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Research Article

Comparison of Isolation of Mycobacterium tuberculosis from various clinical samples using liquidculture (MGIT) and PCR targeting IS6110 gene

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ABSTRACT

Tuberculosis, one of the oldest recorded human afflictions, still one of the biggest killers among the infectious diseases, despite the worldwide use of a live attenuated vaccine and several antibiotics. The Aim of this study was to isolate Mycobacterium tuberculosis from various clinical samples using liquid culture (MGIT) and confirm the growth as MTB using Polymerase chain reaction (PCR).

Material and Methods: All 85 samples were received from clinically suspected cases of pulmonary and extrapulmonary tuberculosis from the various clinical departments of SGRRIM&HS, Dehradun over a period of one year. All samples were processed by ZN staining, culture in LJ medium & MGIT, rapid card (BD MGIT TBcID) test and PCR done by using IS6110gene.

Result: Out of total 85 samples, 35 (41%) came out to be positive by PCR and 50 (59%) cases were found to be negative. Correlation of ZN smear with MGIT culture: 2 cases (2%) were positive in both ZN stain and MGIT culture but 24 (28%) cases showed positivity in MGIT culture. Correlation of MGIT culture with PCR: 24 cases (28%) showed positivity in both MGIT culture and PCR.11 cases (13%) showed positivity in PCR but negative in MGIT culture. The positivity rate was 28% (24/85) in MGIT culture & 41% (35/85) in PCR.

Conclusion: Our study clearly reflects that PCR finding are more positive as compared to MGIT and ZN staining. Because of high specificity and sensitivity of PCR few MTB DNA can be easily detected which is not possible in MGIT and ZN staining. Techniques like PCR is highly valuable for detection of Mycobacterium tuberculosis but the limitation is that it can't differentiate between live and dead bacteria.

Keywords: Mycobacterium, MGIT, PCR, LJ medium, IS6110 gene, Extra-pulmonary TB.

INTRODUCTION

Tuberculosis (TB) is the most ancient disease of mankind. The causative agent, *Mycobacterium tuberculosis* was discovered in 1882 by Robert Koch. Now days it has become critical health problem among millions of people worldwide and recognized as second leading cause of death globally due to multi drugs resistant strains and co-infections.[1,2] Tuberculosis (TB), one of the oldest recorded human adversities, is still one of the biggest killers among the infectious diseases, despite the worldwide use of a live attenuated vaccine and several antibiotics as humans are the only reservoir for the tubercular bacilli.[3, 4] TB infection begins when the mycobacteria reach the pulmonary alveoli, where they invade and replicate within endosomes of alveolar macrophages.[5, 6] The primary site of infection in the lungs, known as the "Ghon focus", is generally located in either the upper part of the lower lobe, or the lower part of the upper lobe.[7, 8] Tuberculosis of the lungs may also occur via infection from the blood stream. This is known as a "Simon focus" and is typically found in the upper lobe of the lung.[9] This haematogenous transmission can also spread infection to more distant sites, such as peripheral lymph nodes, the kidneys, the brain, and the bones.[10] The life time risk of breaking down to disease among those infected with TB is 10-15%, which gets increased to 10% per year amongst those co-infected with HIV. Other determinants such as diabetes mellitus, smoking tobacco products, alcohol abuse and malnutrition also increase the risk of progression from infection to TB disease.[11] Tuberculosis (TB) is a global health concern for both developing and developed countries and has recently become more complex due to persistence in aging populations and the rise of drug-resistant strains.[12, 13] In clinical practice, rapid TB diagnosis can be difficult, and early pulmonary TB detection continues to be challenging for clinicians. Prompt diagnosis of active pulmonary TB is a priority for TB control, both for treating the individual and for public health intervention to reduce further spread in the community.[14] The diagnostic instruments system has been developed by Becton Dickinson as liquid culture Mycobacterial growth indicator tube (MGIT), for rapid identification of Mycobacterium species. The use of this system considerably improves the isolation and shortens the time needed to detect the Mycobacterial growth. The reliability of this method has been evaluated and reported that it requires 7 to

12 days for identifying growth of tubercle bacilli.[15] Nucleic acid amplification is a rapid and relatively easy method for detecting MTB. Of the various techniques available, polymerase chain reaction (PCR), fully automated platform of real-time PCR, and loop-mediated isothermal amplification platform (LAMP) are important presently.[16,17] NAA tests can detect the presence of *M. tuberculosis* bacteria in a specimen weeks before culture for 80 to 90% of patients suspected to have pulmonary TB whose TB is ultimately confirmed by culture.[18, 19] Polymerase chain reaction is the most commonly used technique of nucleic acid amplification. The most commonly used target for the detection of MTB is the insertion sequence IS6110. This sequence is specific for *M tuberculosis* and offers multiple targets for amplification, being present up to 20 times in the genome.[20] The sensitivity and specificity of PCR using the IS6110 have been found to be 94.74 and 100%, respectively.[21]

