The Journal of Teachers Association
ISSN 1019-8555 (Print) & ISSN 2408-8854 (Online)
Frequency: Bi-Annual
DOI: https://doi.org/10.62469/taj.v037i02.021

OPEN ACCESS
Website: www.tajrmc.com
Email: editor@tajrmc.com
Volume-37 | Issue-2 | Jul-Dec 2024

Role of Real time Polymerase Chain Reaction in the diagnosis of tubercular lymphadenitis

Nazifa Islam[1]*, Shah Md Badruddoza[2], Khadiza khanam[1], Anindita Sarkar[1], Tanshina Afrin[1]

[1] Department of Pathology, Rajshahi Medical College, Rajshahi
[2] Department of Pathology, Islami Bank Medical College, Rajshahi

Abstract: ***Background:*** Tubercular lymphadenitis (TBL) is the most common form of extra pulmonary tuberculosis. Currently the standard diagnostic tool for TBL is culture, which takes more than several weeks to yield results. Recently the use of nucleic acid amplification tests (NAATs), especially polymerase chain reaction (PCR) has gained acceptance for rapid diagnosis of tubercular lymphadenitis. This study is to evaluate the role of real time PCR in the diagnosis of tubercular lymphadenitis. ***Methods:*** This cross-sectional type of descriptive study was conducted in the Department of Pathology, Rajshahi Medical College and Department of Microbiology (BSMMU) over a period of two years from July 2017 to June 2019. Tissue biopsy from the lymph node was fixed with 10% formalin and was processed and stained with haematoxylin and eosin stain and was examined in Pathology Department of Rajshahi Medical College, Rajshahi and Real time PCR was done in Microbiology Department of BSMMU, Dhaka. Out of the 47 cases, 32 cases were histopathologically confirmed as tubercular lymphadenitis and the rest 15 cases were as chronic non-specific lymphadenitis which served as control in the study. ***Results:*** The Real time PCR had (46.87%) sensitivity, (100%) specificity. PPV was (100%) and NPV was (46.87%). The overall diagnostic accuracy of the test was (63.82%). ***Conclusion:*** The study concluded that role of Real time PCR was mildly sensitive and highly specific in the diagnosis of tubercular lymphadenitis.

Keywords: Histopathology, Tubercular lymphadenitis, Chronic nonspecific lymphadenitis, Real time PCR.

Original Research Article

Correspondence:
Dr. Nazifa Islam
Assistant Professor (c.c)
Department of pathology,
Rajshahi Medical College. Rajshahi
E-mail: nazifa79islam@gmail.com

How to cite this article:
Islam, N., Badruddoza, S. M., Khanam, K., Sarkar, A., & Afrin, T; Role of Real time Polymerase Chain Reaction in the diagnosis of tubercular lymphadenitis. Taj 2024;37 (2): 161-167.

Article history:
Received: August 16, 2024
Revised: October 25, 2024
Accepted: November 12, 2024
Published: December 01, 2024

Article at a glance:

Study Purpose: *The purpose of this study was to find out the role of Real time Polymerase Chain Reaction in the diagnosis of tubercular lymphadenitis.*

Key findings: *Real time Polymerase Chain Reaction was highly specific in the diagnosis of tubercular lymphadenitis.*

Newer findings: *Real time Polymerase Chain Reaction is significantly positive in tubercular lymphadenitis.*

Abbreviations: *PCR: Polymerase Chain Reaction, NAAT: Nucleic Acid Amplification Test. TBL: Tubercular.*

INTRODUCTION

Tuberculosis is the major public health problem of developing countries and tubercular lymphadenopathy is the most common extra pulmonary manifestation of tuberculosis.[1] The prevalence rate of all forms of tuberculosis is 404/100000 population in 2014.Extra pulmonary TB constitutes 15-20% of all cases of tuberculosis. Tubercular lymphadenitis is seen in 35% of extra pulmonary cases, cervical lymph nodes being the most common site of involvement (60-90%).[2] The laboratory diagnosis of tubercular lymphadenitis is usually established by histopathology, microscopy for demonstration of acid fast bacilli and mycobacterial culture on biopsy specimen. Each of these diagnostic methods has its own merits and demerits and varies in terms of sensitivity and specificity. Granulomatous lymphadenopathy has a wide differential diagnosis and many other clinical conditions can present the same cytology

and histopathology as tubercular lymphadenitis. Demonstration of AFB by Ziehl-Neelsen (ZN) stain lacks sensitivity.

