Conventional Polymerase Chain Reaction in the Diagnosis of Extrapulmonary Tuberculosis from Formalin-Fixed, Paraffin-Embedded Tissues

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Abstract

Background: Tuberculosis is one of the serious public health problems of developing countries. There is also global rise in the incidence of tuberculosis as well as in Bangladesh. The extrapulmonary tuberculosis is also common. Poverty, malnutrition, low socioeconomic condition, overcrowding and immunodeficiency are the common causes of tuberculosis in Bangladesh. This research was conducted to see the usefulness of conventional PCR in the diagnosis of extrapulmonary tuberculosis from paraffin-embedded tissues.

Methods: This was a cross-sectional observational study where molecular detection of MTB DNA by targeting the gene IS6110 of MTB with the method of conventional PCR was done from paraffin embedded tissue samples. A total of 60 cases of extrapulmonary tuberculosis that were histologically diagnosed from tissue samples on the basis of granulomatous inflammation in the proper clinical context were included in the study. Paraffin block of each case was subjected to Ziehl-Neelsen staining followed by conventional PCR examination. Results of all the cases were collected and tabulated in a data sheet. Statistical analysis was performed on the tabulated data by Fisher's exact test.

Results: Among 60 cases 37(61.66%) cases showed negative PCR examination whereas 23(38.33%) showed positive PCR examination. Among the PCR positive 23(38.33%) cases, 22(36.6%) cases histologically contained typical granuloma. In 1(1.7%) case granuloma was not found but caseous necrosis and Langhans' giant cells were present and PCR examination was positive. Among the PCR positive 23(38.3%) cases, 22(36.6%) cases histologically contained typical caseous necrosis but in 1(1.7%) case caseous necrosis was not found but granuloma and Langhans' giant cells were present and PCR examination was positive. In this study among 60 cases, Z-N stain was positive in 2 cases (3.3%) and negative in 58 cases (96.7%). Among the Z-N stain positive 2(3.3%) cases, PCR was positive in one case and in other case PCR examination was negative. Statistically significant association was not found with PCR examination results with granuloma (P-0.343) and caseous necrosis (p-0.675).

Conclusion: The results of the present study suggested that PCR can be considered as a diagnostic modality in the challenging cases of extrapulmonary tuberculosis from FFPE tissue samples by demonstrating the presence of MTB specific DNA.

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Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains a global health concern. About one-quarter of the world's population is infected, but only 10–15% develop active TB; the rest (85–95%) remain asymptomatic and non-infectious.[1] According to WHO estimates, tuberculosis affects more than one billion people worldwide, with roughly 10 million new cases and around two million deaths occurring each year.[2] TB primarily affects the lungs, but around 15% of cases in low-incidence countries are extrapulmonary—higher in high-incidence areas. Diagnosing extrapulmonary TB is challenging due to its typically low bacterial load.[3] Tuberculosis affecting the lymph nodes, known as tuberculous lymphadenitis, is the most frequently occurring type of extrapulmonary tuberculosis (EPTB).[4] TB can affect the larynx, pleura, intestines, fallopian tubes, skin, brain, kidneys, liver, bones, joints, spleen, and other organs. During primary infection, the bacteria spread via lymphatics or bloodstream, forming granulomas with caseous necrosis and Langhans' giant cells which is classical histopathological features of TB.[5]

