppressed cellular immunity in pulmonary tuberculosis patients. This study highlights the importance of cellular immunity, conducted by T-lymphocytes in outcome of pulmonary tuberculosis.

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CONFLICT OF INTEREST

None

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MILIARY TUBERCULOSIS: A DESCRIPTIVE STUDY IN WEST GUJARAT

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ABSTRACT

Introduction: Almost 10 million patients suffer from tuberculosis all over the world. Military tuberculosis accounts for hardly 1-2 % of total tuberculosis cases but is usually fatal if left untreated. With global epidemic of human immunodeficiency virus (HIV) infection chances of developing tuberculosis disease has also increased. In last few decades MTB has shifted to young adults and elderly who are the most productive members of the society.

Fifteen adults were diagnosed to have military tuberculosis during last one year and their data were analysed. Persons living with HIV(PLHIV) accounted for 46% of all cases of military TB. Twelve out of 15 were males. None had meningeal involvement. There was no mortality during hospital stay or follow up till date.

High index of suspicion in classical clinical settings and early institution of anti-TB treatment can save lives of patients. Sputum for CBNAAT is very helpful in reaching the diagnosis.

Key Words: Military tuberculosis, HIV, CBNAAT, HRCT, Choroid tubercle

INTRODUCTION

Tuberculosis (TB) has existed for millennia and remains a major global health problem. It causes ill-health for approximately 10 million people each year and is one of the top ten causes of death worldwide. For the past 5 years, it has been the leading cause of death from a single infectious agent, ranking above HIV/AIDS (WHO). One-third of the world population is estimated to be infected with *Mycobacterium tuberculosis* (latent TB infection) and 10% of these individuals will develop active TB in their lifetime ⁽¹⁾.

Military TB is a fatal form of disseminated TB that results from a massive lymphohematogeneous dissemination from a *Mycobacterium tuberculosis* laden focus⁽³⁾. Radiologically, the military pattern has been defined as “a collection of tiny discrete pulmonary opacities that are generally uniform in size and widespread in distribution, each of which measures 2 mm or less in diameter” ⁽⁴⁾.

In various clinical studies among immunocompetent adults, military TB accounts for less than 2% of all cases of TB and up to 20% of all extra-pulmonary TB (EPTB) cases ⁽⁵⁾. In late HIV infection, EPTB accounts for more than 50% of all cases of TB and military TB is more frequently encountered. Before the advent of anti-TB treatment, military TB was predominantly seen in infants and children ⁽⁶⁾. But after 1980s a changing epidemiological trend has been observed and military TB is increasingly diagnosed in adults also. Two peaks are evident, one involving adolescents, young adults and

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another later in life among elderly individuals⁽⁷⁾. Miliary, or disseminated, tuberculosis occurs with greater frequency among aging patients; many cases are detected only at autopsy⁽⁸⁾. In both pediatric as well as adult patients, male gender is more frequently affected by military TB⁽²⁾.

On physical examination, choroidal tubercles in the eye are considered pathognomonic for miliary TB⁽⁹⁾. The rate of choroidal tubercles was reported between 2% and 21% in miliary TB series. In study conducted by Mert A et al⁽¹⁰⁾, this figure was found to be 8%. Choroidal tubercles are reported to be less frequent in adults than autopsy probably because of not performing a routine eye examination and not using midriatics during this examination. Previous studies reported an underlying disorder making the host vulnerable to miliary TB (HIV infection, collagen-vascular disorder, diabetes mellitus, neoplasm, chronic renal failure, pregnancy, steroid use, and alcoholism) in nearly half (30%–66%) of the cases. The mortality rate is 25% (14%–30%), and seemed to remain unchanged during last 25 years⁽³⁾.

Hyponatraemia and an elevated ALT on admission were associated with an unfavourable clinical course. The causes of hyponatraemia in those with miliary TB are numerous, but are likely to include brain injury as well as adrenal and pituitary dysfunction⁽¹¹⁾.

Miliary TB still remains a treatable cause of death which is more true in HIV+ve patients. Having two very busy big ports in Kachchh district, with more migrant population, we expected to have more cases of HIV and TB. It generated an interest to look into this dual problem so we analysed military TB cases retrospectively.