AFB culture is highly specific and sensitive but takes 4-12 weeks to give results with the drug susceptibility.[3] A meta analysis that was done among 50 studied cases with ZN stain showed 50% sensitivity and 90% specificity. Histopathology showed 80% sensitivity and 90% specificity and PCR showed 100% sensitivity and 90% specificity.[4] PCR recently has been used for the identification of Mycobacterium tuberculosis in formalin fixed tissue. It may replace acid fast staining as the preferred screening method since it is more sensitive test.[5] PCR detects not only nuclear DNA in live bacilli but also DNA molecules isolated from the killed bacilli and deposited in stroma or in the phagocytic cells.[6] PCR can be used for identifying genes related to mycobacterial species or drug resistance strains.[7] PCR can limit the steps, costs, amounts of samples needed, risk of missing mycobacterial DNA and lower the possibility of cross contamination.[8] In tubercular granuloma, mycobacterial staining and culture are often negative but PCR on paraffin embedded tissue seems to be highly sensitive.[5] So the application of PCR along with histopathology help early diagnosis, treatment and rapid outcome in terms of prognosis.

MARTERIALS AND METHODS

This cross-sectional study was conducted in the Department of Pathology, Rajshahi Medical College and Department of Microbiology, BSMMU over a period of two years from July 2017 to June 2019. A total 47 cases of sample were included in the study. Specimen of tissue was fixed with 10% formalin and stained with haematoxyline and eosin stain was examined. Histopathological diagnosis was done in the Rajshahi Medical College and Real Time PCR was done from the Department of Microbiology, BSMMU. Statistical analysis was performed by using SPSS-version 25. The sensitivity, specificity, positive and negative predictive values and diagnostic accuracy were computed. The level of significant was set at 5% and p value 0.05 was considered significant.

RESULT

The this study was intended to find the role of Real time polymerase chain reaction in the diagnosis of tubercular lymphadenitis. A total number of 47 were clinically suspected cases of tubercular lymphadenitis. All the cases were subjected to histopathological examination followed by Real time PCR. Out of 47 cases, 32 cases were histopathologically confirmed as tubercular lymphadenitis and rest 15 cases were found as chronic non-specific lymphadenitis. Among 32 cases, 15 cases were confirmed as tubercular lymphadenitis by Real time PCR.

Table 1: Distribution of patients by demographic characteristics (n=47)

Demographic characteristics	Number of patients	Percentage
Age (years)*		
<20	8	17.0%
21-30	26	55.3%
31-40	6	12.8%
41-50	4	8.5%
>50	3	6.4%
Sex		
Male	20	42.6%
Female	27	57.4%
Socioeconomic condition		
Lower class	24	51.1%
Middle class	21	44.7%
Upper class	2	4.3%
Occupation		
Student	9	19.1%
Farmer	6	12.8%

Service holder and retired person	7	14.9%
Businessman	4	8.5%
Housewife	21	44.7%

*Mean age = (30.02± 12.75) years; Range = (07 – 70) years.

This table showed 55.3% of the patients were in between 21-30 years old with mean age of the patients being 30.02± 12.75 years and the youngest and oldest patients were 7 and 70 years respectively. Majority of the patients were female (57.4%) and were in lower class (51.1%). Housewives comprised over 44.7% of the patients. Student, service holder/ retired person, businessman and farmer were 19.1%, 14.9%, 8.5% and 12.8% respectively.

Table 2: Distribution of patients by histopathological findings (n=47)

Microscopic finding	Number of patients	Percent
Necrotizing granulomatous inflammation	25	53.2%
Granuloma (non-necrotizing)	7	14.9%
Chronic non-specific lymphadenitis	15	31.9%
Total	47	100%

This table showed that on histopathological examination 25 (53.2%) of patients had necrotizing granulomatous inflammation, 7 (14.9%) of patients had non necrotizing granuloma and 15 (31.9%) of patients had chronic non specific lymphadenitis respectively.

Table 3: Distribution of patient by the finding of Real time PCR for mycobacterium tuberculosis from formalin fixed paraffin embedded tissue specimen (n=47)

Real time PCR	Number of patients	Percent
Positive	15	31.91%
Negative	32	68.08%
Total	47	100.0%

This table showed 15(31.91%) of patients had positive Real time PCR and 32(68.08%) of patients had negative Real time PCR.