Traditionally, TB diagnosis in tissues used histopathology, smear microscopy, and culture. In recent years, FNAC has become useful for diagnosing extrapulmonary TB, especially in peripheral lymphadenopathy. Diagnosis relies on Ziehl-Neelsen staining for AFB and culture, though these methods often show low detection rates.[4] In patients with confirmed tuberculosis, the acid-fast bacilli (AFB) detection rate ranges from 15% to 47%, influenced by whether necrosis is present.[6] Likewise, studies have shown that mycobacterial cultures from fine needle aspirates yield positive results in 35% to 65% of cases.[7,8,9] Furthermore, the culture process requires six to eight weeks, leading to a significant delay in starting treatment.[4] Rapid and sensitive detection of *M. tuberculosis* in tissues is vital for diagnosis. PCR, a type of nucleic acid amplification techniques (NAAT), is widely used due to its speed, high sensitivity, and specificity.[3] PCR is effective in detecting *M. tuberculosis* DNA in formalin-fixed tissues and is useful for confirming diagnosis in cases with granulomatous inflammation, even when culture is inhibited by preservatives.[10] In addition, PCR testing can serve as an effective diagnostic tool for quickly identifying rifampicin resistance in FFPE tissue samples.[5] PCR has the potential to quickly distinguish Mycobacterium tuberculosis from non-tuberculous mycobacteria. [11] EPTB diagnosis can be challenging due to difficulty in sample collection, insufficient material, absence of caseous necrosis, or presence of foreign body granulomas. Z-N staining and culture are often negative. Hence, PCR offers a more useful tool for early diagnosis and management.

Objectives

This study was conducted to examine the usefulness of conventional PCR in detecting *Mycobacterium tuberculosis* from formalin-fixed, paraffin -embedded extrapulmonary tissue samples.

Methods

This cross-sectional study was conducted at the Departments of Pathology and Microbiology, Mymensingh Medical College, from March 2021 to February 2023. Data were purposively collected from 60 histologically diagnosed EPTB cases. The study was approved by the IRB of Mymensingh Medical College.

Data were recorded using a structured questionnaire after obtaining informed written consent.

Cases with available FFPE tissue blocks of EPTB diagnosed at the Department of Pathology, Mymensingh Medical College,

Mymensingh were included. Cases with inadequate, poorly fixed, or poorly processed tissue blocks were excluded. Calculated sample size was 288.

According to the statistics of Mymensingh Medical College Hospital (MMCH) average of 3 tuberculosis biopsy samples are obtained per month in Pathology Department of Mymensingh Medical College (MMC). Therefore, it could be deducted theoretically total of 72 specimens would be available during study period of two years. Since the samples were obtained from a relatively small finite population the final sample size was calculated using finite population correction formula (Israel, 1992). Accordingly, a total of 60 samples following selection criteria were included in this study.

Histopathological Evaluation

All 60 cases were collected from MMC. Fresh specimens underwent grossing, processing, and histopathological examination at the Department of Pathology, MMC. H&E slides were reviewed to confirm anatomical site and granulomatous inflammation consistent or compatible with or suggestive of tuberculosis in the proper clinical context. Presence of epithelioid cells, caseation necrosis, and Langhans' giant cells were recorded.

Z-N staining for detection of acid-fast bacilli (AFB)

One new section from each paraffin block was stained with Z-N stain as per standard protocol at MMC. Slides were examined for AFB, recorded in the data sheet. AFB appeared as red beaded rods on a blue background.

Molecular detection of MTB from FFPE tissue by conventional PCR: It includes several steps:

DNA extraction

Up to 8 sections were cut from each paraffin block using a Leica blade and placed in 1.5 ml Eppendorf tubes for DNA extraction. To prevent contamination, the blade was cleaned with xylene and ethanol after each sample. DNA was extracted using the Qiagen kit (LOT-172023109) following the manufacturer's protocol.

MTB gene amplification by PCR

A pair of primers was selected targeting the IS6110 gene of MTB. This primer pair was considered for this study because the IS6110 gene was the most frequently detected target gene in MTB species.[13]

Then the master mix for the PCR detection of the IS6110 gene of MTB was prepared (1 sample 20 µl) according to the standard protocol followed at the Molecular microbiology laboratory of Mymensingh Medical college.

PCR thermal cycler was run in a batch of six samples initially. The PCR tubes were labeled with respective case number. In each tube, calculated volume of master mix and respective extracted DNA (5.0 µL) were added keeping the tubes on ice. All the reagents were thoroughly mixed by flicking to ensure optimum distribution of the DNA.

The PCR tubes were placed in the thermal cycler (INFINIGEN and DLIN) and the reaction was run according to the thermal profiles described for IS6110 gene in the research article.[14]

Statistical Analysis of Data

Data were compiled in Excel to create a master sheet, then analyzed using IBM SPSS Statistics version 26 (Armonk, NY, USA). Categorical variables (e.g., sex, site of involvement) were presented as frequency and percentage. Continuous variables (e.g., age, duration, PCR results) were summarized by frequency, percentage, mean, and standard deviation. Chi-square test was used to

compare categorical variables, with $p < 0.05$ considered statistically significant.

