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EDITORIAL

State of Art

Original Articles

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EDITORIAL

State of Art

Original Articles

AIMS AND SCOPE:

The SAARC journal of Tuberculosis, Lung Diseases and HIV/AIDS is the official journal of the STAC. The Journal's main aim is the continuing education of personnel and the dissemination of the most up-to-date information in the field of tuberculosis, lung diseases and HIV/AIDS. It is devoted to dissemination of knowledge concerning various aspects of tuberculosis, lung diseases and HIV/AIDS. All articles relevant to the practice of this journal and quality health research are published. The journal is an appropriate forum for the publication of articles concerning the social, economic, public health, epidemiology, diagnostics, genetics etc. in the area of tuberculosis, lung diseases and HIV/AIDS. The scientific manuscripts presenting the results of public health importance are encouraged. The novel case reports which adds to the existing knowledge and consistent with the scope of Journal will be considered for publication. The Journal accepts review/mini-review, case report, short communications, and letters to editors within the scope of the journal.

DISCLAMER:

Any opinions expressed or policies advocated do not necessarily reflect those of the STAC.

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EDITORIAL

The article in this issue of the Journal (A Study on challenges in diagnosis of Drug Resistant TB by Gene-Xpert in Bangladesh -2016) which was conducted by National TB Control Programme Bangladesh in collaboration with SAARC TB and HIV/AIDS Centre (STAC) provides challengers which encountered during application of the Gene-Xpert and key lessons learned in Bangladesh. According to this study, main challenger while operating these machines in Bangladesh has been a frequent module failure, which require longer time period to replacement. Also the study revealed that, frequent errors are shown due to poor quality of sample, unstable electricity supply or poor skill of machine operators. Authors concluded that proper training of operators and proper sputum transport system is urgently needed for efficient use of these machines.

Tuberculosis (TB) remains a major public health problem, as evidenced by the estimated 9 million incident cases, 300 000 multi-drug resistant (MDR) cases and 1.5 million deaths worldwide in 2015. However, only 58% of the new incident cases were bacteriologically confirmed by smear, culture, or Xpert® MTB/RIF (Xpert), while the remaining 42% were diagnosed clinically, including by X-ray.

Xpert® MTB/RIF (Xpert) is an automated molecular test for simultaneous detection of tuberculosis (TB) and rifampicin resistance, recommended by the World Health Organization as the preferred diagnostic method for individuals presumed to have multi-drug resistant TB (MDR-TB) or HIV-associated TB. In 2010, the World Health Organization (WHO) endorsed the Xpert test and recommended its use as the initial diagnostic test for people with HIV-associated TB or presumptive multidrug resistant TB. Three years later the recommendation was extended (conditional on availability of resources) to cover initial diagnostic testing for all adults presumed of having TB.

Early treatment of tuberculosis (TB) is hindered by the lack of rapid, accurate diagnostic modalities that can be applied in resource-limited settings Sputum smear microscopy which is the cheapest and the most available method of TB diagnosis identifies TB in less than half of patients with HIV/TB co-infection. Access to mycobacterial culture is limited and where available results are often delayed by several weeks. Xpert MTB/RIF test (GeneXpert) is a promising innovation in routine TB diagnosis in developing countries owing to the its high sensitivity, specificity and rapid turnaround time of only two hours.

Laboratories play an important role in the NTPs, primarily in the detection of tuberculosis cases, thus ensuring effective treatment and cure by periodic examination of sputum specimens by smear microscopy. Over the years, SAARC TB and HIV/AIDS Centre (STAC) has been conducted quality assurance for smear microscopy in National Reference Laboratories in all the SAARC member states and results showed very good performances.

The Xpert test has been shown to improve TB case finding especially among HIV infected, pediatric age group, extra-pulmonary TB. This and other molecular tools which are fast followers of Xpert and other Point of Care tests has potential to replace microscopy as the first tool for screening and is likely to be the subject of interest in which the SAARC laboratory as the Supra-national reference laboratory could play a key role to validate and generate evidence for wider use of this test in the SAARC region.

In addition, good quality C&DST laboratories are to be strengthened to evaluate the level of dug resistance in the region.

State of Art

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

Haq Rouseli, Bichha R.P., Uddin Md. Ashraf, Modak Pronab Kumar, Rahman Md. Mojibur

Abstract

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

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

Results: Xpert MTB/RIF was first introduced in Bangladesh in March 2012. Till December 2015, a total of 61 Xpert MTB/ RIF machines were functioning at 43 sites in the country. In 42 sites the GeneXpert machines are placed in a separate room and in one site it is placed along with other general pathological laboratory activities. It was found that in all sites necessary physical support like air-cooler, dust control, UPS as well as regular electricity and water supply were available. Out of 43 sites, 38 have 4-module and 5 sites have 16-module machine. In totall, 55 four module machines and 6 sixteen module have been established. Out of these only one 4modul machine was non-functioning.

In about half (48.8%) of the sites machines are run twice a day and in 17 sites (39.5%) only once while in 5 sites (NGO supported sites) machines are run 3 times a day. In 19 (44.2%) sites 1-4 samples are tested per day and in 16(37.2%) sites 5-8 samples/day. Per day 9-20 samples are tested in 4(9.3%) centres while in other 4 centres 21-30 samples are tested daily. In all the centres a total of about 300 samples are tested in a single working day. In total, 38 sites informed about problems they faced in operating the machine. The most common problem was module failure (67.4%), followed by delay in maintenance support (46. 5%). Inadequate cartridge supply and load shading were faced by 16.3% and 11.6% respectively. In 42 sites there were needs for support. The most common support they need is refresher training (93%), followed by maintenance training by 79.1%, and Software training by 18.6%.

The responses from both FDG and in-depth interviews were as follows:
Most common problem faced by the heath workers were lack of timely maintenance of Machines, false result of Rifampacin Resistance due to low bacterial load, module failure, no proper sputum transport mechanism, lack of appropriate centrifuge machine for processing of samples of EP cases and inadequate man power. From the past experience the group provided some valuable suggestions and comments as follows;

A well maintained sputum transportation mechanism to be established. Stable power supply is absolutely necessary as discontinuation of electricity even for a fraction of second will cause erroneous test result. More machines need to made available for easier access. Machine operator needs refresher training including training related to day- to-day maintenance and software system. Good quality sample in sufficient amount to be ensured for Xpert testing to produce accurate results.

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

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Introduction:

Tuberculosis (TB) is a major global health problem. It causes ill-health among millions of people each year and ranks alongside the human immunodeficiency virus (HIV) as a leading cause of death worldwide. Bangladesh is among 30 countries with the high burden of TB and MDR-TB. The estimated incidence rate of all forms of tuberculosis was 225 per 100, 000 population in 2015. In 2015 a total of 206,915 new and relapse cases were notified, among them 4% cases aged less than 15 years. The overall male female ratio was 1.5 in 2015. The treatment success rate among new and relapse cases (all forms) is above 90% since 2007. In 2014 cohort the overall treatment success rate was 93% and among bacteriologically confirmed cases it was 94%. [1] NTP Bangladesh has conducted countries first nationwide drug resistance survey in 2010-2011. According to this survey report the proportion of new TB cases with MDR-TB is 1.4% and that of retreatment cases with MDR-TB is 28.5%. On this assumption the estimated total number of MDR-TB cases in 2015 in the country is 4662 (2198 among new PTB cases and 2464 among retreated PTB cases). [1]

People with MDR-TB or RR-TB are eligible for second-line treatment with MDR-TB regimens. Globally, a total of 111 000 people were started on MDR-TB treatment in 2014, an increase of 14% compared with 2013. Only 50% of patients on MDR-TB treatment were successfully treated, largely due to high rates of mortality and loss to follow-up [2–4]

The SAARC region, with an estimated annual incidence of 3.1 million TB cases, carries 32% of the global burden of TB incidence. Three of the eight Member Countries in the Region are among the 30 TB and MDR-TB high burden countries, with India accounting for 23 % of the world's TB cases.

In 2014, there were an estimated 3.1 million incident cases of TB, equivalent to 185 cases per 100 000 population in the region. This carries 31% of the global burden of TB incidence. The absolute number of incident cases is falling slowly, from 2000 to 2014. [2]

The MDR TB cases in the region range from less than one to four percent (1-4%) among new TB cases and it ranges from less than one to almost 35 percent among the retreatment TB cases.

MDR-TB situation in Bangladesh [3]

The results of the first national DRS completed in 2012 confirmed a low proportion of new TB cases that have MDR-TB (1.4%, confidence intervals 0.7–2.5), but the proportion among retreated cases was revised upwards (28.5%, confidence intervals 24–34). The total number of estimated MDR-TB cases among notified cases in 2015 was 4662. Coverage of routine surveillance of drug resistance is still low. [3]

Issues related to conventional method of diagnosis TB:

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

The main reasons for these gaps are inadequate diagnostic capacity and an over reliance on chest radiography and/or sputum smear microscopy as diagnostic tools. Patients with HIV-associated TB, those with sputum smear-negative and/or extra pulmonary disease, and drug-resistant TB patients are particularly affected by the failure of microscopy as a primary diagnostic tool. The "classical" diagnosis of HIV- associated and drug-resistant TB is complex, expensive, slow and technically demanding, relying on conventional culture and drug susceptibility testing (DST). The long delay (up to several weeks) required to obtain results has devastating consequences for patients who go undiagnosed (and therefore untreated or inappropriately treated), or are diagnosed too late [7]. Detecting more cases, detecting them early and rapidly identifying drug resistance are essential for improving individual patient health and avoiding transmission in the community. This requires universal access and early detection using contemporary tools and innovative strategies [8, 9, 10]

The past decade has seen unprecedented growth in the TB diagnostic pipeline and accelerated efforts to establish the necessary laboratory infrastructure [10]. Nevertheless, although recommended by WHO, the latest generation liquid culture diagnostics and molecular line probe assays for rapid detection of MDR-TB have not yet solved the diagnostic dilemma in most resource-limited settings, largely due to the need for expensive laboratory infrastructure, extensive bio-safety precautions and specialized staff. [10]

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

shift in the diagnosis of TB and drug-resistant TB by simultaneously detecting Mycobacterium tuberculosis and Rifampicin resistance-conferring mutations in a closed system suitable for use outside conventional laboratory settings in less than 2 hours, directly from sputum samples [11, 12]

Field demonstration studies revealed that,

Xpert MTB/RIF detected 90.3% of the culture-confirmed TB cases, compared with 67.1% using microscopy. In sputum smear- negative, culture-positive TB patients Xpert MTB/RIF test sensitivity was 76.9% and specificity was 99.0%. Sensitivity for rifampicin resistance was 94.4% and specificity was 98.3% [13]

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

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

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

In the previous studies, the sensitivity of the MTB/ RIF test for detecting RIF resistance was 94.4 to 100% and the specificity was 98.3 to 100% [14].

Operational and logistical issues:

The available evidence confirmed the robustness of the Xpert MTB/RIF assay under varying temperature and humidity conditions, the need for minimal staff training, basic bio-safety requirements (as for sputum smear microscopy) and high levels of user satisfaction. Operational challenges included the requirement for an ambient temperature < 30^0 C (necessitating air conditioning in hot climates), and uninterrupted and stable electrical power supply (requiring generators in several sites). Storage space and conditions (28^0C for cartridges, waste generated (considerably more than for microscopy), and the 12-month shelf-life of cartridges were listed as main operational challenges (15,16) Cost, affordability and cost-effectiveness analyses Using Xpert MTB/RIF for the diagnosis of smear-negative pulmonary TB was deemed cost-effective compared with existing diagnostic strategies in India, South Africa and Uganda, and within WHO acceptable incremental cost effectiveness ratios (14,15,16)

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

Xpert MTB/RIF was first introduced in Bangladesh in March 2012 with the USAID supported TB CARE II project. Till December 2015, Xpert MTB/ RIF machines were functioning at 43 settings (including 5 sites supported by icddr,b) in the country. Since inception of this method, no evaluation the challenges in diagnosis of TB and drug resistant TB in Bangladesh was done in a systematic way. Hence according to the decision of the 25th Governing Board Meeting of the STAC this study was conducted in Bangladesh form 06 January to 05 April 2017 to achieve the following objective.

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

Methodology;

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

Study Design:

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

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

Three FGDs were carried out with i) National level officials related to TB control and are involved in GeneXpert procurement, establishment, functioning

and supervision/ monitoring ii)Microbiologists/ Laboratory Technicians who are involved in Gene X- Pert Procedure

iii) Medical Officers/health workers who are currently treating/managing MDR TB patients. A set of standard questionnaire was used for conducting FDG.

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

Verbal informed consent was obtained from participants before starting interviews.

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

Findings

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

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

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

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

Other key parameters/characteristics of the Machine operators:

Regarding general education, more than one- third of the operators were only SSC pass and less than one-fourth of them were HSC pass. About 87% (36/43) of them have lab related formal institutional educational background (e.g. Diploma in Medical Technology Lab) and seven has no such background or even no formal lab related training. Professional designations are not harmonized among government and non-government organizations. More than 50% (22/43) has professional designation as MT Lab (under GOB) , while 14 (32.6%) are designated as Technical Asst. Lab (under NGO). Other designations are health worker (4), senior lab technician, research assistant and lab attendant (each one). All MT labs are qualified with Diploma in MT Lab and are Supported by Government of Bangladesh. On the other hand most of the operators supported by NGO (BRAC and ICDDR,B) are not diploma holder.

Length of Service: More than 60% (26) have been working for 30-40 months, 14% (6) for 50-59 months, 3 for 2 months and 8 (18.6%) for less than I year. All except two(2) have received 3 day training on GeneXpert machines (36 from form NTP and 5 from ICDDR,B). The other 2 have working experience with trained colleagues and will also receive formal training. (Table 5). Length of service varies because of phase wise installation of machines and training also provided phase wise by NTP with the support of NTP and Challenge TB USAID.

Type and number of machines:

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

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

On an average in about half (48.8%) of the sites machines are run twice a day, in 17 sites (39.5%)

only once while in 5 sites (NGO supported sites) machines are run 3 times a day (Table 11).

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

In total, 38 sites informed about problems they faced in operating the machine. The most common problem was module failure (67.4%), followed by delay in maintenance support (46.5%). Inadequate cartridge supply and load shading were faced by 16.3% and 11.6% respectively (Table 13).

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

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

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

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

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

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

Conclusion:

- Availability of GeneXpert Machine are inadequate in comparison to need.
- Though almost 100% of the available machines are functional, there is lack of adequate support for timely maintenance.
- Module failure is a common problem and their replacement takes longer time. (> 18% of total modules are found nonfunctional).
- Frequent errors are shown that might be due to poor quality of sample, unstable electricity supply or poor skill of machine operators. (only 3 days training received). Also might be due non existence of well established sputum transport mechanism.
- Non-existence of EQA
- Strong monitoring and supportive supervision is key to improve the situation.

Recommendation:

- Rapid expansion of GeneXpert facility is required to enhance detection of TB and MDR TB
- Diagnostic algorithm needs to be revised to testing all presumptive TB and DR TB
- System to be developed for timely maintenance of machines
- Refresher training for Machine operator needs to be arranged
- To ensure quality EQA system to be established.
- Supervision/monitoring to be strengthened.