METHODOLOGY

This was a descriptive study conducted at GAIMS, Bhuj. Adult patients admitted and diagnosed as miliary tuberculosis during Jan 2019 to Dec. 2019 were included in this study. Patients were diagnosed on the basis of clinical presentation, radiological and /or microbiological basis. All the patients were subjected to CXR, HRCT, sputum for AFB and CBNAAT, routine hemogram, LFT, KFT, S. electrolytes, HIV testing, RBS and urine

examination and their results were analysed. Since it was a retrospective study, consent from the patients was not taken. However adherence to the guidelines of the Declaration of Helsinki was always kept.

RESULTS

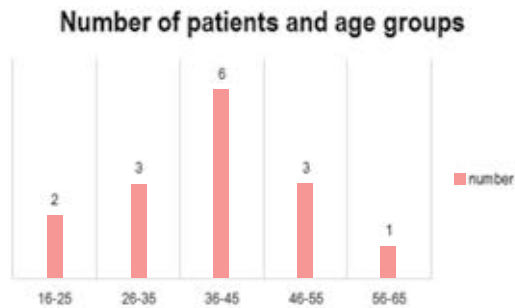
In total 15 adult patients were analysed who were diagnosed as miliary TB on clinical, radiological (CXR and HRCT) and /or bacteriological basis. During the same period a total of 470 adult patients were diagnosed with all forms of tuberculosis in Kachchh district of Gujarat bringing the incidence of miliary tuberculosis to 3.12%. During this period of time 380 patients were diagnosed as new cases of HIV. Thirty-seven patients had HIV-TB coinfection meaning thereby 10.3% of HIV patients had some form of tuberculosis and 7 of them had miliary tuberculosis (18.9%, which is very high in comparison to immunocompetent adults who had 8/433 or 1.84%). The median age of patients of miliary tuberculosis patients was 40 yrs. Twelve of 15 patients were in age group of 26 to 45 yrs, which shows a dramatic shift in age-prevalence of miliary TB. There were 3 females and 12 males. Out of 15 patients 7 were HIV positive and another one had diabetes mellitus. Thirteen patients were anemic (86.6%). Ten out of 15 patients had hyponatremia (67%) that is less than 135 mEq of Na+. Only 2 patients had choroid tubercle (2/15) on fundus examination, one was HIV+ve and the other HIV-ve.

Sputum examination for CBNAAT was positive in 8 patients (4 HIV+ve and 4 HIV -ve). One patient was a contact case of MDRTB and his sputum for CBNAAT also showed rifampicin resistance. One patient who was HIV+ve also had intracranial tuberculoma. Another HIV+ve patient was reactive to syphilis also. One HIV+ve patient had pleural effusion and another HIV+ve had cervical lymphadenopathy.

One female patient of 30 yrs age with no immunocompromised status had abdominal TB, Potts spine and psoas abscess along with miliary TB signifying some other undiagnosed immune deficiency. No patient had tubercular meningitis and only one had tuberculoma in brain who was HIV+ve.

All patients had BCG scar.

All patients were given anti-tubercular treatment according to NTCP guidelines and there was no mortality either in ward or during follow-up till date.



DISCUSSION

Incidence of military TB among patients of all forms of TB was 3.12% probably due to high number of HIV+ve patients in our series. If we exclude HIV+ve patients it comes to 1.84 % which is in accordance with other reports. As with other reports⁽¹²⁾ we also noticed male predominance. The changing age prevalence of the disease was also noticed in our study^(3,8).

Forty-six percent of the patients were HIV+ve and all of them had CD4 count <200 with mean being 80. If we include diabetic also 53.3% patients were in immunocompromised category.

The presence of choroid tubercle in 2 (13.3%) patients was similar to other studies⁽¹⁴⁾.

In a large case series of 263 patients of military TB from various centres in Turkey, Mert A, et al⁽¹⁰⁾ found anemia in 86.3 % of patients which was almost similar to our study (86.6%). They also found lymphopenia in 48% and we found it in 40% of our patients. Anemia was very common in Saudi Arabian series also. Al-Zahdali et al⁽¹³⁾ found anemia in 66% and lymphopenia in 89% of cases.