Table 4: Distribution of patient by histopathological findings of tubercular lymphadenitis and findings of Real time PCR. (n=47)

Histopathological findings	Real time PCR		Total
	Positive	Negative	
Necrotizing granulomatous inflammation	14(29.80%)	11(23.40%)	25(53.18%)
Non necrotizing granulomatous inflammation	1(2.10%)	6(12.80%)	7(14.9%)
Chronic non-specific lymphadenitis	0(0.0%)	15(31.90%)	15(31.9%)
Total	15(31.90%)	32(68.10%)	47(100.0%)

$\chi 2$=14.707, df=2, P value=0.001

This table showed 25(53.18%) patients had necrotizing granulomatous inflammation in which 14(29.78%) of patients had PCR positive and 11(23.40%) PCR negative result. 7(14.9%) patients had non necrotizing granulomatous inflammation in which 1(2.1%) of patients had positive PCR and

6(12.8%) had negative PCR results. 15(31.9%) patients had chronic non specific lymphadenitis in which all the patients had PCR negative result. The relationship between histopathological findings and Real time PCR was found statistically significant (P< 0.01).

Table 5: Accuracy of Real time PCR in diagnosis of tubercular lymphadenitis from chronic non-specific lymphadenitis (n=47)

Real time PCR	**Histopathological diagnosis**		Total
	Tubercular lymphadenitis	**Chronic non-specific lymphadenitis**	
Positive	15 (31.90%)	0(00%)	15 (31.90%)
Negative	17 (36.20%)	15 (31.90%)	32 (68.10 %)
Total	32 (68.10%)	15 (31.90%)	47 (100.0%)

$\chi 2$=10.327, df =1, P value=0.001

This table showed 68.10% patients had tubercular lymphadenitis on histopathological examination in which 31.90% patients had PCR positive results and 36.20% patients had PCR negative results. This table also showed 31.90% patients had chronic non- specific lymphadenitis on histopathological examination but all the patients had PCR negative results. The sensitivity of Real time PCR was 46.87% and specificity was 100%. The positive and negative predictive values of the test were 100% and 46.87% respectively. The overall diagnostic accuracy of the test was 63.82%.

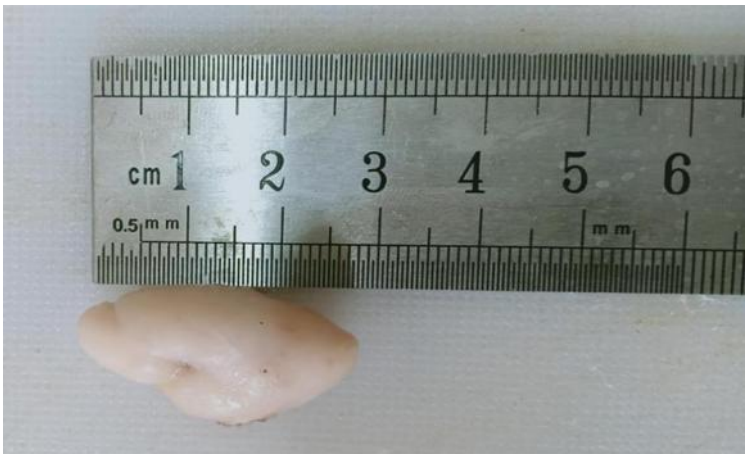

Figure 1: Gross appearance of tubercular lymphadenitis

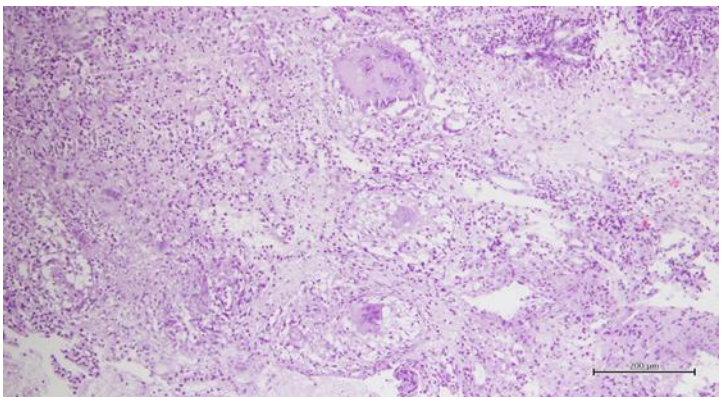

Figure 2: Histological section showing typical granuloma with langhans giant cell.