MATERIAL AND METHODS

This study was conducted at the department of Microbiology and Immunology, Shri Guru Ram Rai Institute of Medical & Health sciences, Dehradun. Samples were collected from suspected cases of tuberculosis, during the period of study from various clinical departments after obtaining written informed consent from patient. The duration of study was one year; studies were started after taking approval from Ethical Committee of SGGRIM&HS Dehradun. A total of 85 samples were included in the study.

Sample collection and transport[21]

Sample was collected in sterile, wide mouth, disposable, leak proof containers without any preservatives.

Clinical details of patient were recorded in case recording form. The samples were collected and processed as soon as possible.

Inclusion criteria

Clinical specimens of patients with suspected tuberculosis, from various clinical departments were included in the study.

Sputum, bronchoalveolar lavage, pleural fluid, CSF, endometrial tissue, body fluid, pus, lymph node, suction tip

Exclusion criteria

Samples less than 2 mL, Samples consisting mainly of saliva, Swab, Samples preserved in formalin

Sample processing[22, 23]

Sample were first subjected to decontaminated process and divided into 3 parts for carrying out the following test smear, culture and PCR.

The materials employed in present study were procured from Hi media Pvt. Ltd, Mumbai.

Preparation of smears

Smears were prepared on a new, clean, unscratched glass slide over an area of 1 by 2 cm, heat fixed and stained.

Ziehl-Neelsen (ZN) staining

- Smear flooded with 1% filtered Carbol-fuschin and the slide heated intermittently to steaming for 5 minutes and washed with water.
- Decolourization was performed with 25% sulphuric acid (H_2SO_4).
- The smear was washed with distilled water again and decolourization step was repeated until the smear appeared light pink in color.
- Counterstain was done with Malachite green for 1 to 2 minutes.
- The slide was rinsed, drained and air dried for 1 to 2 minutes.

The smear was examined under oil immersion and reporting was done as per RNTCP guidelines.Smear was prepared on a new, clean, unscratched glass slide over an area of 1 by 2 cm, heat fixed and stained.

Culture

Solid culture

Lowenstein – Jensen (LJ) medium was procured from Hi Media Pvt. Lmt. Mumbai for putting as a control for culture.

Liquid culture

- *BACTEC™ MicroMGIT™ system*

The Mycobacterium Growth Indicator Tube (BBL MGIT) contains modified Middle brook 7H9 broth base. When supplemented with MGIT growth supplement and antibiotics polymyxin B, amphotericin B, nalidixic acid, trimethoprim, azlocillin (PANTA), it provides an optimum medium for growth of a majority of Mycobacterial species. The MGIT Mycobacteria growth indicator tube contains 7 mL of modified Middle brook 7H9 Broth Base. The complete medium, with 0.5 mL OADC enrichment and 0.1 mL of PANTA antibiotic mixture, is one of the most commonly used liquid media for the cultivation of mycobacteria. In our study the respiratory specimens were inoculated into MGIT for primary isolation of Mycobacteria. The specimen was processed according to SOP manual provided by Becton Dickinson Company.

Polymerase chain reaction analysis

Polymerase chain reaction (PCR) was done for all 85 samples which were received as suspected samples for tuberculosis in the department of microbiology.

Sample preparation (according to manufacturer's protocol)

- Sample was transferred to screw cap 50 mL centrifuge tube.
- Supernatant was decanted and 20 to 30 mL Tris buffer was added and centrifugation was done at 6000rpm for 10 minutes.
- Supernatant was decanted.
- Lysis buffer 1250 µL was added.
- Sample was ready for DNA extraction.

DNA extraction (as per Qiagen DNA extraction kit instructions)

- 200 µL of proteinase K and 200 µL sample was added.
- It was vortexed and incubated at 65°C for 30minutes.
- Centrifugation at 10000rpm for 10 minutes as done.
- 200µL of the lysed sample and 200 µL of lysis buffer 2 was mixed.
- Contents were mixed carefully
- Incubated at 70°C for 10 minutes was done.
- 200 µL of 96 to 100% ethanol distilled water was added and vortexed thoroughly.

