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ORIGINAL ARTICLE

Diagnostic Accuracy of GeneXpert in Comparison with Histopathological and Microbiological findings in Children with Suspected Tuberculous Lymphadenitis

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ABSTRACT

Objective: To determine the diagnostic accuracy and relationship between GeneXpert MTB/RIF assay, histopathological findings, and microbiological results in children with suspected tuberculous lymphadenitis.

Study Design: Cross-sectional study.

Place and Duration of Study: Pediatric Department, Gulab Devi Hospital, Lahore, Pakistan, from April 2023 to September 2023.

Material and Methods: A total of 50 children aged 2–14 years with suspected tuberculous lymphadenitis were enrolled using non-probability consecutive sampling. Clinical data were recorded using a structured questionnaire. Lymph node samples were obtained through fine needle aspiration cytology or biopsy and subjected to histopathological examination. Microbiological evaluation included acid-fast bacilli smear microscopy, and molecular testing was performed using GeneXpert MTB/RIF assay. Chest X-ray was carried out in all patients. Data were analyzed using SPSS version 25. Chi-square and Fisher's exact tests were applied to assess associations between variables, and independent sample t-tests were used to compare diagnostic modalities. A p-value of <0.05 was considered statistically significant.

Results: The majority of patients were aged 10–13 years (76%). Fever was present in 76% and lymph node enlargement in 88% of cases. AFB smear was positive in 6% of patients, while GeneXpert detected Mycobacterium tuberculosis in 58% of cases. Histopathological examination revealed granulomatous inflammation with caseous necrosis in 26% and without necrosis in 24% of cases, while 12% showed features suggestive of malignancy. Chest X-ray findings were suggestive of tuberculosis in 48% of patients. Statistically significant associations were observed between GeneXpert, microbiological findings, and histopathological outcomes ($p<0.05$).

Conclusion: GeneXpert MTB/RIF assay demonstrates higher diagnostic yield compared to smear microscopy and shows significant correlation with histopathological findings in pediatric tuberculous lymphadenitis. A combined diagnostic approach using molecular, histopathological, and microbiological methods improves diagnostic accuracy and helps avoid unnecessary empirical treatment.

Keywords: Tuberculous lymphadenitis, GeneXpert MTB/RIF, Fine needle aspiration cytology, Extrapulmonary tuberculosis, Pediatric tuberculosis.

INTRODUCTION

Tuberculosis (TB) is a significant health issue across the world with the low- and middle-income countries being overburdened. Pakistan is one of the countries with a high TB burden that contributes to the disease load on the global and regional level. Around 61 percent of the TB in the World Health Organization (WHO) Eastern Mediterranean Region is attributed to the country, which is also one of the leading nations in terms of prevalence rates of multidrug-resistant tuberculosis (MDR-TB)[1]. Although there is an access to uniform treatment regimens, the delays in diagnosis and gaps within the health system provision remain contributors to the spread of the disease and unfavorable outcomes.

Pakistan has close to 510,000 new infections of TB every year, and about 4.2 percent of the infections are multi-drug resistant[2]. Drug-resistant TB presents a major challenge to TB control program because it has a long course of treatment, it is very toxic, expensive, and the success rate of treatment is low. Some of the factors that have led to development of drug resistance include late diagnosis, improper or poor treatment, absence of supervision of treatment, and ineffective social support systems of the vulnerable populations[3].

The percentage of extrapulmonary TB (EPTB) is particularly high in children, and the most frequent form of this disease is tuberculous lymphadenitis (TBLA)[4]. TBLA often occurs with chronic lymphadenopathy and constitutional symptoms, although it has a close resemblance to other infectious, inflammatory and malignant conditions. Such an overlap can make it difficult to diagnose in time and correctly and may consequently require empirical anti-tuberculosis therapy (ATT), thus exposing the patients to unnecessary drug treatment and causing improvement in diagnosis of other pathologies, such as malignancies,[5].

Microbiological confirmation in TBLA is complex because of the paucibacillary condition of the disease as well as the complications encountered in obtaining sufficient tissue samples. Minimally invasive diagnostic methods such as fine needle aspiration cytology (FNAC) and lymph node biopsy are typically used, with the option of being subjected to the microbiological analysis of histopathology, and microbiological testing such as acid-fast bacilli (AFB) smear and culture[6]. Although the mycobacterial culture is the best method of TB diagnosis, its common use is constrained by the long turnaround time that is usually between two and four weeks and thus may act as a bottleneck in the treatment decision.

AFB smard microscopy, despite being fast and cost-effective, is poorly sensitive in extra pulmonary specimens, especially in children[7]. Granulomatous inflammation with or without caseous necrosis is suggestive, but not pathophysiological findings of tuberculosis and can be observed in other infectious and non-infectious diseases. Because of this, relying solely on histology may cause diagnostic uncertainty particularly in cases of culture-negativity.