The presence of sputum CBNAAT positivity in our series was very significant (53.3%) and it should be the first investigation. The presence of one MDR military patient signifies importance of history taking and performing sputum CBNAAT.

Jonathan U et al⁽¹¹⁾ found significant correlation between mortality and hyponatremia and raised ALT. Although two-thirds of our patients had hyponatremia but only 3 had Na+ <125 mEq. and none of them died.

Han Y et al⁽¹⁴⁾ found that pre-treatment Neutrophil-Lymphocyte Ratio (NLR) at admission may be a useful biomarker for mortality and development of Acute Respiratory Distress Syndrome (ARDS) in patients with military TB but we could not find this correlation. Our patients had a highly varied NLR ranging from 0.93 to 30 and none of them developed ARDS or died of military TB.

None of our patients had tubercular meningitis (TBM). Only one had brain tuberculoma who was HIV+ve. Tanrikulu C et al⁽¹⁵⁾ found TBM in one fourth of their patients.

No mortality was reported either during hospital stay or follow-up, probably because anti-tubercular treatment was started early, even on CXR evidence of military pattern. Maartens G et al⁽¹⁶⁾ noted 24% mortality and one of the causes of this was treatment delay. Further, they also found lymphopenia to be very common (in 87%).

CONCLUSION

In endemic country (of TB) like India, military TB should be a high consideration in all cases of prolonged fever, which is more true among HIV+ve patients. Besides radiology, a simple test sputum for CBNAAT, available now everywhere, should be recommended at the earliest as it is positive in more than half of the patients. Timely diagnosis with high index of suspicion and early start of anti-tubercular treatment can save the lives of patients who otherwise would definitely succumb to the disease and this was the single most important conclusion of our study.

CONFLICT OF INTEREST

None

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IMPACT OF COVID-19 PANDEMIC ON TB CONTROL PROGRAMME IN NEPAL: AN OVERVIEW

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ABSTRACT

COVID-19 pandemic is affecting worldwide. TB and COVID-19 both are infectious and primarily affect the lungs and show similar symptoms thus, it is essential to diagnose cases. Accurate diagnostic tests are essential for both TB and COVID-19. COVID-19 has reversed minimum five years of progress in fighting TB. The study is based on review of all available reports, literature, published studies in peer reviewed journal related to COVID-19 pandemic impact on TB control program in Nepal. PubMed, Google scholar, HINARI are the major search engines and or databases used for the review. COVID-19 led lockdown had a significant impact on TB services in Nepal. There is decline in the identification of new cases and had impacted on sputum courier, enrolment, diagnosis and follow up. People centered delivery of TB prevention, diagnosis, treatment and care services should be ensured in tandem with the COVID-19 response. Health authorities should maintain support to essential TB services during emergencies.

Key Words: COVID-19, Pandemic, Tuberculosis, Nepal

INTRODUCTION

Tuberculosis is an infectious disease, caused by *Mycobacterium tuberculosis* and spread through the air via respiratory droplets where, COVID-19 caused by SARS-CoV-2 virus. Both are infectious and primarily affect the lungs and show similar symptoms such as cough, fever and difficulty in breathing which might perplex peoples. Tuberculosis is a contagious disease with an estimated 10 million active TB cases with almost 4,000 daily deaths globally⁽¹⁾. A quarter of the world's population has latent TB infection⁽²⁾. Treatment coverage and success rate are on going challenges for low- and middle-income countries^(3,4). TB care and prevention programs

specially in high burden countries are facing disruption to their routine services⁽⁵⁾. TB remains a public health challenge in Nepal, with an estimated 69,000 new cases per year and more than 50% of cases estimated being missed⁽⁶⁾.

COVID-19 pandemic (SARS-CoV-2) was first detected in the Hubei province of China in December 2019 which took less than a week to reach Nepal; the first case was detected on 23rd January, 2020. We had no inkling of the crisis that awaited us where a number of cases began to exaggerate and upset the public health system thus, government of Nepal declared public health emergency⁽⁷⁾. The COVID-19 pandemic has disturbed the delivery of health care and placed unprecedented demands and pressure on the health system⁽⁸⁾.