- Mixture was transferred in spin column in 2 mL collection tube.
- Centrifugation at 8000 rpm for 3 minutes was done and was discarded.
- Sample was ethanol mixed and was run through column.
- 500 µL of wash buffer1 washed.
- Spinning at 8000 rpm for 3 minutes was done.
- Flow through was discarded and 500 µL of wash buffer2 was added.
- Spinning at 12000 rpm for 3 minutes was done
- Flow through was discarded. Empty spin column was spined for2minutes.
- Spin column was placed in new labeled 1.5 mL Eppendorf tube.
- About 100 µL pre warmed (50°C) elution buffer was added. It was incubated for 5 minutes.
- Centrifugation at 10000 rpm for 1 to 2 minutes to elute DNA was done.
- Sample was then ready for amplification.

DNA Amplification

(As per manufacturer's instructions, GeNei, Bangalore) Amplification was done in thermal cycler (Bench TOP 9600)

Target genes

IS6110

First amplification master mix

- 1st amplification pre mix(purple cap) .....8.2 µL.
- Taq DNA polymerase (Genei hot start) .....0.33 µL
- Uracil DNA glycosylase (UDG) .....0.50 µL

About 9 µL amplification master mix was aliquoted into each of the labeled vials.3 µL of each extracted DNA was added to the vials.3 µL of positive control DNA to PC vial was added. Sample was added first then PC (positive control) for preventing false positive. Initial denaturation at 94°C for 5 minutes, denaturation at 94°C for 30 sec, annealing at 68°C for 1-minute, extension at 72C for 1-minute and final extension at 72°C for 2 minutes.Amplified product was stored at 2 to 8°C.

Amplification master mix 2

- 2nd amplification pre mix (red cap).....14.7 µL.
- Taq DNA polymerase (Genei hot start).....0.33 µL

About 15 µL of the amplification master mix 2 was added to each tube after PCR is completed.Second PCR program was performed

Table 1: Sample wise distribution of suspected cases of pulmonary as well as extrapulmonary tuberculosis (n = 85)

Types of samples	*No. of cases*	*Percentage (%)*
Bronchoalveolar lavage	42	50
Endometrial tissue	15	17
Pus	8	9
Sputum	6	7
Pleural fluid	4	5
Pericardial fluid	3	4
Lymph node	3	4
CSF	2	2
Suction tip	1	1
Synovial fluid	1	1

RESULT

This study was carried out in the department of Microbiology Sri Guru Ram Rai Institute of Medical and Health Sciences Patel Nagar Dehradun Uttarakhand. The duration of study was one year. Total number of samples received from the clinically suspected cases of tuberculosis were 85, out of which 35 (41%) came out to be positive by PCR. Numbers of negative cases were found to be 50 (59%).

As per table above out of the total 85 samples obtained from clinically suspected cases of pulmonary and extrapulmonary tuberculosis, maximum number received were of bronchoalveolar lavage 42 (50%) followed by endometrial tissues 15 (17%), pus 8 (9%),sputum 6 (7%) , pleural fluid 4 (5%), pericardial fluid 3 (4%), lymph node 3 (4%), CSF 2 (2%), suction tip 1 (1%) and synovial fluid 1 (1%) (Table 1 and Fig. 1).

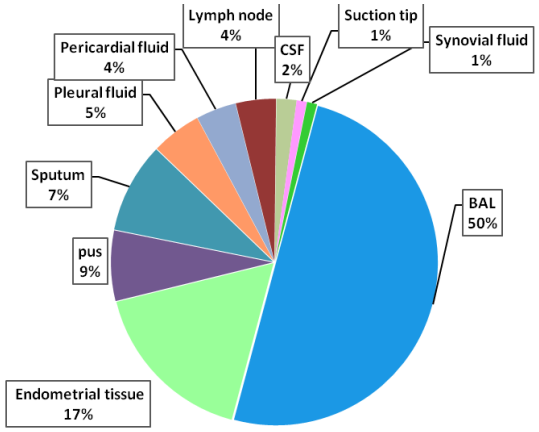

Fig. 1: Sample-wise distribution of suspected cases of pulmonary as well as extrapulmonary tuberculosis (n = 85)

Table 2: Sample-wise distribution of suspected cases of pulmonary tuberculosis (n = 48)

Sample type	*No. of pulmonary cases*
Bronchoalveolar lavage (BAL)	42 (88%)
Sputum	6(12%)

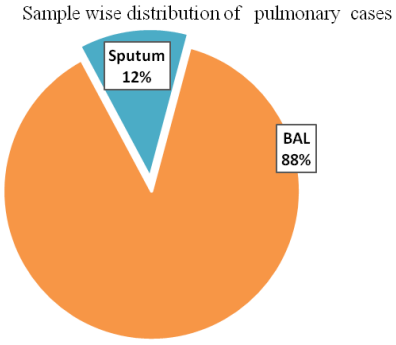

Fig.2:Sample-wise distribution of suspected cases of pulmonary tuberculosis (n = 48)