Results

A total of 60 histologically diagnosed EPTB cases with granulomatous inflammation were analyzed. Ziehl-Neelsen staining detected AFB in only 2 cases (3.3%), while conventional PCR confirmed TB in 23 cases (38.3%).

Demographic data, clinical details, histopathological findings, and results of Z-N staining and PCR were recorded and analyzed statistically.

Table I: Distribution of the patients according to age

Age (year)	Frequency	Percent
0-10	4	6.7
11-20	10	16.7
21-30	20	33.3
31-40	9	15.0
41-50	9	15.0
51-60	6	10.0
61-70	2	3.3
Total	60	100.0
Mean ± SD (Min-Max)	32.57 ± 16.17 (2-70)	

*SD= Standard Deviation

In case of age distribution among 60 cases, 23 (38.3%) cases were male and 37 (61.7%) cases were female with a male-female ratio of 1:1.6.

Table II: Distribution of the patients according to anatomical site of tissue sample

Site	Frequency	Percent
Colon tissue (biopsy/ resected colon 4/5=9)	10	16.7
Abscess wall	13	21.7
Brain tissue	1	1.7
Lymph node (Cervical/Supraclavicular/ Axillary /Inguinal /Mesenteric =17/1/2/1/2=23)	23	38.3
Omental tissue	2	3.3
Skin biopsy (Tuberculosis verrucosa cutis)	1	1.7
Tissue from epiglottis	1	1.7
Tissue from wound site	2	3.3
Sinus tract	7	11.7
Total	60	100.0

Among 60 cases, 49 cases (81.7%) presented with clinical symptoms for less than 12 months and the remaining 11 cases (18.3%) presented with clinical symptoms for more than 12 months.

Table III: Distribution of the patients according to histological findings and results of Z-N staining and PCR examination

Parameters	Frequency	Percent
ZN stain		
o Positive	2	3.3
o Negative	58	96.7
Granuloma		
o Present	59	98.3
o Absent	1(Caseous necrosis and Langhans' giant cells were present)	1.7
Caseous necrosis		
o Present	57	95.0
o Absent	3(Granuloma and Langhans' giant cells were present)	5.0
PCR examination		
o Positive	23	38.3
o Negative	37	61.7

Table IV: Distribution of the patients according to demographic characteristics by PCR examination (n=60)

Demographic characteristics	PCR examination		p value
	Positive	Negative	
Age (year)			
o ≤30 years	11 (32.35)	23 (67.64)	0.284[b]
o >30 years	12 (48.0)	13 (52.0)	
Sex			
o Male	8 (34.78)	15 (65.21)	0.656[a]
o Female	15 (40.54)	22 (59.45)	
Duration (month)			
o ≤12 months	19 (38.77)	30 (61.22)	0.884[b]
o >12 months	4 (36.36)	7 (63.63)	

[a]Chi-square test was done to measure the level of significance.
[b]Unpaired t test was done to measure the level of significance.
Figure within parenthesis indicates in percentage.

In this study among the PCR positive 23 cases, granuloma was present in 22 cases (36.6%)

Table V : Association of granuloma with PCR examination results

Histopathological findings		PCR result			
		Positive	Negative	Total(Granuloma)	p value
Granuloma	Present	22 (36.6%)	37 (61.7%)	59(98.3%)	
	Absent	1 (1.7%)	0	1 (1.7%)	0.383
Total		23 (38.3%)	37 (61.7%)	60 (100%)	

*Fisher's exact test was done to measure the level of significance.

In this study among the PCR positive 23 cases, caseous necrosis was present in 22 cases (36.6%).