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

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

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

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

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

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

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

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

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

parameters	Frequency	%	Remarks
Education (general):			
SSC	29	67.4	
HSC	10	23.3	
Bachelor	3	7.0	
Master	1	2.3	
Total	43	100%	
Education (technical):			
None	7	16.3	
Laboratory related	36	83.7	
Total	43	100%	
Professional Designation			
MT Lab	22	51.2	
Technical Asst. Lab	14	32.6	
Senior Lab Technician	1	2.3	
Lab attendant	1	2.3	
Research Assistant	1	2.3	

Health worker	4	9.3	
Total	43	100%	
Working Duration			
< 1 year (2-23 months)	8	18.6	
1-2 year (24 months)	3	7.0	
30-48 months	26	60.4	
>4 years (50-59 months)	6	14.0	
Total	43	100%	
Training Received on GeneXpert			
Yes	41	95.3	
No	2	4.7	
Total	43	100%	
Training Received from			
NTP (NTRL)	36	83.7	All received 3 days training
ICDDR,B	5	11.6	
No formal Training	2	4.7	On the Job training form Trained operator who has left the job

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

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

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

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

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

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

Table 9 Functional status of Machines

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

Table 10 Functional status of modules by sites

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

Table 11. number of times machines run per day

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

Table 12. Average number of samples test per day

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

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

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

Table 14. Type of support needed

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

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

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

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

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

Table 17 Response from the respondents on these 3 areas (multiple challenges)

Problem /challenges faced	Frequency
Sputum transport mechanism is not well established	2
Lack of timely maintenance of Machines	8
Module failure	3
Frequent error	9
False result of Rif Resistance due to low bacterial load	3
Lack of appropriate centrifuge machine for processing of samples of EP cases	3
Inadequate manpower	1
Lesson Learned	
Availability of GeneXpert is very less in comparison to presumptive cases	4
Diagnostic algorithm has limited indication for diagnosis of TB and MDR TB	2
GeneXpert is a very useful tool for quick and accurate diagnosis of TB and MDR TB	15
Selection criteria for referring to GeneXpert has limited scope	3
Incremental yield for Bacteriological cases	1
Ambient temp and regular cleanliness (at least once in a month) of filter to keep the machine well functioning.	3
Well oriented staff can operate the machine nicely	2
Error rate is higher in samples of EP TB due to low bacterial load and appropriate centrifuge machine is required for processing	3

As only one sample is tested, good quality sample is required	2
Regular maintenance and calibration should be in place for smooth implementation of GeneXpert	4
"Dedicated well trained staff should be there to operate the system for ensuring quality lab services"	1
Stable power supply is absolutely necessary. Discontinuation of electricity even for a fraction of second will cause erroneous test result	3
"The module replacement process kills lot of time as we have to acquire the replacement modules from Cepheid, Netherland"	1
Suggestions (if any) for further improvement	
A well maintained sputum transportation mechanism to be established	2
Diagnostic algorithm needs to be revised to testing all presumptive TB and DR TB	2
More machines need to made available for easier access	5
Ensure regular and timely maintenance	5
Machine operator needs refresher training including training related to day- to-day maintenance and software.	5
Ensure god quality sample in sufficient amount for Xpert testing to produce accurate results	2
Diagnostic criteria should be more broadened so that more presumptive TB and DR TB can be tested.	2

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TUBERCULOSIS AND HUMAN IMMUNODEFICIENCY VIRUS CO-INFECTION: CLINICO-DEMOGRAPHIC DETERMINANTS AT AN ANTI-RETROVIRAL THERAPY CENTER IN NORTHERN INDIA.

Giri Om Prakash, Giri Vishal Prakash, Vishwakarma Kirti, Datta Debranjan

ABSTRACT

Background:

In India, Tuberculosis (TB) is endemic and Human immunodeficiency virus (HIV) infection is epidemic in few states. The risk of developing TB in people living with HIV (PLHIV) is about 19 (27-22) times greater than those without it. TB is major cause of death in HIV-TB co-infected patients. Globally 0.4 million deaths occur annually due to HIV-TB disease.

Material & Methods:

The present observational study was conducted at Darbhanga Medical College and Hospital ART (Anti-retroviral therapy) center during period from January to June 2017. Data of HIV-TB co-infected patients was collected from HIV-TB register and entered into Microsoft Excel sheet for analysis using Statistical Package for the Social Sciences.

Results:

Young persons mostly from the labouring class working in other states were most affected. Pulmonary tuberculosis (sputum smear positive) was most common co-infection. Baseline CD4 cell count at the time of presentation was observed to be low (less than 200 cells/µL) in 46.64% HIV-TB co-infected patients.

Conclusion

Rural young people working as migrant labourer need focus of health interventions. They should be educated and screened for HIV and TB. Baseline CD4 cell count should be done in all PLHIV cases to assess their immune status.

Keywords: Tuberculosis; HIV infection; CD4 Lymphocyte Count; Treatment Outcome

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INTRODUCTION

Thirty seven percent of HIV infected patients who initiate ARV (antiretroviral) have advanced HIV infection (CD4 count < 200 cells/μL). These patients are at high risk of death. With advent of newer antiretroviral that have greater effectiveness, improved tolerability and fewer adverse effects, survival of these patients have been prolonged and hence HIV infection has evolved from a probable death sentence to a chronic disease.[1, 2] HIV infection is an important cause of death among adults. Hence it is a matter of major health concern. HIV evade and invade innate immune system.[3]

First-line antiretrovirals recommended by World health Organisation (WHO) for the treatment of HIV infection are Tenofovir disoproxil fumarate (TDF) and Lamivudine (3TC) or Emitricitabine (FTC) and either the nonnucleoside Efavirenz (EFV) or the intergrase inhibitor Dolutegravir (DTG). The second-line treatment recommendation is two nucleos(t)ide analogues and a boosted protease inhibitor either Lopinavir/ Ritonavir (LPV/R) or Dorunavir/ Ritonavir (DRV/R).[4]

Human immune system consists of ancient innate immune system and acquired adaptive immune system. The principal functions of immune system are recognition and elimination of foreign antigens. In addition, it is also responsible for formation of immunological memory and development of tolerance to self antigens. T-lymphocytes mediate cellular immunity and B-lymphocytes mediate humoral immunity. These provide adaptive immunity and work in close collaboration with innate immune system.

CD4+ T cells are part of adaptive immune system. These are crucial in for regulated and effective immune response to pathogens. Their proper functioning is vital for survival of human beings. They play a major role in mediating immune response through secretion of specific cytokines. They are also responsible for activation of cells of the innate immune system, B-lymphocytes, cytotoxic cells as well as suppression of immune reaction.[5]

HIV causes immunosuppressant that leads to diseases caused by opportunistic pathogens in HIV infected persons. These opportunistic infections (OIs) include Pneumocytic pneumonia, Toxoplasma gonadi encephalitis, cryptosporidiosis, microsporidisis, Mycobacterium tuberculosis infection and disease, Mycobacterium avium complex (MAC) disease, bacterial respiratory disease, bacterial enteric disease, bartonellosis, syphilis, candidiasis, mycoses ,aspergillosis, cytomegalovirus disease, non-cytomegalo virus herpes, JC virus infection, hepatitis B & C infection, and miscellaneous diseases (malaria, leishmaniasis, cryptopsoriasis, penicillosis marneffei, chagas disease). OIs adversely accelerate HIV progression and increase transmission of HIV .[6]

The role of CD4 cell count testing in people living with HIV (PLHIV) is changing with a shift away from using it to decide when to start treatment (ART) and how to monitor treatment. ART can be started irrespective of CD4 cell count and CD4 cell count monitoring can be stopped in PLHIV who are stable on ART as ART efficiency can be monitored by viral load. At present, CD4 cell count testing is an important tool in the management of advanced HIV disease. Cryptococcal antigen (CrAg) and urine lateral flow-LAM assay for TB diagnosis are recommended for all patients with a CD4 cell count less than 100 cells/ μL .[7-9]The present study was designed with the aims to evaluate demographic profile and baseline CD4 cell count in the patients of HIV co-infected with pulmonary and extra-pulmonary tuberculosis.

MATERIAL AND METHODS

The present cross-sectional, observational study was undertaken with objectives of finding out demographic profile, CD4 cell count, type of TB and treatment outcome of HIV-TB co-infected patients who attended ART center, Darbhanga Medical College and Hospital during the period from 1st January 2017 to 30th June 2017. The data from HIV-TB register were entered in the computer and analyzed statistically.

The HIV serological testing was done as per National AIDS Control Organization (NACO) guidelines. HIV seropositivity was diagnosed by CombAids- RS kit, Meriscreen HIV 1-2 WB kit and Trispot kit supplied by NACO to integrated counseling and testing centre (ICTC), Darbhanga. Cy Flow counter was used for CD4 cell count [Figure 1].

Pulmonary tuberculosis (Tuberculosis of lung parenchyma and tracheobronchial tree) was

Diagnosed when either sputum smear was positive by fluorescent microscopy or when clinical features and/ or radiological imaging (x-ray chest/ CT scan chest) were suggestive of pulmonary tuberculosis. Extra-pulmonary tuberculosis (tuberculosis of organs other than lung parenchyma or tracheobronchial tree) was diagnosed by culture of FNAC/ biopsy specimen on solid media (LJ media) or histopathological examination (epitheloid granuloma with caseous material) or radiological imaging or clinical judgement.

The statistical analysis was done using Statistical Package for the Social Sciences (SPSS) (Version 20.0. Armonk, NY: IBM Corp.). Descriptive statistics and chi-square tests were performed.

RESULTS

A total 508 (five hundred eight) HIV-TB co-infected patients were studied. 278 (54.72%) were observed to be in the age group of 35-59 years, out of which 150 (29.52 %), 19 (3.74%) and 109 (21.45%) were HIV-PTB smear positive, HIV-TB smear negative and HIV-EPTB respectively. Next age group maximally affected was 15-34 years 203 (39.96%) followed by less than 14 years 17 (3.34%). Patients of age group 60 and above were least affected 10 (1.96%). Gender distribution of all patients revealed 339 (66.73%) males and 169 (33.27 %) females. Thus males outnumbered females. The mean ± SD age of HIV-TB co-infected patient was observed to be 32 ± 11.7 years [Table 1].

Two hundred fifty six (50.39%) patients had CD4 cell count less than 200 cells/μL. Out of which 149 (29.33 %) had HIV-TB smear positive status. 14 (2.75 %) were HIV-TB smear negative and 93 (18.31%) suffered from HIV-EPTB 2. Relation to CD4 count less than 200per cells/μL with pulmonary and extra-pulmonary TB was calculated using ANOVA and it was found to be insignificant (F= 0.31, p=0.74). Yates corrected degrees of freedom were used for interpretation of the result. 252 (49.60%) patients had CD4 cell count more than 200 cells/μL. The mean CD4 cell count observed amongst HIV-PTB smear positive, HIV-PTB smear negative and HIV-EPTB patients was 227.6, 270.15 and 228.58 respectively. Relation to CD4 count more than 200 cells/μL with pulmonary and extra-pulmonary TB was also not significant (F= 0.45, p=0.64). [Table 2].

Mortality was recorded 25 (4.92%) cases, out of which maximum 17 (3.34%) had HIV-TB co-infected with smear positive pulmonary tuberculosis (PTB) and 8 (1.57%) had EPTB [Table 3].

Table 1: Characteristics of patients with HIV-TB co-infection

Age group (in years)	Total number of patients (n=508)	Pulmonary TB sputum smear(+) (n=278)	Pulmonary TB sputum smear (−) (n=42)	Extra Pulmonary TB (n=188)
≤ 14	17	13	1	3
15-34	203	108	21	74
35-59	278	150	19	109
60 and above	10	7	1	2
Gender				
Male	339	191	27	121
Female	169	87	15	67

Statistic is 2.0928. The p-value is 0.553371. The result is not significant at $p < 0.05$.

There was no correlation between age group and type of tuberculosis (pulmonary and extra-pulmonary). The chi-square statistic is 4.5787 .The p-value is 0.205377. The result is not significant at $p < 0.05$

Table 2: CD4 count in relation to pulmonary and extra-pulmonary tuberculosis.

CD4 Count (cells/μL)	Pulmonary TB sputum smear (+) Mean±SD (n)	Pulmonary TB sputum smear (−) Mean± SD (n)	Relation to CD4 count with Pulmonary TB Sputum smear (+) and sputum smear (-) Significance (p-value)	Extra -Pulmonary TB Mean± SD (n)	Relation of CD4 count with pulmonary TB and extra-pulmonary TB Significance (p-value)
≤ 200	111.83 ± 49.57 (149)	118.46 ± 58.16 (14)	0.64	116.45±49.26 (93)	0.74
>200	356.35 ± 147.86 (129)	346 ±140.23 (28)	0.73	339.34 ± 109.89 (95)	0.64
Mean CD4 count	227.6 (278)	270.15 (42)		228.54 (188)	

Correlation is significant at the 0.05 level. n=Number of patients, SD= Standard deviation.

Relation of CD4 count < 200, with pulmonary TB sputum smear (+) and sputum smear (-) reveals 95 % CI -21.147 to 34.3070, t-statistic: 0.471, DF 161. Relation of CD cell count > 200, with pulmonary TB sputum smear (+) and pulmonary sputum smear (-) reveals 95% CI -70.7090 to 50.0090, t- statistic: - 0.339, DF 15.

Table 3: Treatment outcome of the patients with HIV-TB co-infection

Treatment Outcomes	Pulmonary TB Sputum smear (+) (n=278)	Pulmonary. TB sputum smear (−) (n=42)	Extra-Pulmonary TB (n=188)
Treated	27	1	12
Ongoing/ Follow up	172	39	164
Defaulter	1	1	1
Transferred/LAMA	61	1	3
Death	17	0	8

n= number of patients.

Complete cure was observed more among HIV pulmonary TB sputum smear (+) patients than HIV pulmonary TB sputum smear (-) patients. Found statistically significant (p value < 0.05).

Follow up was major treatment outcome among HIV pulmonary TB sputum smear (+) patients and HIV pulmonary TB sputum smear (-) patients. Statistically significant (p < 0.05).

Death occurred more among HIV pulmonary TB patients than HIV extra -pulmonary TB patients. Found statistically significant (p value < 0.05).

DISCUSSION

The present study revealed that males are more susceptible to HIV-TB co-infection than females by acquiring 66.73% of total figure. The reasons being that men of our state bear the disappropriate burden of poverty and migrate to other developed states of India in search of job (e.g. driver, hotel worker and guards etc), live alone and return home after 6-12 months after earning money for the family. This is supported by studies conducted by Dahiya et al and Chandwani et al. The present study finding not in accordance with Chandra et al, Aturaka et, Said et al and Olowe et al , who reported susceptibility of females in 55.62%, 58.60%, 72.30% and 54.40% of HIV-TB co-infected patients.[10-14,17]

According to the age wise distribution, 40.75%.of the individuals belonged to the age group of 35-59 years. Similar to the finding reported by Chandwani et al (35.61%), Aturaka et al (47.6%), Said et al (43%) and Kavya et al (54%).[11,13,14,15]

Dahiya et al reported 25-34 age group to be maximally (60.60%) affected by HIV-TB co-infection.[10]

The mean age of the present study population was found to be 32 years, which is comparable to the findings made by Dahiya et al (31.18 years) and Chandra et al (36.67 years).[10,12]

In this study pulmonary tuberculosis was predominantly (66.99%) found in HIV-TB infected cases. This is consistent with findings reported by Chandra et al (60.58%), Kamath et al (60.20%) and Olowe et al (94.30%) [12,16,17] but contrasting results have been found in studies conducted by Dahiya et al, Chandra et al and Kavya et al [10,12,15] with higher incidence of extra-pulmonary TB than pulmonary TB among HIV-TB co-infected patients.

A higher percentage of patients with HIV-PTB (32.38%) were observed to have CD4 cell count less than 200 cells/μL as compared to HIV- EPTB (30.90%). This is not consistent with the reports of Chandwani et al and Kavya et al who observed CD4 cell count less than 200 cells/μL in higher number of patients with EPTB cases, the figures being 58.16% and 61.25% respectively.[11,15]

Maximum number of cases (50.39%) had CD4 cell count less than 200 cells/μL. Our study results correlates with Chandwani et al, Yasmin et al and Sidheshwari et al study reports of 59.17%, 60.38% and 60% respectively.[11,18,19] This figure points out that with decrease in CD4 cell count there is increased chance of TB in HIV- infected patients.

Mean CD4 cell count in HIV-TB patients noted in the present study is 227.6. This observation is in accordance with observations made by Dahiya et

al (199.0), Chandra et al (218.32), Kamath et al (147.47), Rajbhandari et al (123.70) and Singh PK (166.7).[10,12,16,20,21]

Out of total 320 PTB cases in our study 278 (54.72%) had sputum smear positive PTB and 42 (8.27%) sputum smear negative PTB. 149 (26.33%) smear positive cases and 14(2.75%) smear negative cases have been noted in the present study to have CD4 cell count less than 200 cells/μL. 93(18.36%) EPTB cases had CD4cellcount less than 200 cells/μL. Thus in our study advanced HIV infection was noted in higher percentage of sputum smear positive PTB cases followed by EPTB and least in sputum smear negative cases.

Jyostna et al observed that higher number of patients who had CD4 cell count less than 200 cells/μL had EPTB followed by smear negative PTB and least in sputum positive PTB.

We observed mortality of 25 (4.92%) HIV-TB co-infected patients, out of which 17 (3.35%) had HIV co-infected sputum smear positive PTB and 8 (1.57%) EPTB.

Large proportion of HIV-TB co-infected patients die in hospital due to invasive bacterial infections as these patients are at high risk of nosocomial infections due to immunosuppressant, frequent invasive procedures and multispectral

CONCLUSION

HIV infection is the most potent risk factor for tuberculosis and HIV-TB co-infection is a serious public health concern. The independent risk factors for unfavorable outcome found in this study were low baseline CD4 cell count (less than 200 cells/μL), rural background of the patients, young age and sputum smear positive pulmonary tuberculosis. Based on these finding we recommend that stakeholders involved in HIV and TB management should focus on these risk factors. Grass-root awareness among the rural population and working class about the disease may be the key in prevention and early detection the disease.

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TUBERCULOSIS AMONG YOUNG PEOPLE ON RISE IN SRI-LANKA

(An analysis of trend and associated factors)

Kapilawanse Saman, Bichha R.P., Samaraweera Sudath, Pallewatte Nirupa, Vitharana Harshni, Jayakody Wasantha, Gangathesewaran, M.