Table 3: Sample-wise distribution of positive cases (n = 35)

Types of sample	*Total no. of cases (n = 85)*	*Positive cases (n = 35)*	*Percentage (%)*
BAL	42 (50%)	19	(19/35) 54%
Endometrial tissue	15 (17%)	4	(4/35) 11%
Sputum	6 (7%)	4	(4/35) 11%
Pus	8 (9%)	3	(3/35) 9%
Pleural Fluid	4 (5%)	2	(2/35) 6%
Lymph node	3 (4%)	1	(1/35) 3%
CSF	2 (2%)	1	(1/35) 3%
Synovial fluid	1 (1%)	1	(1/35) 3%

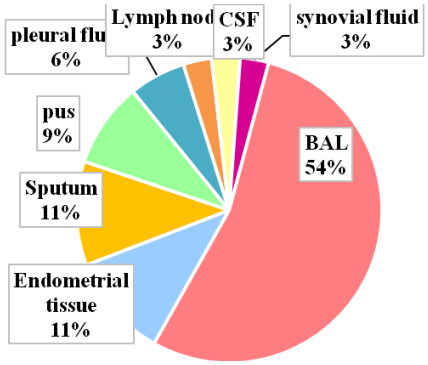

Fig. 3: Sample-wise distribution of total no. positive cases (n = 35)

Table 4: Gender-wise distribution of positive cases (n = 35)

Gender	*No. of cases*	*Percentage of Positivity*
Male	19	54
Female	16	46

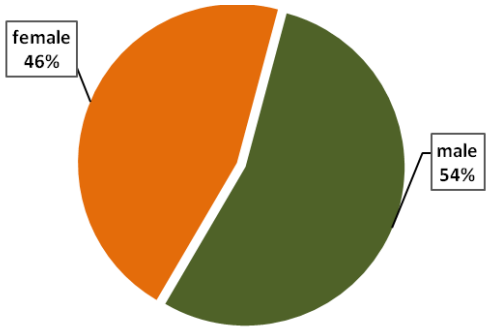

Fig. 4: Gender-wise distribution of positive cases (n = 35)

Table 5: Gender-wise distribution of total cases (n = 85)

Gender	*No. of cases*	*Percentage (%)*
Male	45	53
Female	40	47

Table 6a: Distribution of cases according to age group (n = 85)

Age (in years)	*Male (45)*	*Female (40)*	*Total (%)*
0–10	0 (0%)	0 (0%)	0 (0%)
11–20	2 (5%)	0 (0%)	2 (2%)
21–30	5 (11%)	9 (22%)	14 (16%)
31–40	7 (15%)	3 (7%)	10 (12%)
41–50	4 (9%)	7 (17%)	11 (13%)
51–60	10 (22%)	5 (13%)	15 (18%)
61–70	9 (20%)	11 (28%)	20 (24%)
>70	8 (18%)	5 (13%)	13 (15%)
Total	45	40	85

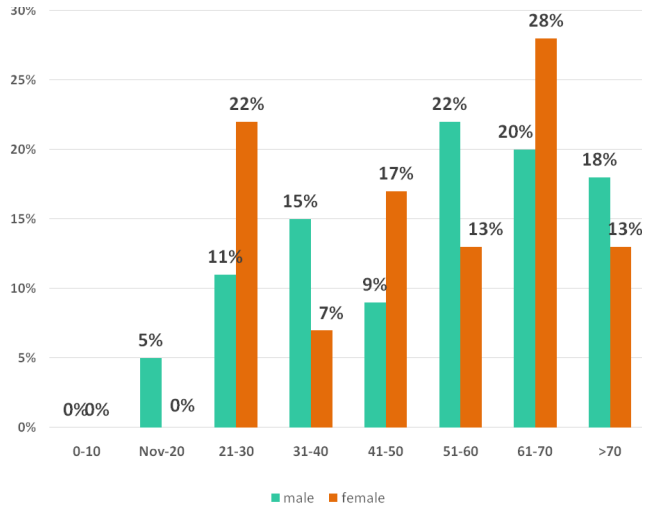

Fig. 5: Distribution of cases according to age group (n = 85)

As per table out of the total 48 samples obtained from clinically suspected cases of pulmonary tuberculosis, maximum number received were of BAL 42 (88%) and sputum 6 (12%).(Table 2 and Fig. 2)

As per the table bronchoalveolar lavage (BAL) being the most predominant sample in our study the positivity rate was 19/35 (54%). Among the endometrial tissue samples positivity rate was 4/35 (11%),sputum 4/35 (11%), pus 3/35 (9%), pleural fluid 2/35 (6%), lymph node 1/35 (3%), CSF 1/35 (3%) and synovial fluid 1/35 (3%), respectively (Table 3 and Fig. 3).