Table VI: Association of caseous necrosis with PCR examination results

Histopathological findings		PCR result		Total (Caseous necrosis)	p value
		Positive	Negative		
Caseous necrosis	Present	22 (36.6%) (Extensive area mostly)	35 (58.3%) (Focal area mostly)	57((95%)	0.675
	Absent	1 (1.7%)	2 (3.4%)	3 (5%)	
Total		23 (38.3%)	37 (61.7%)	60(100%)	

*Fisher's exact test was done to measure the level of significance.

As Z-N stain was positive only in 02 (two) cases, so no comparison table was given and both granuloma and caseous necrosis were present in these two cases.

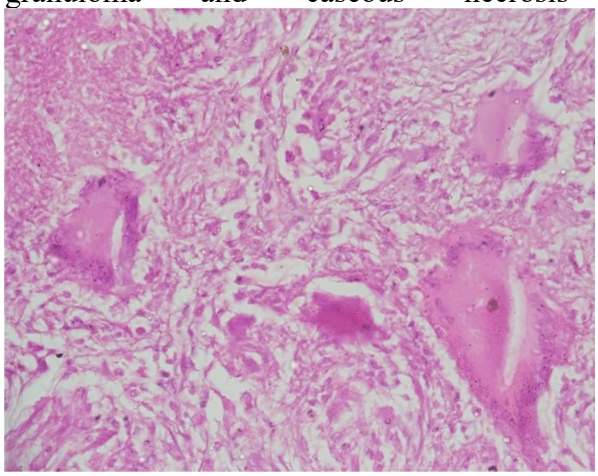


Figure 1. Photomicrograph showing multiple Langhans' giant cells in Tuberculosis (Case no-1, H&E x 400, Inguinal lymph node)

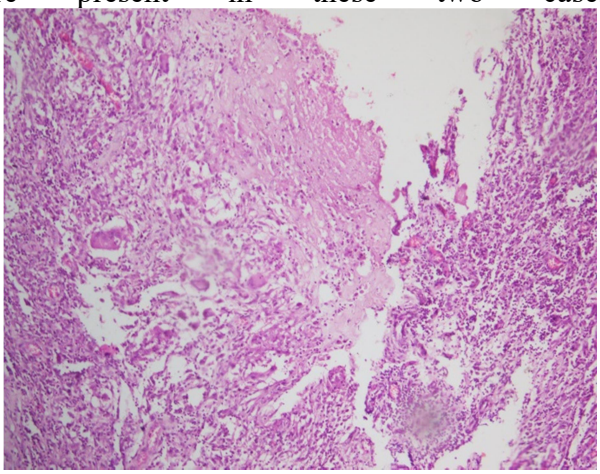


Figure 2. Photomicrograph showing Caseous necrosis & Langhans' giant cells in Tuberculosis (Case no-13, H&E x 100, Sinus tract)

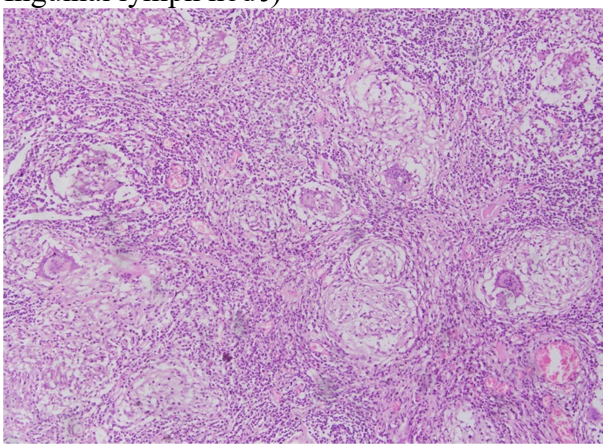
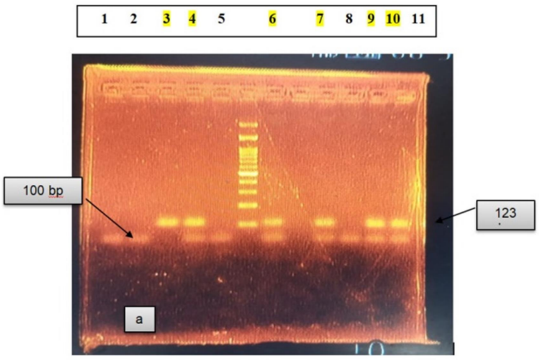
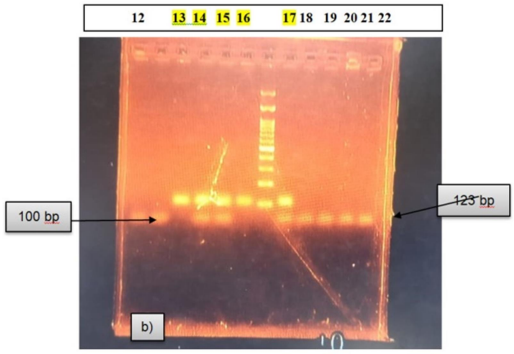