ABSTRACT

Introduction: A descriptive cross sectional study was carried out in 5 randomly selected districts i.e Gampaha, Kandy, Badulla, Nuwara Eliya and Ratnapura during the period of March to September 2014. The general objective of the study was to describe the trend of TB among young population over past 6 years in Sri Lanka and to determine contributory factors associated with TB among young population.

Methodology: The study was carried out in two stages. The first stage was analysis of the trend of TB over the 6 year period of 2008-2014. The analysis of the trend was carried out using the existing secondary data at the Medical Records Division of the National Programme for Tuberculosis Control and Chest Diseases (NPTCCD). Trend was analyzed for new cases of TB patients of 15--34 years of age and separately for 15-24 and 25-34 age groups and for each type of new TB cases. The second stage was a descriptive cross sectional study which was carried out to determine the associated factors. All the patients (new/ retreatment)in age group of 15-34 years and registered at the selected district chest clinics with confirmed TB were selected as the study subjects. Operational Definitions were used to identify confirmed TB cases ie, Sputum smear positive TB, smear negative pulmonary TB and Extra pulmonary TB. The data were collected using a pre tested interviewer administered questionnaire by the District Tuberculosis Control Officers attached to the relevant District Chest Clinics.

Result: The main findings of the study were,

The two peaks of TB incidence were observed in the trend across age groups and fist one was in the age group of 25-34 years second peak in the age group of 45-54.Overall trend of TB in the country is more towards the older age groups (over 45 years), but shift to younger age groups were observed in 15 out of 16 districts in the country. A total of 223 patients were included in the descriptive study. The highest proportion (34%) of study population was from the district of Gampaha while least proportion (10%) of it was from Nuwara Eliya. Nearly 53% of the study population was above 25 years of age with the sex ratio of 1:1. A majority (68%) of the study population was Sinhalese while nearly 70% of the study population has passed the O/L, A/L, Diploma or a degree. About one third of the study population was unemployed. Nearly half (50.4%) of the study population was having monthly income of about Rs. 20,000.00 or above. 31% of the study population were current or past alcohol consumers and 29% were smokers and 7% of the study population were current or past cannabis consumers. Majority (77%) of the study population were under-nourished while 14% of them were less than 15 kg/m^2. Nearly three fourth (72%) of the study population have been diagnosed by a consultant. Statistically significant associations were found between the age category and more than 3 family members (χ^2=5.063, df=1, p<0.017), ethnicity (χ^2=4.229, df=1, p<0.04), employment category (χ^2=13.859, df=3, p<0.003). Statistically significant associations were not found between the age category and residing district (χ^2=5.962, df=4, p<0.202) and type of house (χ^2=1.875, df=1, p<0.110),

Conclusion: The study recommends more detailed assessment regarding the dietary habits of the patients, Emphasis on community awareness regarding nutritional aspects. Improving the nutritional status among adolescents and young adults. Modification of risky life style factors, Improvement of socio economic status through enhancement of financial stability, minimizing overcrowding and improvement of housing conditions and involving of non NTP health care providers and health professionals for TB diagnosis and management through capacity building and awareness.

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INTRODUCTION

Tuberculosis (TB) is a multi systemic disease with myriad presentations and manifestations. It ranks alongside HIV as a leading cause of death from an infectious disease related mortality worldwide[1].

Despite the encouraging progress towards control of TB, the global TB burden remains enormous. According to the WHO Global Report, in 2015, there were an estimated 9.6 million new cases of TB (range,9.1 million–10.0 million) globally, equivalent to 133 cases per 100,000 population in 2014[1] .The estimated prevalence of TB in 2014 was 13 million. There were also 1.5 million deaths due to TB (1,100,000 deaths among HIV-negative cases of TB and 400,000 deaths among people who were HIV positive[1].

The estimated prevalence and incidence rates of all forms of tuberculosis in Sri Lanka in 2015 were 99 and 65 per 100 000 population respectively. The notification rate of new and relapses of TB were 44.5 per 100,000 population, showing slight increase when compared with previous years. Treatment success rate for all forms of TB was 83.2% in 2015 (Unpublished data, NPTCCD).

Sri Lanka is not among the 22 high burden countries of tuberculosis. However, Tuberculosis remains a widespread problem and poses a continuing threat to the health and development of the people. The estimated annual risk of TB infection (ARTI) is 0.4% (0.17%-0.72%)[2]. The highest rates of infection have been found in the most densely populated areas, such as Colombo and other urban areas.

World incidence of TB increased with population density and urban development. People in lower socio economic groups are more prone to get TB and it also affects young adults in their most productive years. Micro epidemics among adolescence and young adults have been reported since 1980. An outbreak of Pulmonary TB was reported among young adults in Australia and majority of the patients were of Asian origin[3]. Outbreaks of TB were also reported among School children in Milan, Italy[4], and South Carolina, USA[5] also. Clinical practice in Sri Lanka clearly showed significant change of Age distribution of TB and more and more young adults are presenting with TB without any known risk factors. But scientific evidence is lacking in this regard and the present study is designed to identify trend of TB among young population and to determine contributory factors for development of TB.

METHODOLOGY:

This is a descriptive cross sectional study. It was carried out in two stages.

First stage –Analysis of the trend of TB over the past 6 years.

The trend analysis was carried out using the existing secondary data at the Medical Records Division of the National Programme for Tuberculosis Control and Chest Disease Diseases (NPTCCD). Trend was analyzed for new cases of TB disease among patients of 15--34 years age category for past 6 years (2008 to 2014) and separately for 15-24 and 25-34 age groups and for each type of TB.

Second stage - Descriptive Cross Sectional Study to determine the associated factors

Study setting

The study was carried out in 5 selected districts in Sri Lanka namely, Gampaha, Kandy, Badulla, Nuwara Eliya and Ratnapura.

Study subjects

All (new/ retreatment) the patients in age group of 15-34 years and registered at the selected district chest clinics with confirmed TB were selected as the study subjects. Operational Diagnostic Criteria for confirmed TB is defined as

- **Sputum smear positive TB-** A patient with at least two sputum smears are positive for AFB by direct smear microscopy or a patient with at least one sputum smear positive for AFB by microscopy and sputum culture is positive for M. tuberculosis.
- **Sputum smear negative pulmonary TB -** A patient with at least three sputum smears are negative for AFB by microscopy with chest X-ray abnormalities consistent with active pulmonary tuberculosis and no response to a course of broad-spectrum antibiotics or a patient whose initial sputum smears were negative for AFB, but the sputum culture is positive for M. tuberculosis.
- **Extra pulmonary TB -** Tuberculosis of any organ of the body other than the lung parenchyma either bacteriologically confirmed or clinically diagnosed.

Inclusion criteria

All the patients who fulfill the above criteria and are residing in the selected districts were included in the study

Exclusion criteria

Patients below 15 years of age or 35 years or above and patients who do not residing in the selected districts were excluded from the study

Study Period – Three months starting from 1-08-2013

Sample size.

A formal sample size was not calculated for the study. All the patients registered during the above period and who fulfill the inclusion criteria will be included in the study.

Data collection

Interviewer administered, structured questionnaire was used to collect information. Information was gathered on demographic, socio-economic, environmental conditions, life style factors past and present medical history, contact history, family history, history of medication, and diagnostic variables. The questionnaire was prepared in English and then translated into local languages.

Data collection was done by a pre trained data collectors (preferably pre interns)

Questionnaire was pre-tested in the Chest clinic Colombo.

Data management and analysis

Data entry was carried out and analyzed in the SPSS software. Frequencies, percentages and proportion were calculated for categorical variable while mean was calculated for continuous variables.

Chi square test was used to determine association between categorical variables and 95% confidence interval was also established.

Ethical Considerations

Data security/confidentiality was ensured and no unauthorized persons were allowed to access the data. Ethical approval was obtained from the Ethical Review Committee of the University of Sri Jayawardanapura.

RESULTS

First Stage -The Analysis of the trend of TB over the period of 2008-2014.

The trend of TB was analyzed over the period of years from 2008 -2014 for new cases, new sputum positive, new sputum negative and EPTB cases and for all 26 districts for the age categories of 15-34years, 15- 24 years and 24 -34 years. In addition trend of TB for males and females were assessed separately.

Trend of TB in All New cases –

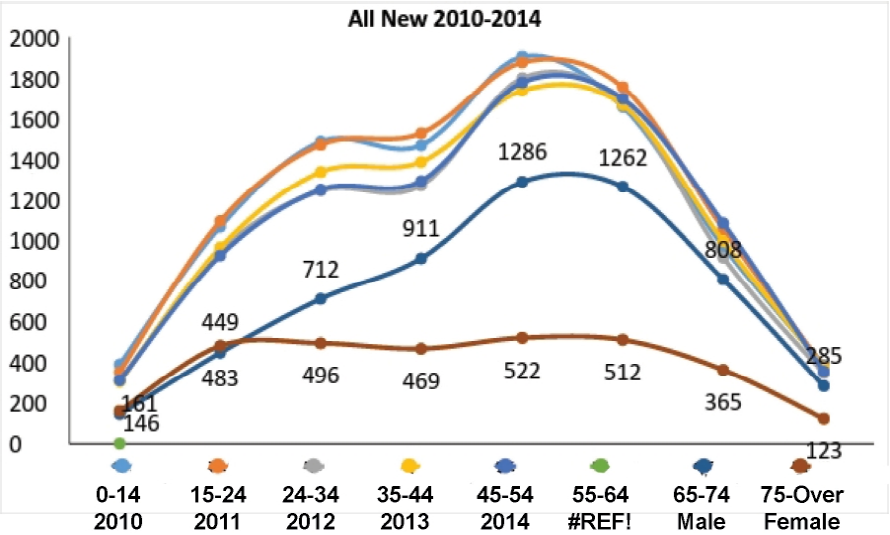
Around one fourth (25%) of the patients detected with TB during the 2010-2014 belongs to the age group of 15-34 years. The two peaks of TB incidence were observed in the trend across age groups and fist one was in the age group of 25-34 years second peak in the age group of 45-54. However, overall trend for all new cases was more towards the older age groups (over 45 years).

The incidence of TB was more among males and number of cases was increased up to 45-54 age group and there was a gradual decrease of cases in older age groups. The number of cases more in young adult females of 15-24 than males in the same age group and it remained more or less static in all age groups except in extreme ages. Present medical history, contact history, family history, history of medication, and diagnostic variables. The questionnaire was prepared in English and then translated into local languages.

Data collection was done by a pre trained data collectors (preferably pre interns)

Questionnaire was pre-tested in the Chest clinic Colombo.

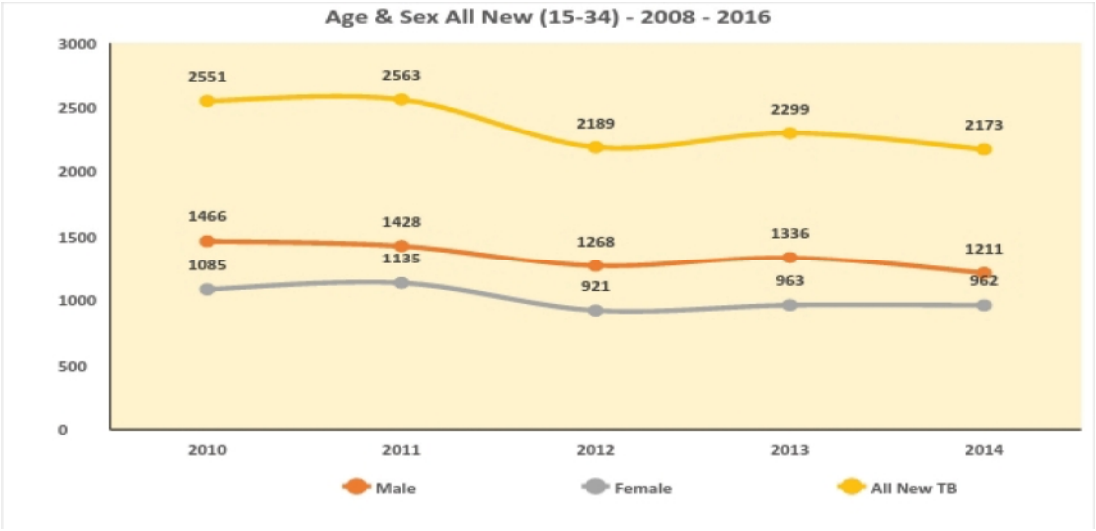
Figure 1 Trend of all new cases of TB for the 2010-2014 period



There was a slight but decreasing trend of new TB cases among the 15-34 years age group. The similar pattern was observed among males of the same age group. However increasing trend was observed among females in year 2010-2011 and 2012 to 2014 time periods.

The pattern of trend similar to the 15 to 34 age group was observed in trends of 15-24 and 24-35 age groups

Figure 2. Overall trend of TB among all case of age group of 15-34 years



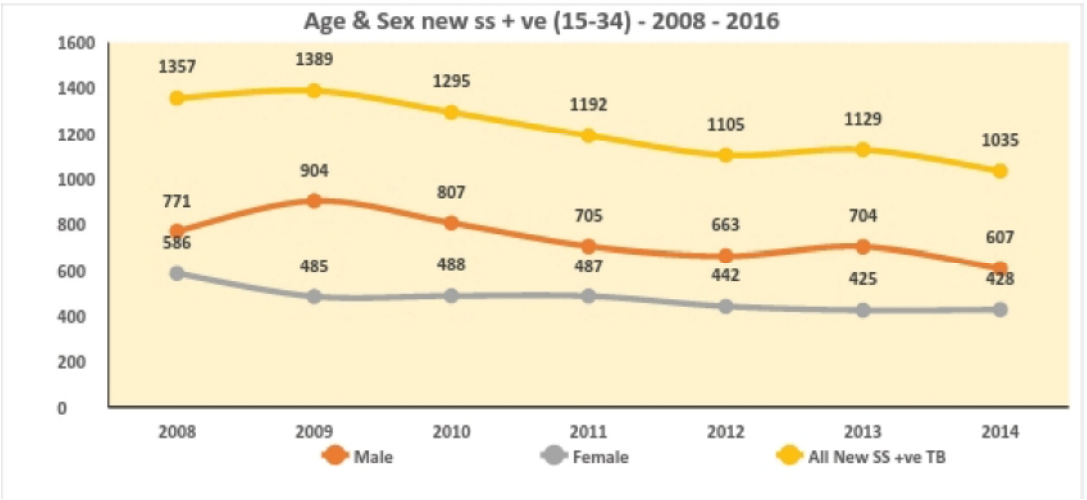
Trend of TB in New Sputum Positive Cases of 15-34 y age group

There were two peaks in the incidence of TB in 2009 and 2013 over the 6 year period of from 2008 – 2014. But the peak was more in 2009 (36 cases than in 2013 (24 cases)when compared to preceding years. A decreasing trend of TB was observed from 2009. A similar pattern was observed among male sputum sm+ve patients.

The decrease of cases was observed among females in 2009 when compared to 2008. However gradual slight increase of cases has been observed from 2009-2011and 2013 to 2014.

The trend similar to 15-34 age group was observed among 24- 34 age group and 15-24 age groups. But the there was no marked difference in cases over the considered period.

Figure 3 . Overall trend of TB among new sputum positive cases of age group of 15-34 years



Trend of TB in New Sputum Negative Cases

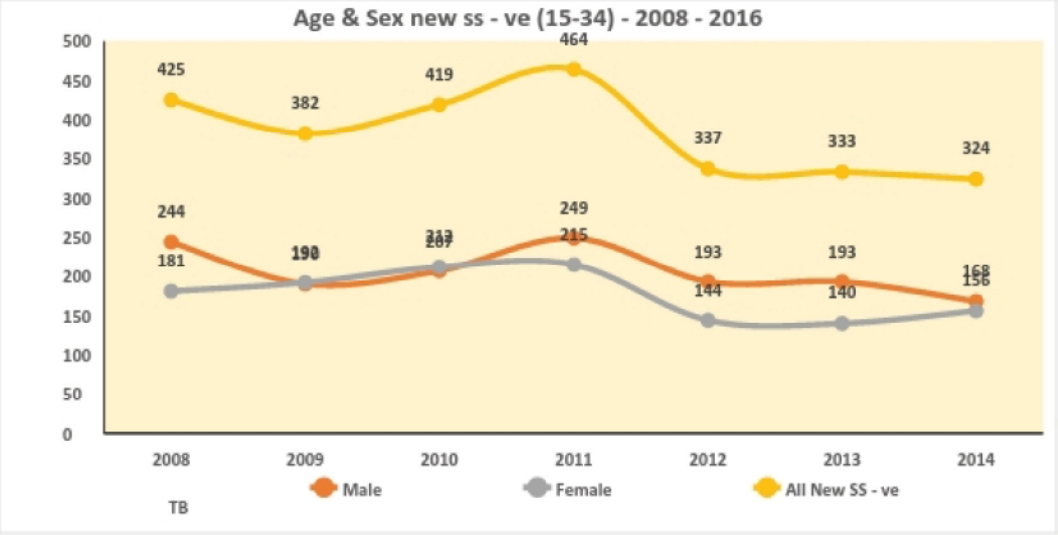
There was a decrease of new sputum negative cases in 2009when compared to 2008 but there was a significant .increase cases from 2009 to 2011. There was a decline in 2012 by 121 cases and the almost static trend was observed in subsequent years.