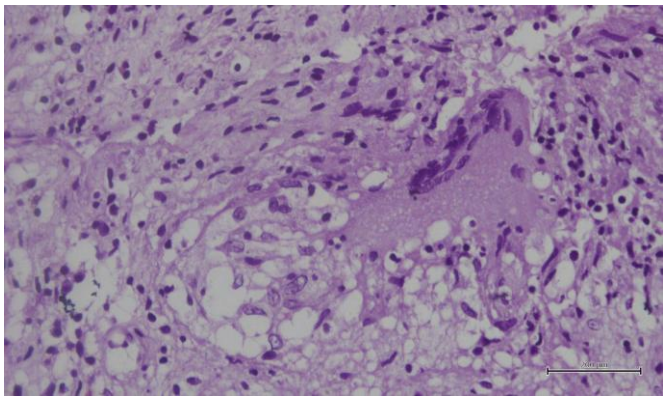

Figure 3: Histological section showing tubercular lymphadenitis with epithelioid cells.

DISCUSSION

Nucleic acid amplification techniques (NAAT) including PCR have a considerable compact on disease diagnosis on account of their specificity and sensitivity. PCR application to the diagnosis of tubercular lymphadenitis has the potential to resolve one of the foremost challenges facing a diagnostic laboratory.[9] Despite a low sensitivity, the real time PCR application might still be of interest in case of non diagnostic histopathological analysis because of the much shorter time to results. When likelihood ratios were taken into account, the positive real time PCR result would significantly raise the probability of TBL, while the negative result slightly lowered the probability of TBL.[10] In this study an attempt was made to assess the role of Real time PCR in the diagnosis of tubercular lymphadenitis. In this study 47 samples were collected in which only 32 samples were diagnosed as tubercular lymphadenitis and 15 were as chronic non specific lymphadenitis by histopathological examination. In this study most common age group was 21-30 years and age range was 7 to 70 years. Mean age was 30±12.75. This results was agreed with Sreenidhi et al.[1] which was 30±11.32 and disagreed with Abdulkader et al.[11], which was 33.9 years.

Most common sex distribution of this study was female (57.4%) which was disagreed with Mohammad Shah Kamal et al[2]. which was about 44% and agreed with Sreenidhi et al[1] which were about 55.4%. Common socioeconomic status of this study was (51.1%) which was agreed with Mohammad Shah Kamal et al.[2] which was lower class. But percentage was slightly higher (58.5%). In this study, the most common occupation was housewives (44.7%) which was agreed with Mohammad Shah Kamal et a,[2] which was also common but percentage was little lower and which was about 38.5%. On histopathological examination of this study it was found that 53.2% had necrotizing granulomatous inflammation which was the highest percentage. Other percentages were non necrotizing granulomatous inflammation (14.9%) and as chronic non-specific lymphadenitis (31.0%). This result was agreed with Park et al.[12] who also found necrotizing granulomatous inflammation as highest (65.4%) and disagreed with Sreenidhi et al.[1] which was much higher (72%). In this study, 31.91% cases had PCR positive and 68.08% cases had PCR negative result.

This result was disagreed with Yoo Jin Lee.[13] who had found 17. 5% as positive and 82.4% as negative and P Bhargava[3] who had found 69.2% as positive and 47.38% as negative. Comparison between histopathological examination and PCR showed p value (0.001) which was disagreed with Yoo Jin Lee[13] who also found p value 0.01. In this study specificity was 100% which was totally agreed with P Bhargava [3] which was also 100%. Sensitivity, PPV, NPV and diagnostic accuracy test were 46.87%, 100%, 46.87% and 63.82% respectively which were disagreed with P Bhargava et al.[3] Low specificity was due to short sample size and old tissue block. Real time PCR is a good alternative to detect TB but it may be sufficient sensitive in selected cases. These results correlate with the histological features, suggesting that the specimens with necrosis should be used to identify TB with PCR. To overcome lower diagnostic sensitivity of PCR, large sized tissue samples with typical

histological changes detected in TB, a proper fixation time, recent tissue blocks are needed. Care to reduce contamination and tissue micro dissection are also required.

CONCLUSION

From the findings of this study it was concluded that Real time PCR has mild sensitivity in the detection of tubercular lymphadenitis although its specificity was appreciably high. Real time PCR is especially suitable for identifying bacterial strains and drug resistant bacteria, which are difficult to culture or grow slowly. It remedies the short comings of traditional methods used to detect pathogenic bacteria, meets the requirements for early diagnosis of tubercular lymphadenitis and has good prospects for clinical application. Real time PCR of tissue specimen is a good alternative to detect tubercular lymphadenitis, but it might not be as sensitive as previously suggested. Its reliability might also be influenced by some histological features like necrosis and old tissue block.

Acknowledgment

Contribution of all the stuffs of histopathology laboratory, Department of pathology, Rajshahi Medical College, Rajshahi and Department of Microbiology, BSMMU, Dhaka. The authers express their sincere gratitude for their help.