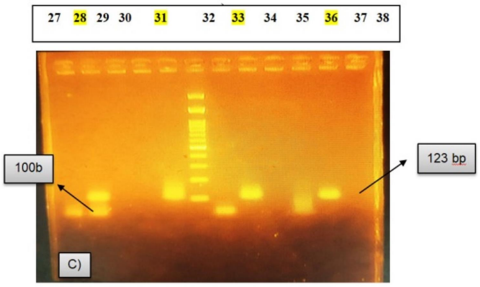
Figure 3. Photomicrograph showing multiple granuloma & Langhans' giant cells in Tuberculosis (Case no-21, H&E x 100, Colonic lymph node)



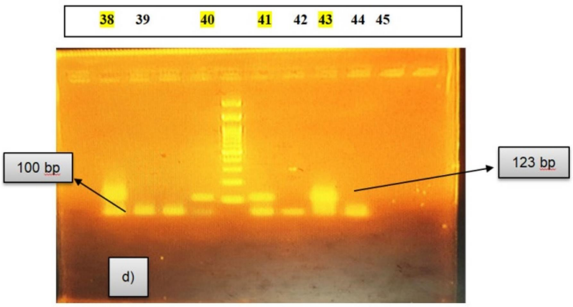
(a) Lane 3,4,6,7,9,10 shows amplified MTB DNA



(b) Lane 13,14,15,16,17 shows amplified MTB DNA



(c) Lane 28,31,33,36 shows amplified MTB DNA



(d) Lane 38,40,41,43 shows amplified MTB DNA

(e) Lane 49,53,54,56 shows amplified MTB DNA

Figure 4. PCR amplification of MTB DNA by IS6110 gene primer(123bp) Base Lane:100bp

Discussion

Tuberculosis (TB) remains a major global health issue, especially in developing countries, due to high morbidity and mortality. Diagnosing extrapulmonary TB (EPTB) is challenging and often requires a combination of direct and indirect methods. Direct tests–include Ziehl-Neelsen staining, fluorescent microscopy, culture methods (Lowenstein-Jensen, BACTEC), PCR, antigen detection, molecular tools, and immunoassays. Indirect methods involve histopathology, cytology, serology, skin tests, interferon release assays, and adenosine deaminase tests. Among these, histopathology combined with ZN staining and culture is preferred for EPTB diagnosis. Imaging tools like CT, MRI, laparoscopy, and endoscopy aid in lesion localization and biopsy collection. Definitive diagnosis depends on detecting *Mycobacterium tuberculosis* by histopathological, microbiological, cytopathological, and molecular approaches.[15]

In this study, patients ranged from 2–70 years, with a mean age of 32.57 years. The 21–30 age group was most affected (33.3%). Similar age trends were noted in other studies.[15,16] Some studies reported mean ages with slight variations compared to the current study[17,18,19]. Regarding sex, females were more affected than males (61.7% vs. 38.3%)[19,1] . While this aligns with some studies, others reported male predominance or equal distribution. [17,20] The reason for female predominance is unclear but may relate to risk factors like malnutrition and immunosuppression.