Focusing on the COVID-19 pandemic, we are ignoring the potential impact of endemic disease to human health which might be more devastating than COVID-19^(4,8). COVID-19 pandemic is having a serious impact and threatens progress on TB

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control program. Due to the pandemic, there is decline in TB cases diagnosis. The signs and symptoms of TB and COVID-19 are similar and both primarily infect the lungs. All the focus is set to COVID-19 and service provision to tuberculosis patients is overshadowed. According to National TB prevalence survey, the burden of TB in Nepal is significantly larger; the preliminary results from the survey suggest the burden of TB is two thirds greater than previously estimated, which increases the number of “missing cases” significantly. As, we already have missing cases which need to be identified, the COVID-19 pandemic has brought disruption in the case diagnosis and notification and we are more far away to achieve END TB strategy⁽⁶⁾

METHODOLOGY

The study is based on review of all available reports, literature, published studies in peer reviewed journal related to COVID-19 pandemic impact on TB control program in Nepal. PubMed, google scholar, HINARI are the major search engines and or databases used for the review. Google was used to find various sources like NTP Nepal and WHO. From these websites, factual information was collected in addition to policy documents and guidelines. PubMed, google scholar, HINARI were used to find relevant abstracts of peer reviewed articles in different journals. The abstracts were further screened and if relevant, full articles were retrieved. Where problems arose with finding full articles, an additional search was done using the HINARI and research-gate search. The National Tuberculosis program of Nepal and WHO world TB report were the major sources for reviewing the pattern and trend of impact of COVID-19 pandemic on TB control program.

RESULT AND DISCUSSION

Globally, tuberculosis case detection decreases by an average 25% after COVID 19 pandemic. An estimated additional 190 000 TB deaths are predicted (13% increase) which bring total to 1.66 million TB deaths in 2020⁽⁹⁾.

COVID 19 has reversed minimum five years of progress in fighting TB⁽¹⁰⁾. Looking towards the global level of TB Mortality 2015, a serious setback in the progress towards the End TB strategy

milestones and targets need to be emphasized⁽⁹⁾. Despite global and national efforts to end TB and availability of cost-effective medicines to treat and cure, still too many people continue to suffer from TB⁽¹¹⁾. Different programs were implemented in different level to end TB by 2035 which includes active case finding screening camp, Childhood TB management , Sputum transportation, Contact investigation, Private Public Mix related activities, Preventive therapy for children, TB screening among migrant and prisoners and FAST strategy in major hospitals to decrease the risk and intensity of TB infection in OPD which were disturbed by COVID-19 pandemic⁽¹²⁾. Because of restrictions in movement, lock down, psychological fear of contacting the disease in health care facilities, diversion of health care workers for containment and management of COVID-19, utilization of diagnostic test for COVID has disrupted TB programme. Moreover, government focused and response to only COVID 19 and channelling most of financial and human resource to fight against pandemic has disrupted TB program⁽⁸⁾.

Case diagnosis: After the COVID-19 led nationwide lockdown, mean number of sputum collection for diagnosis of TB reduced by 67.3%⁽¹³⁾. Due to travel restrictions and fear of contracting COVID-19, about 26.9% of TB patients had postponed or missed going for their follow-up examinations⁽¹⁴⁾. Additionally, decreased incidence may be reported because of the under-diagnosis of TB⁽³⁾. GeneXpert technology was used for diagnosis of TB but in this COVID pandemic it has been used for COVID-19 testing which ultimately affected in the diagnosis of TB⁽¹⁵⁾.

Case notification: COVID-19 has resulted in an estimated 21% reduction in TB notification and 0.5 million additional TB deaths. Simply, we can say COVID-19 pandemic has erased all our gains and efforts made during last decade⁽¹⁶⁾. Tuberculosis programmes have contributed significantly to the COVID-19 response, as both diseases present with respiratory symptoms, and similar infrastructure, skills and expertise are needed to response COVID-19. Services were diverted to COVID 19, which affect program resources and TB services that led to decreased case notification drastically^(11,17).