As per table in the present study, total numbers of male cases were 45 (53%) and female cases were 40 (47%) (Table 4 and Fig. 4).

As per table in the present study, Amongst the positive cases males 3 out numbered females. The p-value is 0.835397. This result is not significant at $p < 0.05$ (Table 5).

As per table amongst the clinically suspected in male cases, the predominant age group involved was 51 to 60 years 10 (22%), followed by 61 to 70 years of age group 9 (20%), more than 70 years of age group 8 (18%), 31 to 40 years of age group 7 (15%), 21 to 30 years of age group 5 (11%), 41 to 50 years of age group 4 (9%), 11 to 20 years of group 2 (5%). No cases were observed below 0 to 10 years of age group.[Table 6(a) and Fig. 5]

In female cases, the predominant age group involved was 61-70

Table 6b: Gender-wise distribution of cases (Male) (n = 45)

Age (in yrs)	*Male*		*Positive cases n = 19*		
	Total No of cases n = 45	*Percentage*	*ZN smear*	*MGIT*	*PCR*
0–10	0	0/45 (0%)	0 (0%)	0 (0%)	0 (0%)
11–20	2	2/45 (4%)	0 (0%)	0 (0%)	0 (0%)
21–30	5	5/45 (11%)	1 (6%)	3 (16%)	3 (15%)
31–40	7	7/45 (16%)	0 (0%)	3 (16%)	4 (21%)
41–50	4	4/45 (9%)	0 (0%)	1 (6%)	1 (5%)
51–60	10	10/45 (22%)	0 (0%)	1 (6%)	1 (5%)
61–70	9	9/45 (20%)	0 (0%)	4 (25%)	5 (27%)
>70	8	8/45 (18%)	0 (0%)	4 (25%)	5 (27%)

years of age group 11 (28%), followed by 21 to 30 years of age group 9 (22 %), 41-50 years of age group 7(17%), 51-60 &> 70 years of age group were 5 (13%) each, 31-40 years of age group 3 (7%). No cases were observed below 20 years of age group. [Table 6(a)]

As per table above the sample received in male cases maximum numbers of positivity in PCR was in age group 61 to 70 years &>70 years of age group 5 (27%) each, followed by 31 to 40 years of age group 4 (21%), 21 to 30 years age group 3(15%), low positivity was seen in age group 41 to 50 years 1 (5%) and 51 to 60 years 1 (5%) each. No positive cases were observed in less than 20 years. [Table 6(b)]

When comparing the findings with MGIT culture, maximum positivity was shown in the same age group i.e. 61 to 70 years of age &more than 70 years of age group 4 (25%)each, followed by 31 to 40 years of age group 3 (16%) & 21 to 30 years of age group 3 (15%) &low positivity was seen in age group 41 to 50 years 1 (5%) and 51 to 60 years 1 (5%).In rest of the age groups no positive case were observed. When comparing the findings with ZN smear single positive case was shown in age group, i.e., 61 to 70 years of age group. [Table 6(b)]

As per table above the sample received in female cases maximum numbers of positivity in PCR was in age group 21-30 years of age group 7 (44%), followed by 31-40 years of age group 4 (25%), 61 to 70 years of age group 3 (19%), least positivity was shown in 41-50 years & 51 to 60 years age group 1 (6%) each. No positive cases were observed in less than 20 years of age group.

When comparing the findings with MGIT culture, maximum positivity was shown in the same age group i.e. 21 to 30 years of age group 4 (25%) ,31 to 40 years & 61 to 70 years of age group 3 each (19%), low positivity was seen in age group 41 to 50 years 1 (6%). In rest of the age groups no positive case were observed. When comparing the findings with ZN smear single positive case was shown in age group i.e 61-70 years of age group. [Table6(c)]

In table above when comparing ZN smear with MGIT culture, Out of a total of 85 clinically diagnosed cases of pulmonary and extra pulmonary tuberculosis:

- 2 cases (2%) showed positivity in both ZN smear and MGIT culture.
- 22 cases (26%) showed positivity in MGIT culture but negative in ZN smear.
- 61 (72%) suspected cases of pulmonary and extra pulmonary tuberculosis were negative both by ZN smear and MGIT culture.
- 81 (98%) suspected cases of pulmonary and extra pulmonary tuberculosis were negative both by ZN smear and MGIT culture.
- When comparing ZN stain with MGIT culture the *p*- value was 0.000003. This result was found significant at $p < 0.05$.