In this study, lymph nodes were the most commonly involved organ (38.3%) among 60 EPTB cases, with cervical nodes most frequently affected. Similar findings were reported in other studies with lymph node involvement in 50%, 80%, and 36.2% of cases.[21,22,16] Conversely, one study noted skin as the most common site (24.27%, 25/103 cases), including various cutaneous TB forms: lupus vulgaris (11), tuberculosis cutis (8), tuberculosis verrucosa cutis (5), and scrofuloderma (1).[15]

In this study, other sites involved were abscess wall (21.7%), colon (16.7%), sinus tract (11.7%), omentum (3.3%), wound site (3.3%), brain, skin, and epiglottis (each 1.7%). Although bone/joint TB is commonly reported (~11.3%)[23], no such case was found here, possibly due to diagnostic challenges and limited biopsy availability.

Among the 23 PCR-positive cases (38.3%), lymph nodes were the most commonly involved site (11 cases), with cervical nodes accounting for 9. This was followed by abscess wall (5), sinus tract (3), colon (2), skin biopsy (1), and epiglottis (1).

Among 60 cases, 49 (81.7%) had symptoms for <12 months, while 11 (18.3%) had symptoms >12 months. Symptom duration ranged from 20 days (TB abscess) to 6 years (skin TB). Delays in diagnosis were often due to late hospital visits, mainly for economic and social reasons.

Among 60 EPTB cases, PCR from FFPE tissue was positive in 23 (38.3%) and negative in 37 (61.7%). Of the PCR-positive cases, all but one showed granuloma; the exception had caseous necrosis and Langhans' giant cells. Likewise, all but one had caseous necrosis; the necrosis-negative case showed granuloma and Langhans' giant cells.

In one study of 55 cases, 78% showed chronic granulomatous inflammation and 22% had chronic inflammation without definite granuloma. Only 13 cases had classic TB features and were TB-PCR positive. TB-PCR was positive in 89% of poorly formed granulomas with necrosis and 88% of well-

formed granulomas with necrosis. PCR positivity was significantly higher in cases with caseous necrosis (86%), Langhans' giant cells (83%), poorly formed granulomas (69%), and well-formed granulomas (73%). However, TB-PCR was positive in 36% (10/28) of cases with chronic inflammation lacking distinct granulomatous lesions.[20]

In this study, Z-N stain was positive in 2 of 60 cases (3.3%). Similarly, one study found AFB in 2 of 24 cases.[24] In contrast, other studies reported higher AFB positivity: 17/50 cases (34%),[21] 17/53 granulomatous cases (32%) out of 81 blocks,[20] and 27/73 cases (37%) from paraffin-embedded tissues.[25]

Lower Z-N stain positivity in this study may be due to the effects of formalin and xylene, which reduce acid-fast staining.[26] Among the 2 Z-N positive cases, one was PCR positive, while the other was PCR negative—possibly due to non-tuberculous Mycobacterium species.[20]

In this study, uniplex PCR on 60 FFPE tissues showed 37 (61.7%) negative cases, possibly due to using a single primer set targeting the IS6110 (123 bp) sequence. In contrast, another study used three primer sets (IS6110, pab, IS986) and found IS6110 to be the most sensitive.[27]

Another study also conducted PCR targeting the MTB IS6110 sequence due to its high specificity, abundant copy numbers and absent in other mycobacterial species. ¯the study also revealed that lacking the IS6110 sequence in their genomes, resulting decrease in PCR sensitivity.[20]

In another study on 50 suspected TB cases, histology detected 26 (52%) positives, while TB-PCR (targeting IS6110) detected 32 (64%). PCR proved sensitive even in small DNA amounts from FFPE tissue. Classic granulomatous features strongly correlated with PCR positivity. Notably, over one-third of chronic inflammation cases without definite granuloma were PCR positive, possibly due to immunosuppression or small sample size.[21]

Another study detected *Mycobacterium tuberculosis* DNA by PCR in 19 of 22 FFPE tissue samples that were AFSC (Acid Fast Staining and Culture), using a 123 bp IS6110 fragment. PCR was positive in caseating granulomas despite negative AFSC, suggesting conventional methods may miss low bacillary loads. IS6110 is specific to the *M. tuberculosis* complex.[28]

Another study showed that out of the 70 samples confirmed as EPTB-positive through histopathology examination (the gold standard), 34 samples (48.6%) tested positive for MTBC using IS6110-based PCR amplification.[13]

Conclusion

The results of the present study suggest that PCR can be considered as a diagnostic modality in the challenging cases of extrapulmonary tuberculosis from FFPE tissue samples by demonstrating the presence of MTB specific DNA. Although PCR was negative in a substantial number of cases in this study which might have occurred due to the use of uniplex PCR targeting only one MTB gene (IS6110) instead of multiple MTB specific genes. Reduced positivity might also be influenced by some histological features like extensive necrosis without typical granuloma, old tissue blocks, less representative tissue sections, presence of foreign body granuloma etc.