The similar trend was observed among males for the 2008-2013 and there was a decrease of cases by 25 in year 2014.

When considered the females, there was a gradual increase of cases in 2008 to 2011 and 2012 to 2014 period. Number of females with new sputum negative TB was more in 2010.

The pattern similar to 15- 34 was observed in both 15 -24 and 25-34 age groups

Figure 4 . Overall trend of TB among new sputum negative cases of age group of 15-34 years

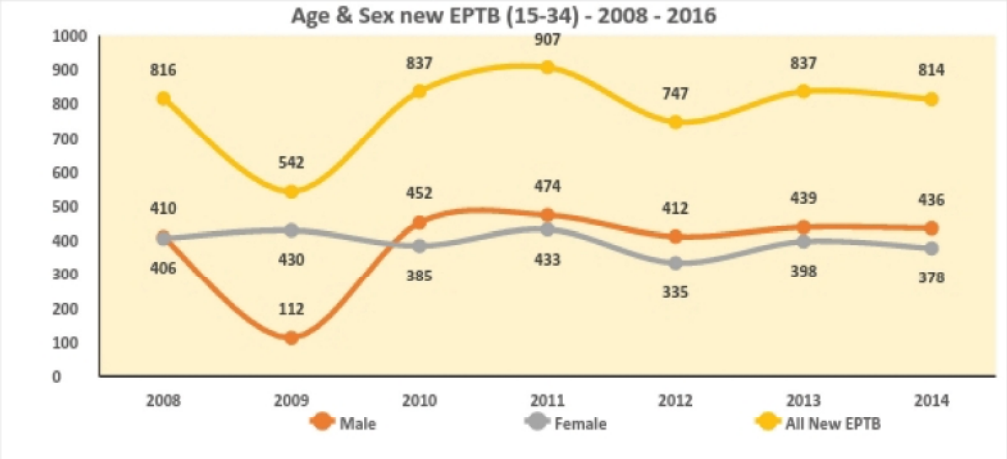


Trend of TB in EPTB Cases

There was a significant decrease of new EPTB cases among the age group of 15-24 in 2009 when compared to 2008. But increasing trend of EPTB cases was observed in 2009 to 2011 period and 2012-2014 period. The similar pattern was observed among males in this age group. EPTB cases among females of the same age group were almost static during the 2008-2014 periods with minor fluctuations. In 2009 New EPTB cases were more among the females than males in the same age group

The pattern similar to the 15-24 age group was observed among 15-24 and 25 -34 age groups.

Figure 5 . Overall trend of TB among EPTB cases of age group of 15-34 years



Trend of TB among new TB cases in the 15-34 age group in Districts

There was a huge variation in trend of new TB cases across districts in 15-34 years age group. A significant increase of all new cases of TB was observed in 2013 when compared to 2012 in 15 districts namely, Colombo, Ratnapura ,Kalutara, Kandy, Polonnaruwa, Kegalle, Baticaloe,, Puttalam, Kilinochchi, Vavnia, Hambantota, Jaffna, Mathale, Nuwaraeliya and Matara . This increasing trend was further continued in districts of Kandy,Mathale, ,Jaffna, Vavnia,,Puttalam and Polonnaruwa Districts. Decreasing trend in subsequent years when compared to 2013 was observed in Colombo Nuwaraeliya, Matara and Baticaloe districts.

In Kegalle District there was a significant reduction of cases in 2012 when compared to previous two years. It was increased by 87 cases in 2013 and subsequent decrease of cases was observed in 2014 and it was mainly due to reduction of cases among males. There was a significant gradual increase of incidence of TB among females in the same age since 2012.

A peak in the trend in 2012 observed in Badulla, Monaragala, Anuradhapura, Kalmunei and Ampara districts followed by a sharp decrease in 2013. In subsequent years , slow but steady increase of TB cases in 15-34 age group was observed in all the above districts except in Ampara, where further decrease was observed. The more or less similar pattern of trend was observed for males and females in the same age group.

Second Stage

Descriptive Study

A descriptive study was carried out in March to September 2014 among 233 patients in 5 randomly selected districts.

For the comparison of the information the study population was divided in to two groups which included age 15 to 24 in the younger group and the age 25 to 34 in the older group.

Table 01: Distribution of study population by their resident district

District	Number	Percentage (%)
Nuwara Eliya	15	9.8
Badulla	22	14.4
Rathnapura	31	20.3
Kandy	33	21.6
Gampaha	52	34.0
Total	153	100.0

Missing value = 80

A highest proportion (34%) of study population was from the district of Gampaha while least proportion (9.8%) of it was from Nuwara Eliya.

Table 02: Distribution of study population by Age

	N	Minimum	Maximum	Mean	Std. Deviation
Age in years	231	14	35	25.9	5.58

Missing value = 2

Mean age of the study population was 26 years with standard deviation of 5.58 and range of 15 – 34 years.

Table 03: Frequency distribution of study population by socio-demographic factors

Characteristic	Number	Percentage (%)
Age (Years)		
15 – 24	109	47.2
25 – 34	122	52.8
Total	231[a]	100.0
Sex		
Male	60	50.4
Female	59	49.6
Total	119[b]	100.0
Ethnicity		
Sinhalese	159	68.2
Tamils	57	24.5
Moor	17	7.3
Total	233	100.0
Level of education		
No schooling	5	2.3
Gr. 1 – 5	10	4.5
Gr. 6 – 10	50	22.5
O/L Passed	87	39.2
A/L Passed	61	27.5
Degree/Diploma Holder	9	4.1
Total	222[c]	100.0

Missing value; a = 2, b = 114, c = 11

Nearly 53% of the study population was above 25 years of age and sex ratio was 1:1 for males and females in the study population. A majority (68%) of the study population was Sinhalese while nearly 70% of the study population has passed the O/L, A/L, Diploma or a degree.

Table 04: Frequency distribution of study population by socio-economic factors

Characteristic	Number	Percentage (%)
Employment		
Unemployed	62	30.2
Unskilled Manual	36	17.6
Skilled Manual	52	25.4
Farmer/Fisherman	1	0.5
Housewife	22	10.7
Sales & Service	12	5.9
Technical	7	3.4
Managerial	1	0.5
Other	12	5.9
Total	205[d]	100.0
Family income (Rs.)		
< 3,000.00	9	4.2

3,000.00 –4,999.00	8	3.7
5,000.00 – 9,999.00	24	11.2
10,000.00 – 19,999.00	65	30.4
20,000.00 – 30,000.00	45	21.0
> 30,000.00	63	29.4
Total	214[e]	100.0

Missing value; d = 28, e = 19

About one third of the study population was unemployed while only less than 4% of the study population was from technical or managerial occupational categories. Nearly half (50.4%) of the study population was having monthly income of about Rs. 20,000.00 or above.

Table 05: Frequency distribution of study population by substance abuse

Type of substance consumed	Number	Percentage (%)
Alcohol		
Yes	33	17.4
Never	130	68.8
Consumed but stopped	26	13.8
Total	189[a]	100.0
Smoking		
Yes	26	13.5
Never	137	71.4
Smoked but stopped	29	15.1
Total	192[b]	100.0
Drugs		
Yes	8	4.3
Never	173	93.0
Consumed but stopped	5	2.7
Total	186[c]	100.0
Cannabis		
Yes	6	3.2
Never	176	93.1
Consumed but stopped	7	3.7
Total	189[a]	100.0
Other		
Yes	5	2.7
Never	179	96.2
Consumed but stopped	2	1.1
Total	186[c]	100.0

Missing value; a = 44, b = 41, c = 47

Substantial percentage of the study population were current or past alcohol consumers (31%) and smokers (29%). However, proportion of the study population who were current or past illicit drug consumers and current or past cannabis consumers were 7% each.

Table 06: Frequency distribution of study population by their BMI

BMI	Number	Percentage (%)
< 15	26	13.8
15 - 18.5	120	63.4
18.6 – 25	40	21.2
25.1 – 30	3	1.6
Total	189	100.0

Missing value; a = 44

Great majority (77%) of the study population were under-nourished while BMI of 14% of the study population were even less than 15 kg/m^2.

Table 07: Frequency distribution of study population by type of their residing house and number of members living in the house

Characteristic	Number	Percentage (%)
Type of the house		
Slums	22	10.2
Semi-detached house	55	25.5
Detached house	124	57.4
Other	15	6.9
Total	216	100.0
No. of family members		
One	71	33.3
Two	75	35.2
Three or more	67	31.5
Total	213	100.0

Missing value; a = 17, b = 20

Nearly 36% of the study population were living in slums or semi-detached house while a third (31%) of the study population were having three or members living together in the family.

Table 08: Frequency distribution of study population by factors related to past medical and surgical history

Characteristic	Number	Percentage (%)
Previous hospitalization		
Yes	72	36.5
No	125	63.5
Total	197[a]	100.0
Past surgery		
Yes	31	16.5
No	157	83.5
Total	188[b]	100.0
Treatment regimen		
CAT I	217	95.2
CAT II	11	4.8
CAT IV	0	0

Other	0	0
Total	228[c]	100.0
Diagnosis made by		
DTCO	35	15.8
GP	8	3.6
Consultant	160	72.1
Other MO	19	8.6
Total	222[d]	100.0

Missing value; a = 36, b = 45, c = 5, d = 11

One third (36%) of the study had hospitalized previously while 16% has undergone a surgery previously. Almost all (95%) study population were on the treatment category of CAT I. Nearly three fourth (72%) of the study population have been diagnosed by a consultant. The percentage treated outside NTP was 12%

Table 09: Association between the age category and their resident district

Characteristic	15 – 24 yrs	25 – 34 yrs	Total	Significance
Nuwaraeliya	7 (46.7%)	8 (53.3%)	15 (100%)	$\chi^2 = 5.962$ df= 4 p = 0.202 Not significant
Badulla	10 (45.5%)	12 (54.5%)	22 (100%)	
Rathnapura	11 (36.7%)	19 (63.3%)	30 (100%)	
Kandy	15 (45.5%)	18 (54.5%)	33 (100%)	
Gampaha	32 (62.7%	19 (37.3%)	51 (100%)	
Total	75 (49.7%)	76 (50.3%)	151 (100%)	

Out of the total, a majority (62.7) of the younger age group resided in Gampaha, while in all other districts majority are from the older age group . But a significant association was not found between the age category and the district they are residing.

Table10: Association between the age category and sex

Characteristic	15 – 24 yrs	25 – 34 yrs	Total	Significance
Male	29 (49.2%)	30 (50.8%)	59 (100%)	$\chi^2 = 0.136$ df=1 p = 0.712 Not significant
Female	27 (45.8%)	32 (54.2%)	59 (100%)	
Total	56 (47.5%)	62 (52.5%)	118 (100%)	

Among males and females, the majority were in the older age group . A significant association was not found between the age category and sex

Table11: Association between the age category and ethnicity

Characteristic	15 – 24 yrs	25 – 34 yrs	Total	Significance
Sinhalese	66 (42.0%)	91 (58.0%)	144 (100%)	$\chi^2 = 4.229$ df= 1 p = 0.040 Significant
Tamils	33 (57.9%)	24 (42.1%)	57 (100%)	
Total	99 (46.3%)	115 (53.7%)	214 (100%)	

A higher percentage (58.0%) of Sinhalese study units was reported from the older age group while that of Tamil study units (57.9%) was reported from younger group. This difference was statistically significant (χ^2=4.229, df=1, p<0.04

Table12: Association between the age category and number of family members

Characteristic	15 – 24 yrs	25 – 34 yrs	Total	Significance
Less than 3	77 (52.4%)	69(47.6%)	146 (100%)	χ^2=5.063, df=1, p<0.017 Significant
3 or more	24(35.8%)	43 (64.2%)	67 (100%)	
Total	101 (46.3%)	112 (53.7%)	213 (100%)	

The majority (52.4%) of study units among families having less than three family members was from younger age group while 64.2% of families having more than three family members were from older age group. This observed difference was statistically significant (χ^2=5.063, df=1, p<0.017)

Table 13: Association between the age category and level of education

Characteristic	15 – 24 yrs	25 – 34 yrs	Total	Significance
Below grade 6	5 (33.3%)	10 (66.7%)	15 (100%)	χ^2 = 1.736 df= 2 p = 0.420 Not significant
Grade 6 – O/L passed	62 (45.9%)	73 (54.1%)	135 (100%)	
A/L Passed and above	36 (51.4%)	34 (48.6%)	70 (100%)	
Total	103 (46.8%)	117 (53.2%)	220 (100%)	

Out of the older age group majority had education below 6th grade while younger group had education .above A/L. But observed association was not significant.

Table 14: Association between the age category and the category of occupation

Characteristic	15 – 24 yrs	25 – 34 yrs	Total	Significance
Unemployed	38 (62.3%)	23 (37.7%)	61 (100%)	χ^2 = 13.859 df= 3 p = 0.003 Significant
Unskilled manual	9 (25.0%)	27 (75.0%)	36 (100%)	
Skilled manual	20 (39.2%)	31 (54.5%)	51 (100%)	
Other	25 (45.5%)	30 (54.5%)	55 (100%)	
Total	92 (45.3%)	111 (54.7%)	203 (100%)	

A majority (62.3%) of unemployed category was from younger study group while 75% of unskilled category was from the older group. This observed difference was statistically significant (χ^2=13.859, df=3, p<0.003).

Discussion

The study conducted in two stages and the trend of TB among s 15-34 age group was analysed over the period of years from 2008 -2014 using secondary data obtained from the medical statistics devision of the NPTCCD. The study was limited to new patients as desegregated data for retreatment cases were not collected through routine system during the period of study. However this was not impacted on the interpretation of the overall trend of the study as retreatment cases are very less. The Trend analysis was limited for a 6 year period as disaggregated data for age was not available. The trend of TB among adolescents (15-19 years) could not be assessed as it was not collected in the routing data collection system.

Around one fourth of the patients detected with TB during the 2010-2015 belongs to the age group of 15-34 year age group. Overall trend for all new cases was more towards the older age groups (over 45years), indicating the good control of TB. However, there is a huge variation in trend of TB among 15-34 age in districts and increasing trend among young age group was observed in 15 out of 26 districts which indicated the further spread of disease. An increasing trend of EPTB was observed in all the years expect in 2009 and may be due to improvement in diagnostic facilities over the

years. Increasing trend of sputum negative cases were observed up to 2011 but it was more or less static over the following years with the expansion of specialist services in subsequent years with improved diagnosis.

The second stage of the study was a descriptive cross-sectional study to identify associated factors. We have randomly selected five districts including Gampaha, Kandy, Badulla, Nuwara Eliya and Ratnapura, for the study with the highest proportion (34%) of study population was from the district of Gampaha while least proportion (10%) of it was from Nuwara Eliya and this is compatible with the population distribution of the districts we selected. Mean age of the study population was 26 years with standard deviation of 5.58.

Nearly 53% of the study population was above 25 years of age with the male female ratio of 1:1. When compared with the male female ratio of 2 : 1 for all TB cases for the country, there was a significant increase of TB cases in the study population. This may be attributed to the exposures outside the family due to life style of this age group.

A majority (68%) of the study population was Sinhalese while nearly 70% of the study population has passed the O/L, A/L, Diploma or a degree. About one third of the study population was unemployed. Nearly half (50.4%) of the study population was having monthly income of about Rs. 20,000.00 or above. This finding is compatible with findings of other studies[16,17] and reflects the link of TB with the social economic status .

Substantial percentage of the study population were current or past alcohol consumers (31%) which was compatible to the alcohol consumption pattern among males (30%) in the similar age group [18] . Twenty nine percent of the study population were smokers while 7% of the study population were current or past cannabis consumers who used illicit drugs in their life time. Presence of these high risk behaviours among the study population might have impacted on the acquiring TB infection.

Nearly three fourth (72%) of the study population have been diagnosed by a consultant and15.8 % was diagnosed by a District Tuberculosis Control Officer and only 12% were diagnosed by the persons outside the NTP and which reflect the need of involvement of other medical practitioners in diagnosis.

Great majority (77%) of the study population were under-nourished while 14% of them were less than 15 kg/m^2. This finding reflects the association between nutritional status on occurrence of active TB and compatible with the findings of other research studies[15]

Nearly 36% of the study population were living in slums or semi-detached house while a third (31%) of the study population were having three or more members living together in the house. But the majority (52.4%) of study units among families having less than three family members was from younger age group while 64.2% of families having more than three family members were from older age group. This observed difference was statistically significant (χ^2=5.063, df=1, p<0.017) and may be attributed to long term exposure among older age groups due to overcrowding.