As per the Table 7 out of a total of 85 clinically diagnosed cases of pulmonary and extra pulmonary tuberculosis, 2 cases (2%) were positive in both ZN stain and MGIT culture.

- 2 cases (2%) were positive in ZN stain.
- 24 (28%) cases showed positivity in both MGIT culture and PCR.
- Total 35 cases (41%) showed positivity in PCR.
- 50 (59%) suspected cases of pulmonary and extra pulmonary tuberculosis were negative by both by MGIT culture and PCR.

Table 6c: Gender-wise distribution of cases (Female) (n = 40)

Age(in years)	*Female*		*Positive cases n=16*		
	Total No of cases (n = 40)	*Percentage (%)*	*ZN smear*	*MGIT*	*PCR*
0–10	0	0/40 (0%)	0 (0%)	0 (0%)	0 (0%)
11–20	0	0/40 (0%)	0 (0%)	0 (0%)	0 (0%)
21–30	9	9/40 (21%)	0 (0%)	4 (25%)	7 (44%)
31–40	5	5/40 (13%)	0 (0%)	3 (19%)	4 (25%)
41–50	7	7/40 (18%)	0 (0%)	1 (6%)	1 (6%)
51–60	5	5/40 (13%)	0 (0%)	0 (%)	1 (6%)
61–70	11	11/40 (27%)	1 (6%)	3 (19%)	3 (19%)
>70	3	3/40 (8%)	0 (0%)	0 (0%)	0 (0%)

Table 7: Comparison of ZN stain with MGIT culture (n = 85)

ZN Smear	*MGIT culture*		*Total*
	Positive	*Negative*	
Positive	2 (2%)	0 (0%)	2 (2%)
Negative	22 (26%)	61 (72%)	81 (98%)

Table 8: Comparison of ZN stain, MGIT culture and PCR (n = 85)

No. of cases n = 85	*ZN Stain*	*MGIT culture*	*PCR*
Positive	2 (2%)	24 (28%)	35 (41%)
Negative	83 (98%)	61 (72%)	50 (59%)

- When comparing ZN stain with MGIT culture the *p*- value was 0.000003. This result was found significant at *p*- <0.05 (Table 8).
- When comparing ZN stain with PCR this result was found significant at *p*- <0.05.

In Table 9 when comparing MGIT culture with PCR, Out of a total of 85 clinically diagnosed cases of pulmonary and extra pulmonary tuberculosis (Fig. 6):

- 24 cases (28%) showed positivity in both MGIT culture and PCR.
- 11 cases (13%) showed positivity in PCR but negative in MGIT culture.
- 50 (59%) suspected cases of pulmonary and extra pulmonary tuberculosis were negative both by MGIT culture and PCR.
- The *p- value* was 0.07635. This result was found not significant at <.05.

DISCUSSION

Tuberculosis (TB) continues to be a major public health problem in India. The increases in the overall age of the population and the rise of drug-resistant, TB has emphasized the need for improvements in rapid diagnosis method and new modalities to detect TB and its treatment.[24] An early and accurate diagnosis of pulmonary and extra pulmonary TB should be established using chest X-ray, sputum microscopy, culture in both liquid and solid media, and nucleic acid amplification. Compared with AFB smear microscopy, the added value of NAA testing greater positive predictive value (>95%) with AFB smear-positive specimens in settings in which non-tuberculous mycobacteria are common and ability to confirm rapidly the presence of *M. tuberculosis* in 50 to 80% of AFB smear-negative, culture-positive specimens.[25] Compared with culture, NAA tests can detect the presence of *M. tuberculosis* bacteria in a specimen week earlier than culture for 80 to 90% of patients suspected to have pulmonary TB whose TB is ultimately confirmed by culture. These advantages can impact patient care and TB control efforts, such as by avoiding unnecessary contact investigations or respiratory isolation for patients whose AFB smear-positive specimens do not contain *M. tuberculosis*.[26]

The present study was planned to Isolation of *Mycobacterium tuberculosis* from various clinical samples using liquid culture (MGIT) & PCR. The worldwide increase in the incidence of tuberculosis and the growing number of mycobacteria in immunocompromised patients require fast and efficient cultivation strategies that can easily be applied in a clinical mycobacteriology laboratory. One of the most recent

Table 9: Comparison of MGIT culture with PCR (n = 85)

PCR	*MGIT culture*		*Total*
	Positive	*Negative*	
Positive	24 (28%)	11 (13%)	35 (41%)
Negative	0 (0%)	50 (59%)	50 (59%)

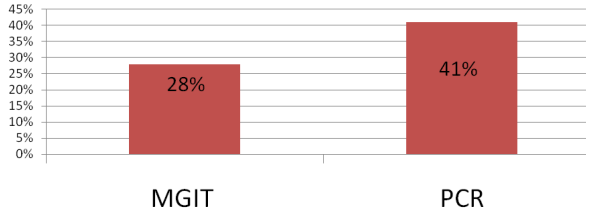

Fig. 6: Comparison of culture positivity in MGIT with PCR (n = 85)

developments, MGIT, points in this direction, it is easy to handle, is non radiometric and at present does not need costly instrumentation.