COVID-19 pandemic has also adversely affected the TB case notification and follow up in China, India^(14,18,19). Study of different countries show that COVID-19 patients with concurrent TB have about three times higher mortality than those without TB⁽¹⁶⁾.

Looking towards the scenario of COVID-19 pandemic impact on TB, there was 45.5% reduction in TB case enrolment and 41.7% reduction in case follow-up which indicates difficulty to achieve End TB by 2035^(8,13).

In 2018, about 32474 TB cases were notified which slightly decreased to 32043 in 2019. In 2020, compared to 2019, there is decrease in TB cases by 13.41 %.⁽²⁰⁾ (Figure 1) TB Case notification was 109 per 100,000 in 2019 which slightly diminished to 93 per 100,000 in 2020. (Figure 2).

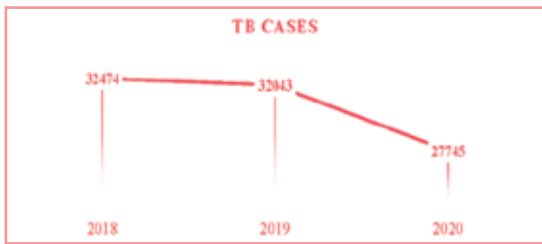


Figure 1 Comparison of TB cases between 2018-2020 COVID pandemic period, Nepal

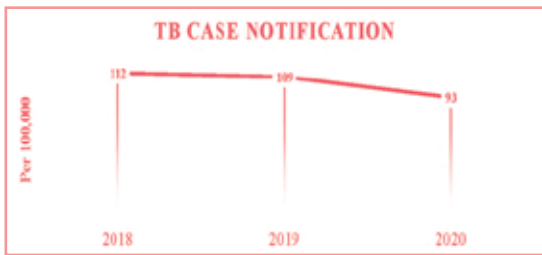


Figure 2 Comparison of TB case notification between 2018-2020 COVID pandemic period, Nepal

TB treatment compliance

COVID-19 pandemic has brought the challenges in drug procurement and supply of drugs. Medical supplies had been stalled due to lockdown and restrictions thus affecting TB patients in drug compliance. Peoples had developed fear of transmission of disease and hesitate to visit health centers for the medicine. The number of patients with registered TB as being on treatment over the month is decreased. TB treatment compliance rate had decreased by 1.41% from 2019 to 2020.

Treatment compliance can effect on tuberculosis program^(20,21).

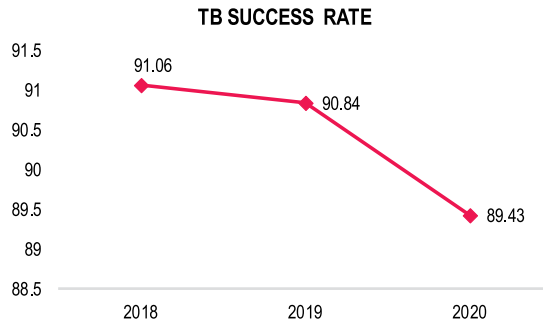


Figure 3 Comparison of TB success rate between 2018-2020 COVID pandemic period, Nepal

Supply of diagnostics kits and chemical

With the COVID 19 pandemic, the tuberculosis diagnostic test platforms such as GeneXperts were used to detect the COVID-19 causal agent, which increases stock outs of the diagnostics kits, chemical. Due to pandemic, bidding for the cartridges for diagnosis TB need to be repurposed and takes a longer time duration in process of purchasing^(8,11,17).

HR management

Tuberculosis programmes have contributed significantly to the COVID-19 response, as both diseases present with respiratory symptoms, and similar infrastructure, skills and expertise were utilized in COVID response. All the human resources of health and health facilities were distracted and assigned a wide variety of events related to controlling the outbreak. The situation of human resources for the tuberculosis program before the pandemic was under-resourced additionally, the existed human resources were also diverted to the COVID 19 response. Due to the scarcity of the health workers focusing on TB, all the services related to diagnosis, awareness, treatment compliance were affected^(16,17).

Nepal turns towards federalism where transitional phase to federalism had brought changes in the structure of health resources. All the experienced district and regional TB staff were transferred to new federal health structure which brought disturbance and discontinuity in the TB services. There was a disruption in the services due to the

transition phase of federalism. Additionally, the COVID -19 pandemic had added burden to TB program as all the services and human resources were diverted to COVID 19 response⁽²²⁻²³⁾.