.A majority (62.7%) of the study units in younger group was from the district of Gampaha compared to the lowest (36.7%) reported from Rathnapura. However these observed differences were not statistically significant (χ^2=5.962, df=4, p<0.202). A higher percentage (58.0%) of Sinhalese study units was reported from the older age group while that of Tamil study units (57.9%) was reported from younger group. This difference was statistically significant (χ^2=4.229, df=1, p<0.04)and may be attributed the differences living habits in different ethnicities which have an impact on spread of the communicable diseases.

A majority (62.3%) of unemployed category was from younger study group while 75% of unskilled category was from the older group. This observed difference was statistically significant (χ^2=13.859, df=3, p<0.003). This may attribute to the spread of TB due to poor living conditions.

Conclusion and Recommendations

According to results there were few areas which need more attention. The study identified low BMI as one of the key risk factors for TB in the study group. Therefore, more detailed assessment regarding the dietary habits of the patients should be taken at the time of diagnosis and nutritional inventions needed to be carried out .Emphasis should be given to community awareness regarding nutritional aspects. Actions should be taken to improve the nutritional status among adolescents and young adults.

Measures should be taken to modify risky life style factors, such as alcohol consumption, smoking and substance abuse which have an impact on nutritional status as well as spread of TB.

The study revealed association between socio-demographic factors such as low income, housing conditions, ethnicity, occupation category and TB in the study population.

Improvement of socio economic status is a must in control of TB. A system must be developed to enhance financial stability of patients and their families. Steps should be taken to minimize overcrowding and improvement of housing conditions.

The study revealed that the diagnosis of tuberculosis as nearly 70% of the patients made by the consultants and only 12 % of cases were detected outside the NTP. This might contribute to the delay in diagnosing and hence more chance to spread. Involvement of non NTP health care providers and health professionals need to be strengthened through capacity building and awareness.

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AN EPIDEMIOLOGICAL STUDY TO FIND OUT RISK FACTORS OF MULTI DRUGS RESISTANCE TUBERCULOSIS IN NEPAL

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ABSTRACT

Introduction:

Drug resistant tuberculosis is a threat to tuberculosis control worldwide. Previous anti- tuberculosis treatment is a widely reported risk factor for multi drug resistant tuberculosis (MDR-TB), whereas other risk factors are less well described. In Nepal National Tuberculosis Control Programme initiated DOTS-PLUS Pilot project from September 2005 using standardized treatment regimen.

Objective:

To explore the risk factors for MDR-TB in Nepal.

Methodology:

Institution based matched case control study with a case: control ratio of 1:2 was carried out in three regions of Nepal. Fifty five cases and 110 controls were selected. Current MDR- TB patients on treatment from DOTS –Plus clinic were enrolled as cases. Controls were age, sex matched cured TB patients and who had completed treatment either from the same centre or any DOTS Centre adjacent to that DOTS Plus Centre. Data was collected by a trained research assistant using interviewer administered structured questionnaire. Matched analysis was done using SPSS 16 version. Confounding effects were controlled by using matching, matched analysis and regression analysis.

Results:

In matched analysis following were the significant risk factors for MDR-TB in Nepal.(1) HIV Sero positivity (OR 15.9, CI 1.9- 133.0) (2) Travel cost more than 50 NRs per day (OR 6.5, CI 2.4- 9.8) (3) Contact history of TB (OR 3.8, CI 2.2- 6.6) (4)Living in a nuclear family (OR 6.0, CI 2.6- 13.9)(5) Non adherence to DOTS (OR 18.6, CI 2.27- 151.0) (6) Distance to treatment centre more than 5 Km (OR 3.9, CI 1.5- 10.) (7)Previous history of TB (OR 12.0, CI 5.4 -26.5)(8) Living in a rural area (OR 4, CI 2.1- 8.5) (9) Unmarried (Crude OR 3.3,CI 1.6- 6.8) (10) Un employment (OR 3.4,CI 1.6-7.6)(11) Living in a rented house (OR 3.5, CI 1.77- 3.67) (12) Single bed room (OR 2.8, CI 1.13- 6.9).Using muti-variate analysis except living in a rented house and single bed room other variables were positive significant predictors for MDR –TB in Nepal.

Conclusions:

Many risk factors were related to the DOTS. Strengthening of DOTS programme to tackle the identified risk factors can reduce the MDR –TB burden in Nepal.

Key Words: MDR TB, Case Control Study, Risk Factor.

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INTRODUCTION

Multidrug- resistant TB (MDR-TB) caused by Mycobacterium tuberculosis resistant to both isoniazid and rifampicin with or without resistance to other drugs is among the most worrisome elements of the pandemic of antibiotic resistance because TB patients that fail treatment have a high risk of death [(1)] . Globally, about three percent of all newly diagnosed patients have MDR-TB.MDR-TB has a lower cure rate and treatment cost being almost 100 times more than treating a drug –susceptible TB patient, it is imperative that this problem be addressed on a priority .

World Health Organization estimated TB prevalence and incidence rate of all forms of TB respectively 215 and 158 per 100 000 populations in 2014. With the introduction of Directly Observed Treatment Short course (DOTS) number of deaths has dramatically reduced from 9,712 (51/100 000) in 1990 to (17/100 000) in 2014. Total 35277 notified new and relapse cases were detected, among the notified new and relapse cases 345 (<1%) cases aged under 15 years. However male female ratio is 1.8 in 2014. Treatment success rate among new smear-positive cases was 91% for the cohort of patients registered in 2013, and has been consistently above the target of 85% since 2001. The success rate among new smear-negative/extra pulmonary and retreatment cases is high.[(2)].

Tuberculosis is one of the major public health problems in Nepal. About 45% of the total population is infected with TB, out of which 60% are in the productive age group. Every year about 44,000 people develop active TB, of whom 20,000 have infectious pulmonary disease. These 20,000 are able to spread the disease to others.

In 1994 the National Tuberculosis Program (NTP) piloted Directly Observed Short Course (DOTS) in four districts with total population coverage of 1.7%. The Tuberculosis program is delivered in the 75 districts of Nepal through the Hospitals, Health Centers and Sub –Health Posts by staff for whom TB treatment is part of their integrated health care activities. In Nepal clinic based ambulatory treatment for Tuberculosis is the norm.

Tuberculosis control is identified as a top priority programme within the Ministry of Health and Population. Full DOTS institutional coverage was reached in the primary health system, including 100% coverage in PHC centers, health posts, and sub-health posts in the country. Decentralization of services, outreach projects and strong community involvement are contributing significantly to increase case-detection and access to TB diagnosis and treatment.

SIZE OF THE MDR- TB PROBLEM IN NEPAL:

Anti tuberculosis drug resistance is a great public health problem, which may become a great challenge for the National TB control program. Of all patterns of drug resistance MDR –TB which is resistant to at least Rifampicin and Isoniazid is the one that focused international attention because of the reduced response to standard Short –Course Chemotherapy (SCC) with first line drugs, leading to higher mortality and treatment failure rates and increased period of transmissibility.

The percentage of TB cases with MDR-TB 2.2% and retreatment cases was 15% in 2014. However, total MDR-TB burden in the country was 1160. National TB Programme has undertaken four national surveys in Nepal as part of the WHO/ IUATLD Global Project on Anti -Tuberculosis Drug Resistance Surveillance. The first survey, in 1996, showed a prevalence of multi drug-resistance (resistance to at least Rifampicin and Isoniazid) around 1.2% among patients never previously treated for tuberculosis. Similarly Drug Resistance prevalence was 3.8% in 1998, 1.3% in 2001 and 2.9% in 2006 and 2.2 in 2010. Nepal was one of the first countries globally to introduce ambulatory MDR-TB case management in 2005 diagnosing and treating Category II failures and other laboratory-confirmed MDR-TB cases under a GLC approved project. According to WHO Anti Tuberculosis Drug Resistance in the World Report No 4 published in 2008 “the Nepal has proven to be the leader in MDR-TB control in the region by establishing the first MDR-TB control programme in the public sector and expanding it’s coverage to 100% of the country by the end of 2006”.[(2)]

Although its causes are microbial, clinical and programmatic, drug –resistant TB is eventually a man made phenomenon. An inadequate or poorly administered treatment regimen allows a drug resistant strain to become the dominant strain in a patient infected with TB. There are many studies conducted in the developed world to find out causes of MDR –TB but in the developing world specially in the SAARC region such studies are scarce. Hence this study was conducted in Nepal to achieve the following objectives.

To determine risk factors for multi-drug resistance in patients with pulmonary tuberculosis in Nepal.

METHODOLOGY:

Study Design

Institution based matched case – control study with a Case: Control ratio of 1:2 was carried out in order to identify the predisposing risk factors of MDR-TB in Nepal.

Study Setting

Selected institutions in Central Western and Eastern region in Nepal where the DOTS PLUS PILOT Project were carried out were the focal point for the study. (Kathmandu valley, Biratnagar and Pokhara) . These areas were selected considering feasibility and case load of MDR –TB patients in each region .

Definition of cases and controls

Definition of cases

A sample of patients who were diagnosed as having MDR TB (sputum culture and sensitivity confirmed) and who is permanent resident of Nepal were recruited for the study as a case.

Exclusion criteria:

MDR-TB patients less than 15 years of age.

Definition of controls

Controls were age -sex matched cured TB patients (who are not diagnosed as having MDR-TB) and who have completed TB treatment either from the same centre or any DOTS centre adjacent to that DOTS Plus centre.

Case: Control Ratio

To enhance the power of the study one case to two controls (1:2) were taken after consideration of the cost and precision of the study.

Matching

Two controls were matched with each case by

a. Age (± 2 year)

b. Sex (same)

Sample size estimation and Sampling method

Parameters used in the calculation of sample size:

The proportion of the general population exposed to risk factors (P_0) and Odds Ratio worth detecting (R) are the parameters needed in calculation of the sample size of a case –control study according to the following equation [3]

$$\frac{N = (1+1/C)\, p^{-1} q^{-1} (Z\alpha + Z\beta)^2}{(P_1 - P_0)}$$

Where

N = Sample size

Zα = Level of significance = 0.05

Zβ = Power of the study = 85% = 0.15

P_0 = Proportion exposed among general population = Prevalence of default from TB treatment = 26 .8% = 25% [4] [5]

C = Number of controls = 2

OR worth detecting = 2

Accordingly

No of cases = 55

No of Controls = 110

In a situation where multiple risk factors are considered, the ideal would be to calculate the sample size using lowest proportion exposed among general population (P_0) and Odds Ratio associated with risk factor since it gives the largest sample size.

After conducting thorough literature review prevalence of default from TB treatment will be taken as 25% for following reason. [4]

It is (defaulter from TB treatment) considered as the one of the most important variable influencing the MDR –TB and this figure is considered relatively small figure compared to the proportion of the exposure due to other risk factors of MDR-TB. (E.g. substance abuse) Also this study is from developing country which is similar to Nepal context.

Therefore the required sample size of 55 subjects was collected as a consecutive sample diagnosed as having MDR-TB in Nepal.

Number of cases = 55

Number of controls = 110

Total number of subjects = 165

Sampling method for cases:

For this study following DOTS Plus centers and DOTS Centers were selected from Central, Western and Eastern region. (Table 1)

Table 1:DOTS Plus Centres and DOTS Centres selected for the study

Region	DOTS PLUS Centre	DOTS Centre	No of Cases	No of Controls
Central	National TB Centre	Thimi Health Post	15	30
Central	GENETUP - Kalimati	GENETUP – Kalimati	10	20
Western	Regional TB Centre – Pokhara	Regional TB Centre – Pokhara	15	30
Eastern	NATA, Morang	NATA, Morang	15	30
Total			55	110

Fifty five MDR-TB cases were randomly selected from above centres using lottery method. Since proportionately higher number of cases are getting treatment from Central region more cases were recruited from Central region.

Sampling method for controls:

Age, sex matched controls were selected randomly from the cured TB patients which registered in the DOTS registry which is kept in the above centers. If the DOTS Plus centre has no DOTS Centre, adjacent DOTS Centre was selected for the controls selection (Ex: NTC- Nepal is only a DOTS Plus centre, hence adjacent DOTS centers (Thimi Health Post) was selected for this study.) Controls were interviewed in their residence with prior appointment. Every effort was taken to preserve confidentiality during interview.

Data Collection Technique

Data was collected by a team of researchers from National TB Programme, Nepal and SAARC TB and HIV/AIDS Centre using an interviewer administered, pre coded structured questionnaire for both cases and controls. Informed consent was obtained from all the cases and controls before interviewing them. The consent of the cases and controls was obtained in the same manner.

Ethical Consideration:

The following ethical issues were considered in the design of the study.

- The cases and controls were briefed regarding the nature, objectives, and method of the study and their voluntary participation acquired.
- Cases and controls were given the option to withdraw from the study at any point of time.
- Total confidentiality with regard to the identification of the cases and controls was assured at all times during and after the study.
- Permission and Consent were obtained from relevant authorities (National TB Control Programme) before commencement of the study.

Data collection instrument

An interviewer administered, pre coded, structured questionnaire was used to collect data. This questionnaire consisted of the following components.

- Socio –Demographic data
- Details of previous and current Tuberculosis status
- Details of previous treatment history and contact history
- Details of previous/present medical and surgical history
- Knowledge and barriers to adhere DOTS
- Quality of professional –patient interaction
- Details of social history

Statistical Analysis:

Statistical analysis was done using SPSS Version 16 soft ware package.

The following steps were followed to analyze the data

1. Basic assessment of the crude risk of MDR-TB by calculating the crude Odds Ratio through univariate analysis.
2. Controlling of confounding effects done by using Matched analysis and Multivariate analysis.

RESULTS:

Table 2 shows the Socio Demographic variables of cases and controls

Variables	Cases	Controls	Odd Ratio(95%CI)	Significant
Other Religion	8 (34.8)	16 (65.2)		
Hindu	47 (33.3)	94 (66.6)	1 (0.4-2.5)	Not significant
Illiterate	13 (26.6)	33 (73.4)		
Literate	42 (35.8)	77 (64.1)	0.65 (0.31-1.38)	Not significant
Unmarried	33 (41.8)	46 (58.2)		
Married	22 (35.6)	64 (74.4)	2.1(1.07-4.01)	Significant
Unemployed	26 (57.7)	19 (42.2)		
Employed	29 (24.2)	91 (75.8)	4.3 95(2.1-8.7)	Significant
Monthly family income Less than NRs 5000	37 (30.7)	63 (63.0)		
Monthly family income 5000 or more NRs	18 (27.7)	47 (72.3)	1.53(0.78-3.03)	Not significant
Nuclear	48 (40.3)	71 (59.7)		
Extended	07(15.2)	39(84.8)	3.8(1.58-9.050	Significant
Place of living (Other's home)	30(51.7)	28(48.3)		
Place of living(Own home)	25(23.4)	82(76.6)	3.5(1.77-3.67)	Significant
Number of bed rooms-one	12(54.5)	10(45.5)		
Number of bed rooms more than one	43(30.0)	100(70.0)	2.8(1.13-6.90)	Significant
Plaace of living -Rural	29(55.8)	23(44.2)		
Urban	26(23.0)	87(77.0)	4.2,(2.1-8.5)	Significant
Current smoker	14(35.0)	26(65.0)		
Not a current smoker	41(32.8)	84(67.2)	1.10(= 0.5-2.4)	Not significant
Regular Alcoholics	43(37.4)	72(62.6)		
No taking alcohol for past 5 years	12(24.0)	38(76.0)	1.89(0.86-3.85)	Not significant

Religion other than Hindu, literate persons current smoking, alcoholics and, monthly family income less than 5000 NRs have higher risk of getting MDR-TB. But these associations were statistically not significant. There was a significant association of getting MDR-TB and unmarried civil status, unemployment living in nuclear family, not living in their own home, living in rural area and only one bed room in the house.

Table 3: shows the comparison of disease related variables among cases and controls

Table 3: Disease related comparison of cases and controls				
Variables	Cases	Controls	Crude Odd Ratio(95%CI)	Significant/not
Previous history of TB-Present	53 (46.0)	62(54.0)		
No previous history of TB	02 (4.0)	48(96.0)	20.5 (4.76-88.2)	Significant
Contact history of TB	33 (52.4)	30(47.6)		
No contact history of TB	22 (21.6)	80(78.4)	4.95 (2.0-7.9)	Significant
HIV Positive	07(87.5)	01(47.6)		
HIV Negative/Unknown	48(30.6)	109(78.4)	15.9(1.92-133)	Significant
Non regularity of TB treatment	08 (88.9)	01(11.1)		
TB treatment taken regularly	45(28.8)	109(71.2)	19.4 (2.27-151)	Significant
Distance to DOTS clinic more than 5Km	13 (61.9)	08(38.1)		
Distance to DOTS clinic less than 5 Km	42(29.2)	102(70.8)	3.95, (1.52-10.2)	Significant
Travel cost to treatment centre more than 50Nrs per day	15(71.4)	06(78.6)		
Travel cost to treatment centre less than 50Nrs per day	40(27.8)	104(72.2)	6.5, (2.36-9.78)	Significant

There was a significant association of getting MDR-TB and previous history of TB, contact history of TB, HIV positive patients, non regularity of TB treatment, distance to DOTS clinic more than 5Km,and travel cost to treatment centre more than 50Nrs per day

Final fitted model for multi variety analysis for the selected risk factors were done. Results are shown in Table 4.