Sample Wise Distribution

Considering samples from suspected cases, majority of them were of Bronchoalveolar lavage (BAL) 42 (50%) followed by endometrial tissues 15 (17%), pus 8 (9%), sputum 6 (7%), pleural fluid 4 (5%), pericardial fluid 3 (4%), lymph node 3 (4%), CSF 2 (2%), suction tip 1 (1%) and least synovial fluid 1 (1%).

A study from Kumar *et al* from Karnataka, India in 2018 reported extra-pulmonary cases as majority of cases of pleural fluid (29.9%), CSF(22.5%) and lymph node (10.7%).[27] Nagpal *et al* from Ludhiana in 2018reported majority of samples from extra-pulmonary cases were of CSF (35%) followed by pleural fluid (16%)& tissue(15%).[28] In 2016, a study from North East India, Bhattacharya *et al* reported pleural effusion (30.04%) was the commonest sample amongst extra pulmonary tuberculosis.[29] In 2012, Lawn *et al*, from South Africa reported tissue (35%) as the commonest sample amongst extra pulmonary tuberculosis.[30] In another study from India Rodrigues *et al* in 2009, reported majority of samples were of sputum (62%) followed by BAL (36%).[31]

Rishi *et al* from Rajasthan, India in 2007 reported majority of samples were of sputum (66%) followed by cerebrospinal fluid (9.8%), pleural fluid (8.2%), bronchial washing (6%), pus (3%) and endometrium (1%).[32] Negi *et al* from Delhi in 2005 reported (36%) sputum samples, followed by BAL (11%),pus (17%), lymph node (2%) and pleural fluid (1%).[33]

Gender wise distribution

In our study the total numbers of male cases were 45 (53%) and female cases were 40(47%) out of 85, in which 19(22%) males and 16(18%) females came out to be positive for tuberculosis.

In another study from Uttarakhand Luke *et al* in 2017 reported (68%) were males and (32%) were females. The results showed that (26.5%) cases of the males while (6.3%) cases of females were positive.[34]

Demographic profile of subjects

Predominant age group involved our study was 61-70 years of age group 20(24%), followed by 51-60 years of age group 15(18%),

21-30 years of age group 14(16%) & least age group involved was 11-20 years of age group 2(2%). No cases were observed below 0-10 years of age group.

Bhattacharya *et al* from Northeast India in 2016 has reported (30.95%) were in the age group 21-30 years, followed by 31-40 years (18.82%), 11-20 years (17.34%) and least common >80 years of age.[29]

Kumar *et al* from Karnataka in 2018 has reported 24% were in the age group <20 years, followed by 53% in the age group of 21-40 years and least common >60 years of age.[27] Tudu *et al* from Jharkhand in 2017, reported higher number of cases in younger age group of 15-25 years (27.7%), followed by 26-35 years age group (27.3%) and 36-45 years age group (21.7%) and least were in age group of more than 65 years (3.3%).[35] In our study, males were more in number (54%) as compared to females (46%) which is similar to other studies like reported by Jiménez-Coron, *et al*. in 2009 (58%)males vs. female (41.1%), Ratnesh *et al* from UP as male (55.9%) vs. Female (44.1%).[36,37] Kumar *et al* from Karnataka in 2018 as male 136 (60.7%) vs female 88 (39.3%). Tudu *et al* from Jharkhand in 2017 as male (70%) vs female (30%) which is quite high as compared to our study.[27,35]

Correlation of ZN smear with MGIT culture

When comparing ZN smear with MGIT culture, out of a total of 85 clinically diagnosed cases of pulmonary & extrapulmonary tuberculosis, 2 cases (1.3%) showed positivity in both ZN stain and MGIT culture. Kabir *et al* in 2018 from Bangladesh reported 3 out of 102 (2.9%) suspected cases of tuberculosis were positive in both ZN stain and MGIT culture, which is quite similar to our study.[38] Bhat *et al* from Mangaluru in 2018, showed that 21 cases out of 100 (21%) suspected cases were positive in both ZN stain and MGIT culture, which is quite high as compared to our finding.[39] Saini *et al* from Uttar Pradesh, India in 2017 reported (15.15%) cases were found to be AFB positive by Z-N staining method and (46.9%) cases positive by MGIT.[40] 24cases (28%) in our study showed positivity only in MGIT culture but negative in ZN stain, whereas Kabir *et al* reported only 12cases (12%) which were positive only in MGIT culture, whereasBhat *et al* from Mangalore in 2018 reported only 2 (2%) cases which were negative in ZN stain but positive in MGIT culture, which is quite low as compared to our finding.[38,39]