CONCLUSION

Tuberculosis remains a global health emergency and needs our attention more than ever, considering the significant resources are now being diverted to COVID-19 management the signs and symptom of TB resembles with COVID-19 thus health workers must be provided with PPE set to avoid the hesitancy to provide TB service. All over the world there is lack of personal protective equipment which ultimately made barriers in service provision. There is an urgent need to increased support in PPE investment, personnel, supplies, tools as well as innovations in programming to offer quality digital and community-based care in TB program.

To minimize the impact of COVID-19 pandemic on TB and get back on track to achieve the END TB strategy by 2035, government need to take immediate measures for continuity of TB diagnostic, treatment and prevention services during restriction period. Within a short period of time, we must focus on the massive catch up activities in identifying missing cases through scaling up innovative approaches. Due to the pandemic there is high number of missing cases which is foremost important to address. All the missed cases should be catch up and offer with TB treatment. Moreover, involvement of private sectors is critical to end TB thus engaging and mobilizing in TB control program is crucial. Staff responsible to conduct all TB related activities in the local and provincial level need to be well trained.

Campaigns, active and intensified case finding, bi-directional screening/testing for people with symptoms of TB, using Gene xpert need to done. Community/home delivery of medicines, community engagement, multi-month drug dispensing, social protection for high-risk groups including nutritional and psychosocial support through community volunteer should be directed. COVID-19 has overshadowed TB, therefore, education programmes on prevention and treatment remains imperative to stop the spread of TB.

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CONFLICT OF INTEREST

None

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Case Study

HIGH FLOW NASAL CANNULA FOR MANAGEMENT OF TUBERCULOUS ARDS IN IMMUNOCOMPROMISED – A VISION OR AN ILLUSION?

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ABSTRACT

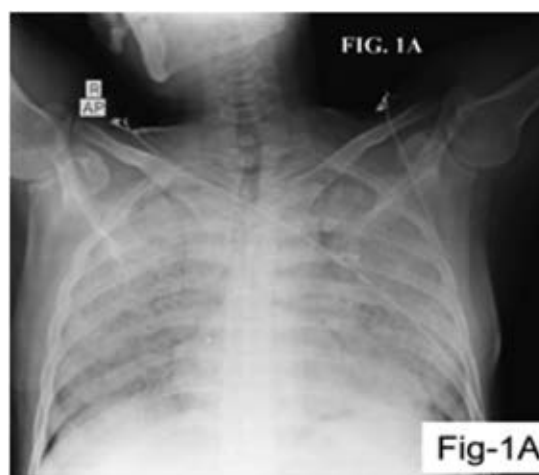
Tuberculosis (TB), although commonly thought to be a chronic pulmonary disease, indeed has protean manifestations. One of its varied acute presentations is Tuberculous ARDS, which is a rare but fatal form of TB with mortality reported as high as 69-80%¹. Here we report a case of sputum smear-positive MDR miliary tuberculosis with tuberculous ARDS in a patient with AIDS managed with HFNC oxygen therapy. Diagnosis of tuberculosis was based on clinical radiological, microbiological and molecular evidence^{2,3}. The diagnosis of ARDS was established as per Berlin definition⁴. The patient was successfully managed with HFNC oxygen therapy along with second line anti tubercular treatment (ATT) and supportive measures.

Key Words: HFNS oxygen therapy, ARDS, Tuberculosis, Immunocompromised

INTRODUCTION

A 34-year-old male, a known case of human immunodeficiency virus (HIV) infection on highly active antiretroviral therapy (HAART) - Tenofovir-300mg / Lamivudine-300mg / Efavirenz-600mg for past 17 months with history of poor compliance to HAART medication, initially presented to a tertiary care hospital with complaints of fever with exertional breathlessness of 1-week duration. Clinically, the patient was tachypnoeic with unremarkable systemic examination findings. On further evaluation, he was found to have polymorphonuclear predominant (79%) leucocytosis (TLC – 11,230/cumm), with raised erythrocyte sedimentation rate (48mm fall in 1st hour). His initial arterial blood gas revealed

respiratory alkalosis with widened alveolar-arterial oxygen gradient (A-a DO₂) (41.1 mmHg). He had mediastinal widening with a bilateral micronodular lesion on chest radiograph. (Fig. 1A).