Table 4: Final fitted model for multi variate analysis for the selected risk factors				
Variable	aOR	df	p	Significant/not
Past Un-employment	4.87	1	0.000	Significant
Un-married	2.24	1	0.016	Significant
Nuclear family	4.5	1	0.002	Significant
Not living in own house	2.1	1	0.608	Not significant
Having only one bed room	1.34	1	0.956	Not significant
Previous history of TB	14.54	1	0.000	Significant
Contact history of TB	3.45	1	0.021	Significant
HIV positive	13.2	1	0.000	Significant
Not adherence to DOTS	16.8	1	0.000	Significant
Distance more than 5Km	3.6	1	0.03	Significant
Travel cost > than 50 NRs	5.8	1	0.002	Significant
Living in rural area	4.1	1	0.04	Significant

oar- Adjusted Odds Ratio,df-Degree of freedom,

Except not living in own house and having only one bed room all other variables which were significant in the univariate analysis were significant in the multy variety analysis.

DISCUSSION:

In the present study, the following factors were found as risk factors for MDR-TB: unemployment, unmarried civil status, those living in nuclear family, previous history of TB, contact history of TB, HIV positive status, non adherence to DOTS during previous treatment, travel cost to DOTS centre (more than 50 Nepali Rs), distance to DOTS centre (more than 5Km), and living in a rural area. A study done by Ahmed et al (2003) in Pakistan identified history of TB treatment, being a male, belonging to 15-25 years age group, having 1-5 years of schooling or having TB patient in the household as a risk factors for MDR-TB [5]. According to the study conducted in Peru by Pablo et al (2003), inadequate treatment regimen, poor adherence to treatment, and HIV infection were the risk factors for MDR-TB [6].

In a case control study conducted in four European countries it was found that IV drug usage, asylum –seeker support as income factor, living in a nursing home, previous TB with pulmonary location, living in a prison, known TB contacts , and Immune suppression other than HIV/ AIDS were the risk factors for MDR-TB[7] .

In the present study HIV infection was found to be as a risk factor for MDR-TB in Nepal. As reported in other studies from Sub Saharan Africa, and the recent Global project on drug resistance, no significant association was observed between the MDR-TB and HIV infection status in the new cases of MDR-TB [8][9][10][11]. Numerous MDR-TB outbreaks have been documented in HIV patients, and in some areas of the world HIV is a risk factor for MDR-TB .[12]

One notable finding in the present study was that difficult and accessibility to health services due to the distance from the health centre being more than 5 Kms. was associated with one getting MDR-TB. The results suggests that patients with MDR-TB coming to the DOTS centre from more than 5Km away are at a great risk of developing MDR TB than patients living nearby to the health services facilities.

High travel cost (more than 50 NRs) may lead to poor patient compliance and be considered as a risk factor for MDR-TB. Previous studies have revealed that the non complaint patients spent more time and cost for traveling to the treatment centre than the compliant patients. The time spent traveling to the centre could be used for other purposes. For those in employment, travel time represents time absent from work.

Hence, non compliance with TB treatment was one of the strongest predictors of MDR-TB. Non compliance with treatment is not only harmful for the patients, but its consequence may be much more severe for the general population.

The substantially higher risk of MDR-TB among unemployed patients is possibly a function of socio economic status. Unemployment may have served as an indirect measure of the patient's functional and socio–economic status. Impoverished populations are well documented in many setting to have higher rates of TB than the general population. The cycle between poverty and TB is broadly recognized [13].

Unmarried civil status was a risk factor for MDR-TB. On the premise that patients are less likely to default if they live with many family members mainly with wife and children who provide encouragement and remind to keep medical appointments than living alone or living with friends,

In the present study those individuals present in rural areas were at high risk for MDR-TB compared to urban areas as the rural inhabitants do not have the same level of access to health and social services as their urban counterpart. Similar findings were reported in study done by Boyte et al 2001 [14].

Patients in the present study who smoked tobacco were considered as at risk for MDR-TB. However it was statistically not found to be risk factor.

Though previous studies have reported that alcoholism is an important risk factor for MDR-TB, our study did not reveal that alcoholism contributed to MDR-TB.[15].

Previous studies reported previous history of tuberculosis treatment is a significant risk factor for developing MDR-TB as in the present study [14].

The present study revealed that previous TB treatment is a risk factor for MDR-TB. In a study of 876 patients in a 6 countries, the re-treatment success rate was 57% and 29% with MDR-TB [16]. Previous resistance surveillance conducted in Nepal also revealed higher rates of MDR-TB among re-treatment group [17] .These results suggest that the higher incidence of MDR-TB were inevitably associated with lower success rate in re treatment group.

Contact with a TB patient at home, in the work place, or elsewhere was a risk factor. These findings might be affected by recall bias because cases were perhaps more likely to remember a history of exposure than controls.

CONCLUSION:

1. Despite a successful implementation of a TB control programme in Nepal, drug resistant TB remains an important issue.
2. HIV sero-positivity (OR=15.9) was significantly associated with MDR-TB in Nepal
3. Travel cost of more than 50 NPR per day (OR=6.5) and distance of more than 5 KM to DOTS centers were significant risk factors for MDR-TB.
4. Previous history of TB, contact history of TB (OR=3.8) and previous non adherence to

DOTS (OR=18.6) were significant risk factor for MDR-TB in Nepal

5. The socio demographic factors such as unmarried civil status (OR=3.3), unemployment (OR=3.4), living in rural area (OR =4) and nuclear family setup (OR=6) were significant risk factors.
6. Many risk factors were related to the DOTS.

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RECOMMENDATION:

1. Analysis of risk factors for MDR-TB are useful for improving programme performance and should be undertaken in other member states also in the SAARC region where prevalence of MDR-TB is high.
2. Further development of MDR-TB should be prevented by sensitizing private practitioners, community members and specialists on issues related to development of drug resistance. This study results can be used as a tool for this.
3. A National HIV prevalence survey among MDR-TB patients should be carried out every two years not only in Nepal but also in other SAARC Countries.
4. Travel cost more than 50 NPR per day and distance to DOTS centre more than 5 KM were significant risk factors for MDR-TB. Accessibility of services should be improved especially in rural areas where the patients have to travel long distance to avail the services. Currently MDR-TB patients are getting some allowance in Nepal to attend the clinic. If possible, in addition, this allowance should also be extended to poor drug sensitive TB patients to enhance treatment adherence and better patient's compliance.
5. DOT for both drug sensitive tuberculosis and drug resistance tuberculosis should be strictly adhered to.
6. Strengthening of DOTS programme to overcome the identified risk factors can reduce the MDR-TB burden in Nepal.

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IDENTIFICATION OF *rpoB, gyrA* AND *embB* GENE MUTATIONS IN *MYCOBACTERIUM TUBERCULOSIS* ISOLATES FROM RETREATMENT TUBERCULOSIS PATIENTS IN NEPAL

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ABSTRACT

Introduction:

Tuberculosis remains one of the major public health problems in Nepal and increasing trend of multi drug resistant and extensively drug resistant tuberculosis (MDR /XDR TB) is a big challenge. Rapid diagnosis and appropriate treatment of MDR/XDR TB is crucial. Identification and comparison of MDR TB using rapid molecular techniques (for *rpob, gyrA, rrs* and *embB* gene mutations) with reference to drug susceptibility test (DST) were the main objectives of this study.

Methodology:

A cross sectional study was carried out in National TB Centre (NTC). Gene Xpert, proportion method and Line Probe Assay (LPA) were used for first and second line drugs susceptibility testing (FLD-DST and SLD-DST). A total of 29 mucopurulent sputum samples were freshly collected from retreatment TB patients (Female 41.4%, Male 58.6%) with median age of 40 years attending to the four MDR TB treatment centres of eastern and central Nepal (via private courier and directly to National TB Reference Laboratory (NRL) at NTC from April 2013 to October 2017.

Results:

Among 29 sputum samples (Female 41.4%; all smear+ve, Male 58.6%; 16 smear+ve and 1 smear-ve), Gene Xpert MTB/RIF assay detected 100% *M. tuberculosis* and rifampicin resistance (*rpoB* gene resistant) of which, 100% were culture positive by conventional Lowenstein–Jensen (LJ) method. FLD-DST was performed on all culture positives of which, 96.6% showed MDR TB and 3.4% showed mono resistance to isonizid only. SLD-DST on 29 MDRTB strains by LPA showed 100%, 58.6%, 44.8% wild type for *rrs*, *gyrA* and *emb B* genes respectively. Mutation for *gyrA* and *emb B* genes was 41.4% and 51.2% respectively, *rrs* genes none. Twelve (Female 6, Male 6) MDR TB strains were identified as pre-XDR-TB. Chi square (χ^2) for trend was used to analyze Gene Xpert, smear, FLD-DST and LPA results.

Conclusion:

From this study, 29(100%) MDRTB were detected from retreatment TB cases by Gene Xpert and FLD-DST. Almost 41.4% MDR TB strains were detected as pre-XDR TB by LPA, which were found to be higher in 15-60 years group of females and males. Samples from retreatment TB patients need to be tested by rapid molecular techniques with reference to culture and DST.

Key words:

Mycobacterium tuberculosis, Gene Xpert, Line Probe Assay, Multi and Extensively Drug Resistance.

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INTRODUCTION

Tuberculosis (TB) is a top infectious killer disease worldwide. Over 95% of TB deaths occur in low- and middle-income countries, and it is among the top 5 causes of death for women aged 15 to 44. Globally in 2014, an estimated 480, 000 (an estimated 3.3% of new TB cases and 20% of previously treated cases) people developed multidrug-resistant TB (MDR-TB) and 190, 000 people died of MDR-TB. An estimated 43 million lives were saved through TB diagnosis and treatment between 2000 and 2014. *M. tuberculosis* strains identified as MDR TB (resistant to isoniazid and rifampicin with or without other first line anti-TB drugs plus any fluoroquinolone and at least one of three injectable second-line drugs is defined XDR TB. MDR TB resistant to either of fluoroquinolones or injectable aminoglycosides is categorized as pre-XDR-TB. An estimated 9.7% of people with MDR-TB have XDR-TB.[1] The SAARC region, with 34% of the global burden of TB, a total 81,142 estimated cases of MDR-TB among notified cases were notified in 2013, of which 59% were previously treated cases.[2]

Tuberculosis (TB) remains one of the major public health problems in Nepal. In 2014, total of 37,025 cases of TB were registered. Most cases were reported among the middle aged group with the highest among 15-24 years of age (20%). TB-HIV co-infection rate in Nepal is 2.4% (HIV among TB) and 11.6% (TB among HIV) based on the sentinel survey, 2011/12. Nationwide, the proportion of multidrug-resistant TB (MDR-TB) was 2.2% among new cases and 15.4% among retreatment cases based on survey carried out in 2011/12. In 2014, total of 349 MDR TB and 25 XDR TB were enrolled for treatment. WHO estimated 4.6 (2.1-7.5) thousand people died from TB in 2014 (NTP Annual report Nepal 2070/71 or 2014).[3] One of the concerned aspects of drug resistance in Nepal is the high level of resistance to fluoroquinolones (26.4%), which leads to heavy burden of pre-XDR and XDR-TB among MDR-TB patients (8% of the cases were found to be XDR among MDR cases in the same survey). To combat the excess mortality related to XDR-TB, it is recommended to perform DST for second line-drugs to all MDR-TB cases at the start of treatment.[4]

Isoniazid (INH) with rifampicin (RIF) forms the cornerstone of short course chemotherapy for tuberculosis and resistance to either drug hampers the complete cure of patients. *M. tuberculosis* strains resistant to at least these two major frontline drugs (INH and RIF) develop multi-drug resistant tuberculosis (MDR-TB).[5]

More than approximately 95% RIF resistant *M. tuberculosis* strains have mutations in an 81 bp hot spot region (codon 507-533) of *rpoB* gene that encodes RNA polymerase beta subunit.[6,7,8,9]

Globally, more than half of all TB cases are not detected the result of health care system weakness and the inadequacy of available technology. If a diagnosis is absent, patients are not treated, transmission may continue, patients suffer needlessly and may eventually die.[10]

Cepheid (Cepheid, Sunnyvale, CA) has recently introduced the GeneXpert MTB/ RIF assay for research use only.[11] The GeneXpert assay is a real-time PCR test that will simultaneously identify *M. tuberculosis* and detect rifampin resistance directly from clinical specimens.[12,13,14] Rifampin resistance can serve as a marker for multidrug-resistant tuberculosis (MDR-TB) and has been reported in 95% of the multidrug-resistant *M. tuberculosis* isolates. The GeneXpert assay detects an 81-bp "core" region of the *rpoB* gene.

The suggested target sites of first line anti-TB drugs and the sites for most frequent mutations occur; for streptomycin, isoniazid, rifampicin, ethambutol, pyrazinamide were described. [15, 16, 17, 18, 19, 20, 21, 22] Similarly, the target sites of second line anti-TB drugs and the most frequent mutations occur for fluoroquinolone, injectable capreomycin and kanamycin were suggested.[23, 24]

The Gene Xpert MTB/RIF assay, conventional culture and FLD-DST and LPA (Genotype MTBDR*sl)* are the choice of DR/MDR TB and XDR TB diagnostics tools. Culture and FLD-DST method takes usually longer time but always being considered as the gold standard that gives the viable organisms and can be used for various research purposes.

The occurrence of MDR TB among retreatment (Cat1 and Cat 2 treatment failures) cases alerts the NTP managers for prompt diagnosis of TB/DR/MDR/ XDR TB using reliable and rapid diagnostics tools based on molecular biological techniques. The main objective of this study was to identify and compare the findings of DR/MDR TB using Gene Xpert MTB/ RIF assay (*rpoB* gene mutations) with reference to culture and drug susceptibility test (DST) and XDR TB (gyrA, rrs and embB gene mutations) by line probe assay (LPA).

METHODOLOGY

The study was descriptive type cross sectional study to identify rifampicin resistant or multidrug resistant tuberculosis (RR/MDR TB) among retreatment TB cases using Gene Xpert MTB/RIF assay and to compare the prospective data obtained with reference to conventional culture and FLD-DST as

well as to identify XDR TB among those MDR TB cases by LPA (Genotype MTBDRs*l* for SLD-DST).

Study site

This study was carried out from April 2013 to October 2017 in NRL at NTC, Thimi, Bhaktapur, Nepal.

Sample size

Twenty nine (29) retreatment tuberculosis patients (Female 41.4%; all smear+ve, Male 58.6%; 1 smear-ve, 16 smear+ve) with median age of 40 years were involved in this study before they were registered for starting second line anti-tuberculosis treatment.

Study population

Retreatment pulmonary TB cases (relapse, treatment after failure, and treatment after loss to follow-up) previously treated with Cat I and Cat II treatment regimen as per National TB Programme (NTP) guidelines based on WHO recommendations were enrolled in this study.

Inclusion/exclusion criteria

Retreatment TB cases (Cat I and Cat II failure) visiting for further diagnosis and diagnosed as sputum smear positive or negative before being registered for and started MDR treatment were included in this study. But the cases already registered and recently undergoing second line anti tuberculosis treatment, blood stained sputum, sputum with food particles, with saliva in greater amount, leaking, dried or if not freshly collected and patients suspected of extra-pulmonary tuberculosis were excluded from this study. The samples showing contamination during culture were not further included in the study.

Sample collection

Twenty nine early morning sputum samples (stuffy and mucopurulent, 3-5ml each) were collected from retreatment TB patients in leak proof, wide mouthed, transparent and sterile 50 ml disposable plastic centrifuge tube (Falcon BD, USA); then were appropriately labeled and stored at refrigerated temperature (2-8^{o}C) until dispatched or processed. Out of total 29 samples 16 were received; 11(F3/M8) from Nepal Anti TB Association (NATA) Biranagar Morang, 5(F4/M1) from BP Koirala Institute of Health Sciences (BPKIHS) Dharan Sunsari of the eastern development region. Similarly, the remaining 13 samples were received; 3(F2/M1) from National TB Centre (NTC) Thimi Bhaktapur, 3(F1/M2) from United Mission to Nepal Hospital (UMN) Lalgarh Janakpur and 7(F2/M5) from National Medical College (NMC) Birgunj Parsa of central development region. The samples from the centres other than NTC were transported through private courier and the duration of sample transportation was not more than 48 hours. Some patients being treated at National TB Centre (one of the MDR TB treatment centres in central development region) submitted fresh samples directly to NRL/NTC.