Authors' Contributions

Dr. Nazifa Islam: Concept and design, data acquisition, interpretation, drafting and final approval. Prof Dr. Shah Md Badruddoza and Prof Dr. Khadiza khanam: Supervision, and helped to shape the manuscript. Dr. Anindita Sarkar and Dr. Tanshina Afrin: Data Acquisition.

Declarations

Funding

The authors received no financial support for the research, authorship and /or publication of this article.

Conflict of interest: authors declared no conflict of interest.

Ethical approval

Ethical approval of the study was obtained from the Ethical Review Committee, Rajshahi Medical College, Rajshahi. All the study methodology was carried out following the relevant ethical guidelines and regulations.

Consent for publications: Taken.

REFERENCES

1. Sreenidhi GM, Nandeeshkumar GN. Clinicopathological study of cervical tubercular lymphadenopathy at KIMS hospital Bangalore. Journal of Evolution of Medical and Dental Sciences. 2013 Nov 4;2(44):8655-67.
2. Kamal MS, Hoque MH, Chowdhury FR, Farzana R. Cervical tuberculous lymphadenitis: clinico-demographic profilesof patients in a secondary level hospital of Bangladesh Pakistan journal of medical sciences.2016 May;32(3):608..
3. Patwardhan SA, Bhargava P, Bhide VM, Kelkar DS. A study of tubercular lymphadenitis: a comparison of various laboratory diagnostic modalities with a special reference to tubercular polymerase chain reaction. Indian journal of medical microbiology. 2011 Oct 1;29(4):389-94.
4. Ahmed OB, Asghar AH. PCR Versus Microscopic Examinations for Detection of Mycobacterial tuberculosis in formalin fixed Histologic Specimens.
5. Hardman WJ, Benian GM, Howard T, Mcgowan Jr JE, Metchock B, Murtagh JJ. Rapid detection of mycobacteria in inflammatory necrotizing granulomas from formalin-fixed, paraffin-embedded tissue by PCR in clinically high-risk patients with acid-fast stain and culture-negative tissue biopsies. American journal of clinical pathology. 1996 Sep 1;106(3):384-9.
6. Hernandez-Pando R, jeyanathan M, Mengistu G, Aguilal D, Orozco H, Harboe M, Rock GA, Bjuine G. Resistance of DNA from mycobacterium tuberculosis in superficially normal lung tissue during latent infection. The *Lancet*, 356(9248), pp.2133-2138.
7. Tevere VJ, Hewitt PL, Dare A, Hocknell P, Keen A, Spadoro JP, Young KK. Detection of Mycobacterium tuberculosis by PCR amplification with pan-Mycobacterium primers and hybridization to an M. tuberculosis-specific probe. Journal of Clinical Microbiology. 1996 Apr;34(4):918-23.

8. Gholoobi A, Masoudi-Kazemabad A, Meshkat M, Meshkat Z. Comparison of culture and PCR methods for diagnosis of Mycobacterium tuberculosis in different clinical specimens. Jundishapur journal of microbiology. 2014 Feb;7(2).
9. Singh KK, Muralidhar M, Kumar A, Chattopadhyaya TK, Kapila K, Singh MK, Sharma SK, Jain NK, Tyagi JS. 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.
10. Dinnes J, Deeks j, Kunst H, Gibson A, Cummins E, Waugh N, Drobniewski F, Lalvani A. A systematic review of rapid diagnostic tests for the detection o tuberculosis infection. Health technology assesment. 2007;11(3).
11. Albasi, AM, El-Siddig, AA, Hussainy AS, Alhujaily AS. Pattern of lymph node pathology in western Saudi Arabia.Asian Pacific Journal of Cancer prevention.2014;15(11):4677-81.
12. Park DY, Kim JY, Choi KU, Lee JS, Lee CH, Sol MY, Suh KS. Comparism of polymerase chain reaction with histopathologic features for diagnosis of tuberculosis in formalin- fixed , paraffin -embedded histologic specimens. Archives pathology and laboratory medicine . 2003 Mar 1;127(3):326-30.
13. Yang YC, Lu PL, Huang SC, Jenh YS, Jou R, Chang TC. Evaluation of the Cobas TaqMan MTB test for direct detection of Mycobacterium tuberculosis complex in respiratory specimens. Journal of clinical microbiology. 2011 Mar;49(3):797-801.

The Journal of Teachers Association
Abbreviated Key Title: TAJ
Official Journal of Teachers Association Rajshahi Medical College