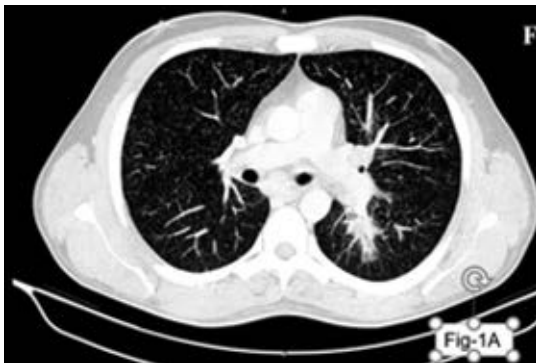
(Fig. 1A) Micronodular lesion on chest radiograph.

He underwent a High-resolution contrast enhanced computed tomography of chest which showed randomly distributed miliary nodular lesions in all segments of both lungs with patchy area of

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consolidation in the superior segment of left lower lobe and nodular lesion arranged in tree – in – bud pattern surrounding the consolidation. (Fig. 2).



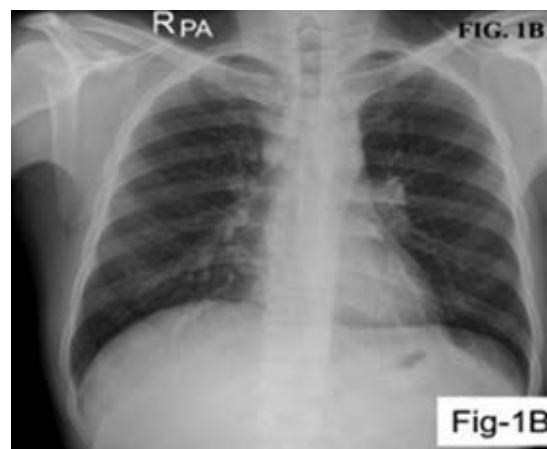
(Fig. 2) Bud pattern surrounding the consolidation.

The patient was provisionally diagnosed as a case of pneumocystis pneumonia and started on a therapeutic dosage of sulphamethoxazole / pyrimethamine and oral steroids. However, the patient continued to worsen clinically and was then transferred to our tertiary care respiratory center in western Maharashtra. At presentation to this hospital, the patient was found to be tachypnoeic and severely hypoxic (Arterial Blood Gas at FiO₂-45%, pH – 7.495, pCO₂ – 25.7 mmHg, pO₂ – 48.4 mmHg, HCO₃- 20 mmol/l, A-a DO₂ – 69. In accordance to Berlin's criteria, the patient was diagnosed as a case of moderate ARDS (PaO₂/FiO₂ -107.5 mmHg). on further evaluation he was found to be sputum smear positive for acid-fast bacilli (3+) and sputum cartridge based nucleic acid amplification test (CBNAAT) showed Mycobacterium tuberculosis (MTB) along with Rifampicin (Rif) resistance following this he was started on second line ATT as per weight band (Inj Amikacin, Tab Levofloxacin, Tab Ethionamide, Tab Cycloserine, Tab Pyrazinamide, Tab Ethambutol). He was commenced on high flow nasal cannula oxygen therapy, AIRVO-2 (Fisher & Paykel, Auckland, New Zealand) at a flow rate of 60L/min and FiO₂ – 80%.