Sample processing

Sputum samples were processed inside a Biological Safety Cabinet class II (BSC-II AIRTECH, Japan) directly by adding twice the volume of 4.0% NaOH digestion method (modified Petroff's method), vortex mixed and left for 15 minutes at room temperature with occasional shaking. Then phosphate buffer (pH 6.8) were added up to level of 45 ml graduation mark, vortex mixed and centrifuged at 3000x g for 15 minutes in a refrigerated centrifuge at 4^{o}C (KUBOTA, Japan).

Culture on Lowenstein Jensen (LJ) medium

The supernatants were discarded and pellets were used for culture; 0.2ml of pellet was inoculated on duplicate LJ media, incubated at 37^{o}C for 4-8 weeks in an incubator (MEMMERT, Germany). The tubes were examined on 7th day for rapid growers and were checked for growth at 2, 3, 4, 5, 6, 7, 8 weeks until negative. If there was any contamination in the culture tube, was recorded.

Gene Xpert MTB/RIF Assay

From the remaining of the pellets, Gene Xpert MTB/RIF tests were performed by following the procedures provided by the manufacturers (Cepheid Sunnyvale, CA, USA, and Gene Xpert IV Cephied, France). Sputum pellets were decontaminated and treated with sample reagent (SR); a mixture of NaOH and iso-propanol. The SR was added at a 3:1 ratio to the sputum pellets and left for 15 minutes at room temperature; 2 ml of the treated samples were transferred to the Gene Xpert cartridges (Cephied, France), which were then loaded into the programmed Gene Xpert modules. Gene Xpert device was kept on and results were observed after the whole process completed (within about 2 hours).

Smear microscopy

A smear of the processed pellets was prepared (size of 2*3 cm), air dried at room temperature (RT), heat fixed, stained by Ziehl-Neelsen method and read under binocular light microscope at the total magnification of 1000X (Olympus, Japan) and reported according to NTP Nepal grading scale that is adopted exactly same scale from WHO/IUATLD grading scale.

Preparation of Bacillary Suspension and inoculation for DST

One looful (4mg approximately) of mycobacterial colonies grown on LJ media was harvested and emulsified with 1ml of sterile distilled water (SDW) in a sterile bijou bottle, vortex mixed and allowed to stand for 15 minutes, supernatant was transferred into a McCartney bottle. Turbidity of supernatant was compared with McFarland Standard No.1 Nephelometer (standardized at 1 mg/ml equivalent to 10^6-10^8 CFU/ml). Made 100 fold dilutions from McFarland Standard No.1 suspension; 1 loopful (nichrome wire loop 24 SWG and 3mm diameter delivering 0.01ml) of bacillary suspension was transferred to 1ml of SDW in bijou bottles and vortexed (10^{-2}: 10,000 CFU/ml), similarly 10^{-4}(l00 CFU/ml) was prepared. One loopful of each dilution (10^{-2} and 10^{-4}) was inoculated on two slopes of plain LJ medium (controls) and one set each of slopes with 4 drugs (streptomycin, isoniazid, rifampicin, ethamputol), incubated at 37°C, read on 4th and 6th week for resistant (growth on drug medium ≥ 1% colonies on control) and final susceptible (no or <1% colonies on control) patterns respectively.

Biochemical identification tests

From the positive growth, identification tests were performed by biochemical methods i.e. growth on PNB containing LJ medium and niacin production tests.

1. *Growth on PNB containing media*

A loopful of neat bacterial suspension (McFarland standard No. 1) was inoculated into one slope of LJ medium and one slope of p-nitrobenzioc acid (PNB) at a concentration of 500 g/ml and incubated at 37°C for each set. Read on 28th day.

M. tuberculosis does not grow but all other mycobacteria are resistant to PNB.

M. tuberculosis H_{37}Rv as negative control (PNB susceptible) and *M. kansasii* as positive control (PNB resistant) were used.

Results and interpretations

No growth on PNB medium: *M. tuberculosis*

Growth on PNB medium: *M. kansasii*

2. *Niacin production test*

BBL Taxo TB Niacin test strips (Becton and Dickinson, USA), absorbent paper strips and TB niacin positive test control paper discs were used according to the manufacturer's instruction. With a sterile transfer pipette, approximately 0.6 ml of the positive culture broth extract was transferred to the bottom of 20 mm × 125 mm screw cap test tube. Negative control was also prepared. The strips were dropped with arrow downward into the tubes. Positive controls, negative controls and test culture tubes were recapped immediately. The colors of the extracts were then compared after 15 minutes.

M. tuberculosis H_{37}Rv as positive control and *M. kansaii* as negative control were used. Niacin accumulation was indicated by vivid appearance of a yellow color in the extract.

Results and interpretation

M. tuberculosis H_{37}Rv: yellow colour (niacin positive)

M. kansaii: colourless (niacin negative)

All the positive cultures have shown PNB negative and niacin positive.

Drug Susceptibility Test on First Line Drugs (proportion method)

Drug susceptibility test (DST) on first line anti tuberculosis drugs (FLD); streptomycin(4.0 µg/ ml), isoniazid(0.2 µg/ ml), rifampicin(40.0 µg/ml), and ethambutol(2.0 µg/ml) (SIRE; manufactured by SIGMA-Aldrich, USA) was performed on all the culture positive samples in duplicated drug tubes as well as two LJ slopes without drug (control) using 1% proportion method. Internal quality control was routinely performed (for each batch of new drug media) using the reference strain *M. tuberculosis* H37Rv (ATCC-27294), which was susceptible to all the 4 drugs. All the inoculated tubes were then incubated at 37°C, resistance pattern of the SIRE was checked at 4th week and 6th week.

Drug Susceptibility Test on Second Line Drugs by Line Probe Assay

The whole process of the LPA was followed as per the guideline provided by the manufacturer-MTBDR*sl* 96 version 1.0 (HAIN Life Science, GmbH Germany) following the steps of DNA extraction, PNM mix, amplification by PCR, hybridization and detection as mentioned below:

1. DNA extraction

Homogenized bacterial suspension was prepared by harvesting 1-2 colonies of organisms from LJ tube with sterile inoculating wire loop inside a BSC-IIA (Micro Flow, Bioquell, UK), re-suspended in 300 µl of molecular grade water in a cryovial (1.5-2ml), mixed by vortexing (SONAR, India), heat inactivated at 95°C for 20 minutes, incubated in an ultrasonic water bath (LABTECH, India) for 15 minutes, centrifuged for 5 minutes at 13000*g (Microfuge, KINTARO) and supernatant containing DNA was transferred to another cryotube and stored at 4°C to -20°C until processed in a refrigerator (SANYO, Japan).

2. Primer nucleotides mix (PNM)

With micropipettes 35µl of PNM, 5µl of 10x buffer (15mM MgCl2), 2 µl MgCl2 (25mM), 3 µl H_2O, 0.2 µl Taq polymerase (Hot star Thermis aqaticus) were added into a cryotube and mixed well carefully. Prepared a master mix for the determined number of samples, mixed and aliquoted (45 µl) in 1.5ml PCR tubes. PNM process was completed inside a LPA Safety Hood (LAB COMPANION). The molecular grade water to bring the master mix to volume was used as conjugate control (CC) and the LPA strip (functions as both the internal "PCR positive control" and the "inhibition positive control) was used as amplification control (AC).

3. Amplification by PCR (Thermal Cycler, USA)

To the aliquoted 45 µl master amplification mix, 5 µl DNA was added (inside a BSC-IIA), gently vortexed to mix, placed into the thermal cycler (Genotype Hot 30 specific progamme). The DNA amplification was performed for 30 cycles following an initial denaturation; 10 cycles of initial denaturation followed by denaturation at 95°C for 30 seconds, chain elongation at 58°C for 2 minutes followed by additional 20 cycles of denaturation at 95°C for 25 seconds, primer annealing at 53°C for 40 seconds and elongation at 70°C for 40 seconds and final extension at 70°C for 8 minutes.

4. Hybridization and Detection

The hybridization buffer (HYB) and stringent wash solution (STR) were prewarmed at 45°C to dissolve the undissolved precipitates, rinse solution (RIN) and DW were prewarmed at RT, freshly diluted Con-C and Sub-C 1:100 in the respective dilution buffer and protected from light. Twincubator set with P1 programme was used for Hybridization and Detection.

4.1 Hybridization probe

Denaturing buffer (DEN) 20 µl was added with 20 µl amplicon, mixed well and incubated for 5 minutes at RT on the shaking platform, 1ml HYB was added using micropipette and filter tips, mixed by tilting the tray back and forth carefully (purple DEN and green HYB mixed well), tray was then placed on the TwinCubator (HAIN Life Science, GmbH Germany). DNA strip was placed in each well (with a forceps) of TwinCubator tray and covered by the liquid. When the temperature reached to 45°C, tray cover was closed and incubated for 30 minutes at 45°C.

4.2 Detection probe

HYB was aspirated completely and 1 ml STR was added and incubated for 15 seconds at 45°C, STR removed completely, 1ml RIN added, incubated for 1 minute at RT, RIN removed completely, 1ml diluted conjugate (10 µl Con-C /conjugate C and 990 µl Con-D/conjugate D) were added and incubated for 30 minutes at RT and conjugate was removed completely. Added 1ml RIN, incubated for 1minute at RT, removed RIN completely and rinsed with H_2O for 1 minute, 1ml diluted substrate added (10 µl Sub-C and 990µl Sub-D), incubated 2-10 minutes at RT and removed substrate completely. Stopped reaction by rinsing twice with H_2O for 1 minute then removed DNA strip from tray and dried it on absorbent paper. Detection process was completed using a TwinCubator (HAIN Life Science, GmbH Germany).

Individual strip after colour development was adhered to the corresponding column of the HAIN Life Science, GmbH Germany provided format and resistance pattern was identified. The original strips showing positive bands were kept for NRL record after being scanned for the present study purpose (Figure 1).

Statistical data analysis

The statistical analysis of the study data were analyzed using SPSS version 16.0 software. The Chi square test was used to compare age and sex wise distribution of negative and positive sputum smear results, smear and culture results, MDR TB identified by Gene Xpert MTB/RIF assay and conventional culture and FLD-DST results, identification of XDR/ pre XDR TB cases by LPA (MTBDR*sl*). The *P*-value <0.05 was considered statistically significant.

RESULTS

The sputum smear microscopy results for female (12/41.4%) were all +ve, whereas for male (17/58.6%); 16 +ve and 1-ve. The age wise distribution of smear results was; 3.4% was smear-ve in 15-29 years and 51.7%, 20.7%, 13.8% and 10.3% in 15-29, 30-45, 46-60 and above 60 years of age groups respectively were smear positives (Table 1).

Table 1: Age (years)/sex wise distribution of retreatment TB cases from different treatment centres

Treatment centre	15-29		30-45		46-60		>60		Total		Remarks
	F	M	F	M	F	M	F	M	F	M	
E+E1	3	2		2		2		2	3	8	*NATA Morang*
E2	2		2			1			4	1	*BPKIHS*
C	2	1							2	1	*NTC*
C1	1	2							1	2	*UMN Lalgarh*
C2	1	2	1	1		1		1	2	5	*NMC Parsa*

A total of 29 freshly collected good quality sputum samples from 5 DR/MDR treatment centres; E+E1: Nepal Anti TB Association (NATA) Morang, 11 (F3/M8) samples and E2: BP Koirala Institute of Health Sciences (BPKIHS) Sunsari Eastern Nepal; 5 (F4/M1) samples. C: National TB Centre (NTC) Kathmandu; 3 (F2/M1) samples, C1: United Mission to Nepal Hospital (UMN) Lalgarh Dhanusha; 3 (F1/M2) samples and C2: National Medical College (NMC) Birgunj Parsa Central Nepal; 7 (F2/M5) samples. There was no significant difference of age and sex wise smear results (p>0.05). All 29 sputum specimens showed rifmapicin resistance (RR/MDR) by Gene Xpert MTB/RIF assay in which 55.2%, 20.7%, 13.8% and 10.3% in 15-29, 30-45, 46-60 and above 60 years age group and 41.4%, 59.6% females and males respectively (Table 2).

Table 2: Age (years)/sex wise distribution of MDR TB cases diagnosed by Gene Xpert from different treatment centres

	15-29		30-45		46-60		>60		Total		Grand total	Remarks
	F	M	F	M	F	M	F	M	F	M		
	3	2		2		2		2	3	8	11	NATA Morang
	2		2			1			4	1	5	BPKIHS
	2	1							2	1	3	NTC
	1	2							1	2	3	UMN Lalgarh
	1	2	1	1		1		1	2	5	7	NMC Parsa
Total	9	7	3	3		4		3	12	17	29	

The age wise distributions of MDR TB by Gene Xpert MTB/RIF assay for 15-29 years, 30-35 years, 46-60 years and above 60 years of age group were 55.2%, 20.7%, 13.8% and 10.3% respectively. Similarly, sex wise MDR TB detection by Gene Xpert was 41.4% and 59.6% for females and males respectively. Similarly, all 29 specimens showed positive growth for all age and sex groups on LJ culture media (Table 3). There was no significant difference of age and sex wise smear and culture results (p>0.05).

Table 3: Age (years)/sex wise Comparison of smear and culture results

Results	Age and sex distribution								Total culture positive		Grand Total culture positive
	15-29		30-45		46-60		>60				
	F	M	F	M	F	M	F	M	F	M	
S+ C+	9	7	3	3	0	4	0	3	12	16	28
S+ C-	0	0	0	0	0	0	0	0	0	0	0
S- C+	0	1	0	0	0	0	0	0	0	1	1
S- C-	0	0	0	0	0	0	0	0	0	0	0
Contamination	0	0	0	0	0	0	0	0	0	0	0

A. χ^2 for trend of age wise smear results=0.842, df=3, P value=0.840 (>0.05), so there was no significant difference of age wise smear results.

B. χ^2 for trend of sex wise smear results=0.731, df=1, P value=0.393 (>0.05), so there was no significant difference of sex wise smear results.

The culture positives were biochemically (PNB-ve, niacin test+ve) identified as *M. tuberculosis* which were then processed for FLD-DST following proportion method. Twenty eight (96.6%; 11F/17M; ss-ve 1M, 11 F and 16M ss+ve) of 29 FLD-DST) showed both INH and RIF resistance, 1 case (3.4%/M) detected as RRTB by Gene Xpert MTB/RIF showed mono resistance to isoniazid only (Table 4).There was no significant difference of age and sex wise MDR TB cases identified by conventional FLD-DST for smear results (p>0.05).

Table 4: Age (years)/sex wise distribution of FLD-DST patterns

DST patterns of FLDs by conventional proportion method											
	15-29		30-45		46-60		>60		Total		Grand total
	F	M	F	M	F	M	F	M	F	M	
Total Tested	9	7	3	3	0	4	0	3	12	17	29
Fully Susceptible	0	0	0	0	0	0	0	0	0	0	0
Any Resistance	1	0	0	0	0	0	0	0	1	1	
Mono Resistance	1	0	0	0	0	0	0	1	1	1	1
S	0	0	0	0	0	0	0	0	0	0	0
I	1	0	0	0	0	0	0	0	1	0	1
R	0	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0	0
Total I+ R Resistance (MDR)	8	7	3	3	0	4	0	3	11	17	28
IR	2	2	0	2	0	0	0	0	2	4	6
IRE	1	0	0	0	0	0	0	1	1	1	2
SIR	1	3	2	0	0	2	0	1	3	6	9
SIRE	4	2	1	1	0	2	0	1	5	6	11

S: streptomycin I: isoniazid R: rifampicin E: ethambutol

A. χ^2 for trend of age wise MDR TB detected by culture and DST results=0.842, df=3, P value=0.840 >0.05, so there was no significant difference of age wise MDR TB detection by culture and DST.

B. χ^2 for trend of sex wise MDR TB detected by culture and DST results=1.467, df=1, P value=0.226 >0.05, so there was no significant difference of sex wise MDR TB detection by culture and DST.