References

1. Shaker AE, Al Kurtas MA, Zalzala H. Agreement Test of Histopathology in the Diagnosis of Extrapulmonary Tuberculosis with Gold Standard Polymerase Chain Reaction Technique: A Step to Overcome False Diagnosis. Open Access Macedonian Journal of Medical Sciences. 2021 Aug 17;9(A):579-82.
2. McAdam AJ, Milner DA, Sharpe AH. The Infectious Disease in Kumar, Abbas AK, Aster JC. South Asia Eds. Vol I. Robbins and Cotran Pathologic Basis of Disease, Mumbai, Reed Elsevier India. 2017: p341-402.
3. Hillemann D, Rüsch-Gerdes S, Boehme C, Richter E. Rapid molecular detection of extrapulmonary tuberculosis by the automated genexpert MTB/RIF system. Journal of clinical microbiology. 2011 Apr;49(4):1202-5.
4. Singh KK, Muralidhar M, Kumar A, Chattopadhyaya TK, Kapila K, Singh MK, et al. Comparison of in house polymerase chain reaction with conventional techniques for the detection of Mycobacterium tuberculosis DNA in granulomatous lymphadenopathy. Journal of clinical pathology. 2000 May 1;53(5):355-61.
5. Polepole P, Kabwe M, Kasonde M, Tembo J, Shibemba A, O'Grady J, et al. Performance of the Xpert MTB/RIF assay in the diagnosis of tuberculosis in formalin-fixed, paraffin-embedded tissues. The International Journal of Mycobacteriology. 2017 Jan 1;6(1):87-93.
6. Lau SK, Wei WI, Hsu C, Engzell UC. Efficacy of fine needle aspiration cytology in the diagnosis of tuberculous cervical lymphadenopathy. The Journal of Laryngology & Otology. 1990 Jan;104(1):24-7.
7. Gupta SK, Chugh TD, Sheikh ZA, Al-Rubah NA. Cytodiagnosis of tuberculous lymphadenitis. A correlative study with microbiologic examination. Acta cytologica. 1993 May 1;37(3):329-32.
8. Dandapat MC, Mishra BM, Dash SP, Kar PK. Peripheral lymph node tuberculosis: a review of 80 cases. Journal of British Surgery. 1990 Aug;77(8):911-2.
9. Radhika S, Gupta SK, Chakrabarti A, Rajwanshi A, Joshi K. Role of culture for mycobacteria in fine-needle aspiration diagnosis of tuberculous lymphadenitis. Diagnostic cytopathology. 1989 Jul;5(3):260-2.
10. Salian NV, Rish JA, Eisenach KD, Donald Cave M, Bates JH. Polymerase chain reaction to detect Mycobacterium tuberculosis in histologic specimens. American journal of respiratory and critical care medicine. 1998 Oct 1;158(4):1150-5.
11. Marchetti G, Gori A, Catozzi L, Vago L, Nebuloni M, Rossi MC, et al. Evaluation of PCR in detection of Mycobacterium tuberculosis from formalin-fixed, paraffin-embedded tissues: comparison of four amplification assays. Journal of Clinical Microbiology. 1998 Jun 1;36(6):1512-7
12. Azad KA, Chowdhury T. Extrapulmonary Tuberculosis (Eptb): An Overview. Bangladesh Journal of Medicine. 2022 Apr 27;33(2):130-7.
13. Meghdadi H, Khosravi AD, Ghadiri AA, Sina AH, Alami A. Detection of Mycobacterium tuberculosis in extrapulmonary biopsy samples using PCR targeting IS 6110, rpob, and nested-rpob PCR Cloning. Frontiers in Microbiology. 2015;6: 675.
14. Barcelos D, Franco MF, Leão SC. Effects of tissue handling and processing steps on PCR for detection of Mycobacterium tuberculosis in formalin-fixed paraffin-embedded