Table-1 -ROX index at 2-hour, 6-hour and 12-hour showing patient's response to HFNC oxygen therapy.				
PARAMETERS →	SpO ₂	FiO ₂	Respiratory Rate	ROX Index
TIME ↓				
2-Hour	99%	60%	34/min	4.85

6-Hour	98%	55%	30/min	5.94
12-Hour	98%	50%	30/min	6.53

Patient was monitored closely as per ROX Index at 2-hour, 6-hour and 12-hour interval (Table 1) to assess the response to HFNC oxygen therapy and foresee the need of invasive mechanical ventilation in event of HFNC oxygen therapy failure. Patient responded well to the treatment with improving Respiratory rate oxygenation (ROX) index at 2-hour, 6-hour and 12-hour. On 3rd day of HFNC his oxygen requirement reduced (flow rate of 30L/min, FiO₂ – 40%) and he was eventually weaned off oxygen on 8th day of hospitalisation. Patient was continued on above mentioned ATT regimen for 6 months after which Inj amikacin was stopped as he attained culture conversion (4th month sputum culture for MTB – No growth) and rest of the ATT drugs were continued for 18 months. Patient had an uneventful recovery and was declared cured of TB after negative MTB Culture post treatment completion. His repeat chest radiograph showed significant resolution of opacities after 10 weeks of therapy. (Fig-1B)



(Fig-1B) Opacities after 10 weeks of therapy.

DISCUSSION

Though TB is a very common disease in the Indian subcontinent, it is a rare cause of ARDS which is often associated with high mortality. The pathophysiology behind this fulminant presentation is a component of mycobacterial cell wall *Lipoarabinomannan*, that acts as an antigen and leads to activation of the inflammatory cascade, which further evolves to ARDS⁵. A high index of suspicion is warranted, especially in the backdrop of identifiable risk factors such as alcoholics,

diabetics, patients on immunosuppression, HIV infection, pregnant women and patients of chronic liver disease.

Over the years, the mainstay of management has been invasive ventilation, however, invasive ventilation in immunocompromised patient is often associated with higher mortality^{6,7}. A case series of three cases from northern India reported successful management of tuberculous ARDS with non-invasive ventilation (NIV)⁸, nevertheless, the cases reported by them were not immunocompromised in contrast to ours. However, another prospective, observational, international multicentre cohort study concluded that NIV seems to be associated with higher ICU mortality in patients with a PaO₂ / FIO₂ lower than 150 mm Hg⁹. Another observational cohort study of 115 immunocompromised patients of non-tuberculous ARDS, revealed that rates of intubation and mortality in ICU to be significantly lower in the HFNC group (35% & 20% respectively) than in the NIV group (55% & 40% respectively)¹⁰. Similarly, in one more multicentric randomised study involving 310 patients of acute hypoxemic respiratory failure, the HFNC group was found to have lower intubation rate (38%), higher no. of ventilator-free days and lower 90-day mortality compared to NIV group (50% intubation rate)¹¹. The outcome of our case was in concurrence with later two studies, having a favourable outcome using HFNC in an immunocompromised patient of tuberculous ARDS. However, a close monitoring is quintessential to predict the outcome of HFNC oxygen therapy.

ROX index provides an objective and standardised tool to foresee the outcome of HFNC oxygen therapy. ROX Index ≥ 4.88 measured at 2, 6, or 12 hours after high-flow nasal cannula (HFNC) initiation is associated with a lower risk for intubation¹². For a ROX Index < 3.85 , risk of HFNC failure is high, and intubating the patient should be discussed. If ROX Index 3.85 to < 4.88 , the scoring could be repeated one or two hours later for further evaluation. ROX index not only prevents unnecessary intubation but also prevents urgent and chaotic intubation. HFNC delivers heated and humidified oxygen at high flow rates generating a low positive end-expiratory pressure, by flushing expired carbon dioxide from anatomical dead space in upper airways, this helps in reducing the work of breathing and dyspnoea whereas heating and humidification help in preventing thick secretions

and atelectasis¹⁰. HFNC is also very convenient and tolerable for patients and it is easy to provide care to these individuals in form of feeding and oral care.

CONCLUSION

HFNC is a promising modality in treating ARDS, and its use as first-line management of tuberculous ARDS may be considered. Review of literature suggests that it has favourable outcome in terms of mortality and ventilator free days vis a vis invasive mechanical ventilation and non-invasive ventilation. When complimented with ROX index, adverse outcome of HFNC in form of delayed intubation can be avoided. Moreover, HFNC is better tolerated by the patients and there are no chances of ventilator-induced lung injury and ventilator-associated pneumonia, especially in immunocompromised patients.

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CONFLICT OF INTEREST

None

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