All the MDR TB cases detected by Gene Xpert MTB/RIF assay and/or FLD-DST were performed for DST on second line anti-TB drugs (SLD-DST) by LPA. During the process of LPA, negative controls were developed at CC and AC bands in the strips. MDR TB strains showed TUB bands formation in the strips. Similarly, 17(58.6%, F5/M12) for *gyrA* WT1, WT2, WT3 probes located in regions from codons 85 to 97 (binding sites for fluoquinonlones / ofloxacin or levofloxacin), 29(100%, F12/M17) for *rrs* WT1, *rrs* WT2 probes located in regions for nucleotides 1401,1402 and 1484 (binding sites for injectable aminoglycoside / capreomycin), and 13(44.8%, F4/M9) for *emb B* WT1 (binding site for ethambutol) gene probes located in regions from codons 306 were found to be susceptible sites for the corresponding drugs. Whereas, the DNA matched with the mutant probes; 12(41.4%, F6/M6) were mutants for *gyrA* gene (*gyrA* MUT1, MUT2, MUT3A to 3D) conferring most frequently mutation occurring codons (A90V, S91P, D94A, D94N/Y, D94G, and D94H), 16(51.2%, F8/M8) for *emb B* (*embB* MUT1A, MUT1B) probes conferring mutations M306V and M306I and none were for AG/CP or *rrs* genes (*rrs* MUT1, MUT2) probes conferring mutations for A1401G and G1484T. Similarly, *gyrA* gene mutations together with *embB* gene that were regarded as fluoroquinolones and ethambutol resistance (*embB* MUT1B) were found in 9 cases (31.0%, F5/M4). Any case showing single *gyrA* gene mutation or *gyrA* gene mutation together with or without other gene mutations was interpreted as pre-XDR TB (Figure 1).

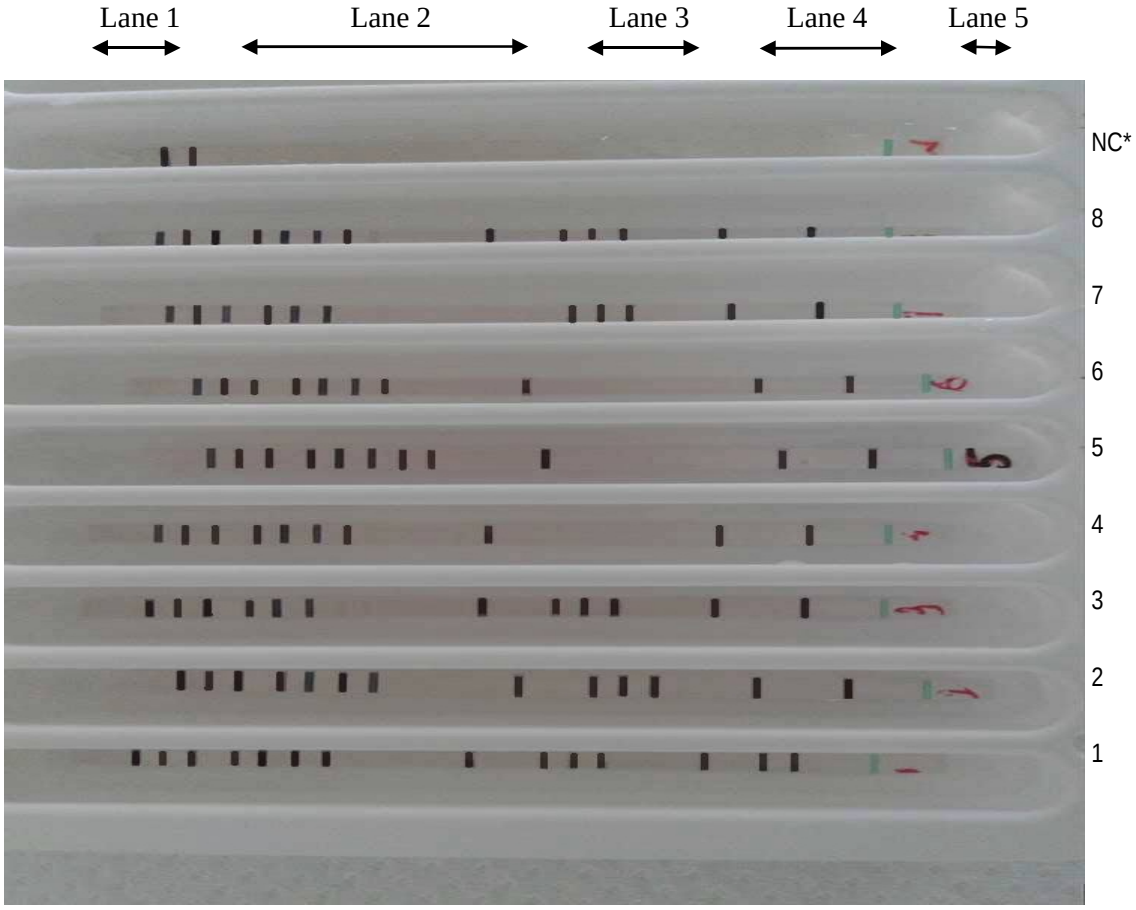

Figure 1: Hybridization process and development of different bands on MTBDR*sl* strips

Lane 1: CC/AC/TUB *(CC: conjugate control, AC: amplification control, TUB: tuberculosis)*

Lane 2: *gyrA* (WT 1-WT 3/MUT 1- MUT 2, MUT 3A- MUT 3D) (*WT: wild type, MUT: mutation)*

Lane 3: *rrs* (WT1/MUT 1-MUT 2)

Lane 4: *embB* (WT 1/MUT 1A- MUT 1B)

Lane 5: M *(Marker)*

A total of 12(43.4%; F6/M6) among 29 MDR confirmed *M. tuberculosis* strains were found to be pre-extensively drug resistant (pre-XDR-TB), but no XDR TB. The age/sex wise distribution of the SLD-DST pattern showed maximum number of pre-XDR TB among 15-45 years of age groups and similar for both females and males (50%; 4F and 2M in 15-29, 33.3%; F2 and 2M in 30-45, 8.3%; M1 in 46-60 and 8.3%; M1 above 60 years). The mutations detected for *emb B* (ethambutol) genes were also similar for both females and males (F8/M8). Twelve cases thus identified as pre-XDR TB were from the MDR TB treatment centres providing treatment to the MDR TB patients were; 4 (F1/M3) out of 11 (F3/M8) from Nepal Anti TB Association (NATA) Biranagar Morang, 1 (F1) of 5 (F4/M1) from BP Koirala Institute of Health Sciences (BPKIHS) Dharan Sunsari of the eastern development region. Similarly, 1(F1) of 3 (F2/M1) from National TB Centre (NTC) Thimi Bhaktapur, 2 (F1/M1) of 3 (F1/M2) from United Mission to Nepal Hospital (UMN) Lalgarh Janakpur and 4 (F2/M2) of 7 (F2/M5) from National Medical College (NMC) Birgunj Parsa of central development region, were found to be pre-XDR TB (Table 5). There was no significant difference between XDR TB detection by LPA on MDR TB identified by Gene Xpert and FLD-DST (p>0.05).

Table 5: Age (years)/sex wise distribution of SLD-DST patterns by Line Probe Assay on MDR TB detected by Gene Xpert and FLD-DST

Description	15-29		30-45		46-60		>60		Total		Remarks
	F	M	F	M	F	M	F	M	F	M	
TUB	9	7	3	3		4		3	12	17	29
gyrA WT	4	5	1	1		3		3	5	12	17
gyrA MUT	4	2	2	2		1		1	6	6	12
rrs WT	9	7	3	3		4		3	12	17	29
rrs MUT											0
emb BWT	4	4		1		2		2	4	9	13
emb BMUT	5	3	3	3		1		1	8	8	16
Treatment centre	Patients tested for SLDST										Remarks
*E+E1	3	2		2		2		2	3	8	NATA Morang
E2	2			2		1			4	1	BPKIHS
**C	2	1							2	1	NTC
C1	1	2							1	2	UMN Lalgarh
C2	1	2		1		1		1	2	5	NMC Parsa
Treatment centre	Patients diagnosed as Pre-XDR TB										Remarks
E+E1	1			1		1		1	1	3	NATA Morang
E2			1						1		BPKIHS
C	1								1		NTC
C1	1	1							1	1	UMN Lalgarh
C2	1	1	1	1					2	2	NMC Parsa

** E+E1, E2: NATA Morang and BPKIHS Eastern Nepal. ** C, C1, C2: NTC, UMN and NMC of Central Nepal.*

From NATA Morang 4 (F1/M3), BPKIHS 1 (F1), NTC 1 (F1), UMN Lalgarh 2 (F1/M1) and NMC Parsa 4 (F2/M2) were found to be pre-XDR TB. χ^2 for trend of MDR TB by Gene Xpert, culture and DST and XDR TB detection by LPA=0.73, df=1, P value=0.393 (>0.05), so there was no significant difference between XDR TB detection by LPA on MDR TB by Gene Xpert and culture and DST.

The age wise distributions of pre-XDR TB by LPA on MDR TB by Gene Xpert MTB/RIF assay and FLD DST for 15-29 years, 30-35 years, 46-60 years and above 60 years of age group were 50.0%, 33.3%, 8.3% and 8.3% respectively. Similarly, sex wise MDR TB detection by Gene Xpert was 50.0% and 50.0% for females and males respectively.

DISCUSSION

Microscopy is still familiar as a main diagnostic technique of diagnosing tuberculosis in resource-limited countries including Nepal. Due to shortcomings of conventional technique, novel molecular techniques are needed that combine the rapidity of microscopy and the sensitivity of culture. They can identify the mycobacterial species, and would help the clinician during the initial treatment of the patient. Though molecular techniques are not used routinely in Nepal, some investigators reported its feasibility (Sapkota et al 2007).[26]

To combat the excess mortality related to XDR-TB, it is recommended to perform DST for second line-drugs to all MDR-TB cases at the start of treatment. To comply with the above suggestion, it seems important for Nepal to strengthen its capacity to perform SLD-DST, either by culture (solid/liquid) or molecular biology e.g. Line Probe Assay (NTP Annual report Nepal 2015).[4] In order to overcome such problems, present study has evaluated a study of sputum specimens collected from retreatment TB cases by Gene Xpert MTB/RIF assay for the rapid diagnosis of DR/MDR TB. All the MDR TB cases detected by Gene Xpert MTB/RIF assay were further verified by conventional culture and DST for first line anti-tuberculosis drugs (FLD-DST). MDR TB diagnosed by both the methods were analyzed for second line drugs (SLD-DST) LPA.

In the present study, despite of smear results (1 ss-ve and 28 ss+ve), Gene Xpert MTB/RIF assay

showed 100% rifampicin resistance (RR/MDR TB), which was high among 15-60 years and in males (58.6%) than in females (41.4%). In previous study, 27 ss+ve and 23 ss-ve specimens were found to be RR/MDR TB detected by Gene Xpert MTB/RIF, whereas 8 cases were negative for MTB among ss-ve. The study report published previously for smear microscopy and Gene Xpert has revealed the similar results.[27]

In this study, concordance of sputum smear results and detection of *M. tuberculosis* along with rifampicin resistance by Gene Xpert MTB/RIF assay for all 29 samples was 100%.

The assay was successful in rapidly detecting *M. tuberculosis* as well as rifampicin susceptibility pattern. Whereas, it was reported in the similar study by Helb et al (2010)[12] from 107 sputum samples in Vietnam that the concordance for ss+ve, Gene Xpert MTB/RIF (RR TB) and culture was 100%(29/29). In the same study, it was described that 64 smear-positive sputa from retreatment tuberculosis cases in Uganda tested by Gene Xpert MTB/RIF assay detected *M. tuberculosis* among 63/64(98.4%) were also found to be culture-positive and rifampin resistance. Similar results were obtained out of total 62 pulmonary TB cases in previous study.[28] But in this study, out of 29 cases detected as MDR TB by Gene Xpert MTB/RIF assay, all the samples were found to be showing positive growth, none were contaminated. Sputum smear +ve and culture positive results were found higher in 15-60 years (even in >60 years) and in males (16) than in females (12). The distribution of ss+ve and culture results was 28(12 F/16M) and 1 male case was ss-ve out of total 29 culture positives.

Twenty eight of 29(96.6%) culture positive samples on drug susceptibility test (DST) showed both INH and RIF resistance, 1 case (3.4%) detected as RRTB by Gene Xpert MTB/RIF showed mono resistance to isoniazid only. Marlowe et al (2011)[14] has reported similar results previously. The age and sex wise distribution of MDR TB by FLD-DST was high in 15-60 years group (even in above 60 years) in males compared to females. It has been reported in one study by Rijal et al (2005)[29] that the MDR TB among previously treated patients was 19.25% (n=161) irrespective of age and sex variation.

Mboowa et al (2014)[30] stated that the resistance was conferred by four different *rpoB* gene mutations in the 81 bp rifampicin resistance detection region (RRDR) of MTB. These were detected by probes A, B, D, and E. It has also been mentioned in previous study that 96.1% *rpoB* gene mutations located in a region of 426-452 amino acid residues (81bp) of MTB *rpoB* gene (RRDR) detected by probes A-E using Gene Xpert MTB/RIF assay.[31] In this study also it can be revealed that all the MDR TB identified by Gene Xpert MTB/RIF assay has detected 100% *rpoB* gene mutations in 81bp RRDR of MTB. Majority of the MDR TB identified by both Xpert and conventional FLD-DST were males. Male dominated MDR-TB results had been described in a similar study previously.[28]

In the present study, all 29 MDR TB cases were performed for DST on second line anti-TB drugs (SLDs) by LPA. As it was mandatory for the two negative controls must be positive only at CC and AC bands in the strips that were clearly formed in the strips used in this study, so the test process was valid. Among 29 *M. tuberculosis* strains confirmed as MDR TB, all were (100%) showing TUB (*M. tuberculosis* complex). It was found that the *M. tuberculosis* probe was 100 per cent specific. All were found to be wild type for *rrs* WT1, *rrs* WT2, 17(58.6%, F5/M12) for *gyrA* WT1, WT2, WT3 and 13(44.8%, F4/M9) for *emb B* WT1 genes, whereas 12 (41.4%, F6/M6) were mutants for *gyrA,* 16(51.2%, F8/M8) for *emb B*) and none were for *rrs* genes. The mutation of *gyrA* gene was detected by the formation of positive band on the nitrocellulose membrane strip (*gyrA* MUT3A and MUT3C) alone and interpreted as fluoroquinolone/FLQ resistance in 12 cases (F6/M6). Similarly, gene mutations of *gyrA* together with *embB* gene in 9 cases (F5/M4). Any case showing single *gyrA* gene mutation or *gyrA* gene mutation together with *embB* gene was interpreted as pre-XDR TB. Seven out of 29 strains (24.1%) were found to be susceptible to all drugs (F3/M4) but none were XDR TB.

There were 12 *gyrA* mutations identified was high among 15-60 years group that were equally distributed among females and males (F6/M6) and 9 *gyrA* and *embB* gene mutations found to be high among 15-60 years group. The result showed that the distribution of pre XDR TB was higher in the male age group of 15-60 years, among which 1 case was ss-ve, which may suggest us to screen ss –ve retreatment TB cases frequently. Whereas, age wise distribution of pre-XDR TB in female was found to be higher in 15-45 years group (6 cases; all ss+ve). The sex wise pre-XDR TB was identified similar in females/males (F6/M6). None of the age or sex group showed pre-XDR TB below 15 years.

Four pre-XDR TB cases identified were from NATA Morang, 1from BPKIHS Sunsari, 1 from NTC, 2 from UMN Hospital Lalgargh and 4 from NMC Parsa. All the 12(41.4%: F6/M6) among 29 MDR confirmed *M. tuberculosis* strains were found to be *gyrA* gene mutations (pre-XDR-TB). The age/sex wise distribution of the SLD-DST pattern showed maximum number of pre-XDR TB among 15-45 years of age groups and was similar for both sexes.

All the 29 MDR TB cases were found to be *rrs* gene wild type. It has been revealed by the previous similar study.[32] It is now clear that the pre-XDR TB is prevalent and scattered in all the MDR TB treatment centres in Nepal.

CONCLUSIONS

Gene Xpert MTB/RIF assay as it is a useful method of simultaneous detecting MTB and rifampicin resistance (surrogate marker of MDR-TB) in both the sputum negative and positive samples along with the culture and DST as reference gold standard.

From this study, out of 29 retreatment TB patients enrolled, 100% were detected RR/MDR TB by Gene xpert MTB/RIF assay irrespective of sputum smear results and all were found to be culture positive. All the culture positive strains were identified as to resist isonoazid and rifampicin with or without remaining drugs resistance. All the 29 MDR TB strains were tested for SLD-DST using LPA (Genotype MTBDR*s*/) and 12 out of 29 MDR TB strains were found to be pre-XDR TB as well. The prevalence of MDR and/or pre-XDR TB was higher in the 15-60 years age group and distributed in both the females and males.

RECOMMENDATIONS

It is recommended that all the samples submitted for microscopic examination should be further processed for culture and DST, if the laboratory setting is capable to do. If not so, all the Cat1 treatment failures as well as Cat 2 treatment failure cases should be tested for RR/MDR TB using Gene Xpert MTB/RIF assay and culture and FLD-DST. Simultaneously, all the MDR-TB cases confirmed by Gene Xpert MTB/RIF assay and culture and FLD-DST should be further tested for SLD-DST by various culture/DST methods and Genotype MTBDR*s*/ to identify pre-XDR and/or XDR TB. This way, prompt diagnosis of TB/DR-MDR TB/XDR TB can be made possible and patient's treatment management as well. Further study should be frequently performed at the national, regional or provincial level on higher samples.

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