samples. Revista do Instituto de Medicina Tropical de São Paulo, 2008;50:321-326.
15. Bisht M, Agrawal R, Mohan N, Kumar P. Clinicopathological spectrum of extrapulmonary tuberculosis in a tertiary care centre of Western Uttar Pradesh with review of literature and special emphasis on the rare sites involved. Indian Journal of Pathology and Oncology. 2016;3(4):721-726.
16. Karim MM, Choudhury SA, Husain MM, Faiz MA. A clinical study on extrapulmonary tuberculosis. Journal of Bangladesh College of Physicians and Surgeons. 2006;24(1): 19-28
17. Abdulkader M, van Aken I, Niguse S, Hailekiros H, Spigt M. Treatment outcomes and their trend among tuberculosis patients treated at peripheral health settings of Northern Ethiopia between 2009 and 2014: a registry-based retrospective analysis. BMC Research Notes.2019;12(1): 1-5.
18. Sreenidhi GM, Nandeeshkumar GN. Clinicopathological study of cervical tubercular lymphadenopathy at KIMS hospital Bangalore. Journal of Evolution of Medical and Dental Sciences. 2013;2(44): 8655-67.
19. Kamal MS, Hoque MH, Chowdhury FR, Farzana R. Cervical tuberculous lymphadenitis: clinico-demographic profiles of patients in a secondary level hospital of Bangladesh. Pakistan journal of medical sciences. 2016 May;32(3):608.
20. Park DY, Kim JY, Choi KU, Lee JS, Lee CH, Sol MY, et al. Comparison of polymerase chain reaction with histopathologic features for diagnosis of tuberculosis in formalin-fixed, paraffin-embedded histologic specimens. Archives of pathology & laboratory medicine. 2003;127(3): 326-330.
21. Ahmed OB, Asghar AH. PCR Versus Microscopic Examinations for Detection of Mycobacterial tuberculosis in formalin fixed Histologic Specimens. 2015
22. Mugulkod P, Chavan S. Serum adenosine deaminase levels and other laboratory parameters in the diagnosis of extrapulmonary tuberculosis: a clinicopathological study. Inter J Res Med Sci. 2017;5(7): 3140-8.
23. Peto HM, Pratt RH, Harrington TA, LoBue PA, Armstrong LR. Epidemiology of extrapulmonary tuberculosis in the United States, 1993–2006. Clinical Infectious Diseases. 2009;49(9): 1350-57.
24. Hillemann D, Galle J, Vollmer E, Richter E. Real-time PCR assay for improved detection of Mycobacterium tuberculosis complex in paraffin-embedded tissues. The International Journal of Tuberculosis and Lung Disease. 2006;10(3): 340-342.
25. Lee HS, Park KU, Park JO, Chang HE, Song J, Choe G. Rapid, sensitive, and specific detection of Mycobacterium tuberculosis complex by real-time PCR on paraffin-embedded human tissues. The Journal of Molecular Diagnostics. 2011;13(4): 390-394.
26. Fukunaga H, Murakami T, Gondo T, Sugi K, Ishihara T. Sensitivity of acid-fast staining for Mycobacterium tuberculosis in formalin-fixed tissue. American journal of respiratory and critical care medicine. 2002; 166(7): 994-997.
27. Özkara HA, Kocagöz T, Özçelik U, Akçören Z, Göçmen A. Comparison of three different primer pairs for the detection of Mycobacterium tuberculosis by polymerase chain reaction in paraffin-embedded tissues. The International Journal of Tuberculosis and Lung Disease.1998; 2(6): 451-455.
28. Tarng DC, Su WJ, Huang TP. PCR diagnosis on formalin-fixed, paraffin-embedded tissues with acid-fast stain and culture negativity in chronic dialysis patients of cervico-mediastinal tuberculous lymphadenitis. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association-European Renal Association.1998;13(6): 1543-1546.