Correlation of ZN smear with PCR

When comparing ZN smear with PCR, out of a total of 85 clinically diagnosed cases of pulmonary & extrapulmonary tuberculosis, 2 cases (2%) were positive in ZN stain. 35 cases (41%) showed positivity in PCR.Kabir *et al* from Bangladesh in 2018 reported Smear positive cases (2.9%) and PCR was positive for 62% cases which is quite similar to our study.[38] Chien *et al*. from Taiwan in 2005 reported (25.9%) cases positive in ZN Stain and (56.7%) cases positive in PCR which is quite high as compared to our study.[41]

Correlation of MGIT culture with PCR

When comparing MGIT culture with PCR, out of a total of 85 clinically diagnosed cases of pulmonary & extrapulmonary tuberculosis, 24 cases (28%) showed positivity in both MGIT culture and PCR.11 cases (13%) showed positivity in PCR but negative in MGIT culture.Nagpal *et al* from Ludhiana in 2018 reported (23%) cases PCR results were positive. MGIT (used in 75 samples) was positive in 21.33% samples.[28] Siddiqui *et al* from Hyderabad in 2013 reported 15 cases which were positive by culture on BACTEC, all 15 (100%) were positive by PCR. In addition, PCR detect 55 out of 85 (64.7%) which were negative by culture on BACTEC.[42] Kabir *et al* from Bangladesh in 2018 reported 14(14%) cases were MGIT culture positive out of 102 samples and 63(62%) cases were PCR positive.[38] Chien *et al*. from Taiwan in 2009 reported (59.6%) patients were positive according to the BACTEC MGIT 960system. Among these (56.7%) were positive by PCR.[43]

SUMMARY AND CONCLUSION

The present study was conducted in the department of Microbiology Sri Guru Ram Rai Institute of Medical and Health Sciences Patel Nagar, Dehradun, Uttarakhand with the aim: Isolation of *Mycobacterium tuberculosis* from various clinical samples using liquid culture (MGIT) & PCR in a tertiary care hospital.

The duration of study was one year ranging from October 2016 to September 2017.

- In this study total numbers of samples received from the clinically suspected cases of pulmonary and extra pulmonary tuberculosis were 85, out of which 41% were positive and 59% negative.
- Out of total 85 suspected cases, the positive cases came out to be 35 (41%) for both pulmonary as well as extrapulmonary tuberculosis.
- Out of total 35(41%) cases males were 19(54%) and females were 16(46%) respectively.
- The maximum numbers of samples received were of BAL 50%, followed by endometrial tissue 17%, pus 9% and sputum 7%.
- Bronchoalveolar lavage (BAL) being the most predominant sample in our study, the positivity rate was 54%.
- Among the endometrial tissue, sputum and pus samples positivity rate was 11%, 11% and 9% respectively.
- The predominant age group involved in male was 51-60 years 22%, followed by 61-70 years 20%. In female cases, the predominant age group was 61-70 years 28% followed by 21-30 years of age group was 22%.
- Out of total number of 85 cases,2 cases (2%) were positive in ZN stain,24 cases (28%) showed positivity in MGIT culture and 35 (41 %) were positive by PCR. 50 cases (59%)of pulmonary and extrapulmonary tuberculosis were negative by PCR.
- When comparing ZN stain with MGIT culture, out of a total of 85 cases 2% was positive in both ZN stain and MGIT culture.28%were positive in MGIT culture and negative in ZN smear. 72% cases were negative both by ZN smear and MGIT culture.
- When comparing MGIT culturewith PCR, out of total of 85 cases 13% was positive only in PCR but negative in MGIT culture. 59% cases were negative both by MGIT culture and PCR.
- The positivity rate was 28% (24/85) in MGIT culture & 41% (35/85) in PCR.
- This study helps in finding the importance of modern technique like PCR in detection of TB.
- High sensitivity and specificity of PCR can be used to detect

MTB DNA in a sample which is coming negative by MGIT and ZN staining.

- Technique like PCR is highly valuable for detection of *Mycobacterium tuberculosis* but the limitation is thatit cannot differentiate between live and dead bacteria.
- In order to conclude, the present study needs to be conducted on alarge number and on various types of samples over an extended period to determine the scenario of pulmonary and extrapulmonary tuberculosis in a tertiary care hospital in Uttarakhand.

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