GeneXpert MTB/RIF assay is a real-time nucleic acid amplification test that has shown to be a promising diagnostic test modality that is able to detect Mycobacterium tuberculosis DNA

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and rifampicin resistance within two hours[8]. Sanctioned by WHO in 2010 and subsequently suggested to use in extrapulmonary samples, GeneXpert has better sensitivity than smear microscopy and has quicker outcomes than the culture. Its application in pediatric TBLA, especially in matching molecular findings with histopathological and microbiological findings, is a field that is under development.

Since the diagnosis of tuberculous lymphadenitis in children is complex and improper diagnosis or treatment might have severe outcomes, there is a necessity to test the diagnostic effectiveness of GeneXpert in the context of clinical routine. The interpretation of the connection between molecular, histopathological, and microbiological data can be useful in improving diagnostic regimens, minimizing the use of unneeded empirical therapy, and patient outcomes.

Objective: To identify the correlation and diagnostic accuracy between GeneXpert, histopathological, and microbiological results of children with suspected tuberculous lymphadenitis.

METHODOLOGY

The study was a cross-sectional study in Pediatric Department of Gulab Devi Hospital, Lahore, Pakistan. It was conducted after receiving permission of the institutional ethical review committee from April 2023 to September 2023.

The population of the study included children aged between 2 and 14 years who had clinical suspicions of tuberculous lymphadenitis. Fifty patients were recruited through the non-probability consecutive method. The criteria used in the inclusion were; children who showed enlargement of the lymph nodes, more than 2 cm in size, lasted a month or more, and may or may not have the constitutional symptoms of fever and weight loss.

The children under 2 years and older than 14 years, those already on anti-tuberculosis therapy, lymph node size less than 2 cm, less than one month of symptoms and those who had been assessed or treated by any other medical institution were excluded out of the study.

Demographic and clinical data were gathered using a structured questionnaire with close-ended questions taking place after seeking informed consent of parents or guardians. All participants were recorded on information about their age, weight, whether they had fever, the swelling of their lymph nodes and weight loss.

Fine needle aspiration cytology or biopsy under aseptic condition was used to obtain the lymph node samples. The samples collected were taken to the histopathology section and classified as granulomatous inflammation with caseous necrosis, granulomatous inflammation without necrosis, reactive lymphoid hyperplasia, chronic non-specific lymphadenitis, or results indicative of malignancy.

Microbiological tests were done using acid-fast bacilli smears microscopy of lymph node specimens to identify Mycobacterium tuberculosis. Molecular testing Molecular testing was performed using the GeneXpert MTB/RIF test to determine the presence of DNA of Mycobacterium tuberculosis and resistance to rifampicin. Chest radiography was done to all the recruited patients to evaluate pulmonary involvement and was categorized as normal or indicative of tuberculosis.

Statistical package of social sciences version 25 was used to enter and analyze data. The quantitative variables age was represented by mean and standard deviation and the qualitative variables like clinical features, microbiological findings, GeneXpert findings, histopathology findings and chest radiograph findings were represented by frequencies and percentages.

Categorical association was determined by Chi-square test and Fisher exact test where the cell count was less than five was used. Independent sample t -tests were employed to compare microbiological, GeneXpert, and FNAC or biopsy results. The p-value below 0.05 was taken to be significant.

RESULTS

The study involved 50 children suspected to have a tuberculous lymphadenopathy. Most of the patients were of age group 10-13 years whereas less were found among the 6-9 years age group. The notable presenting features of the population of the study included fever and enlargement of the lymph nodes.

The microbiological analysis showed that the majority of patients were negative in acid-fast bacilli microscopy by smears. More than half of the cases had Mycobacterium tuberculosis identified by GeneXpert MTB/RIF assay. Histopathological observation revealed a great deal of results such as granulomatous inflammation with or without caseous necrosis, reactive lymphoid hyperplasia, chronic non-specific lymphadenitis, and changes associated with malignancy. The chest radiography revealed almost equal distribution of normal to radiological findings which was suggestive of tuberculosis.

Table 1: Demographic and Clinical Characteristics of the Study Population (n = 50)

Variable	Category	Frequency	Percentage
Age group	6–9 years	12	24.0
	10–13 years	38	76.0
Fever	Yes	38	76.0
	No	12	24.0
Lymph node enlargement	Yes	44	88.0
	No	6	12.0

Table 2: Microbiological, Molecular, and Radiological Findings

Investigation	Findings	Frequency	Percentage
AFB smear	Positive	3	6.0
	Negative	47	94.0
GeneXpert	Detected	29	58.0
	Not detected	21	42.0
Chest X-ray	Normal	26	52.0
	Suggestive of TB	24	48.0

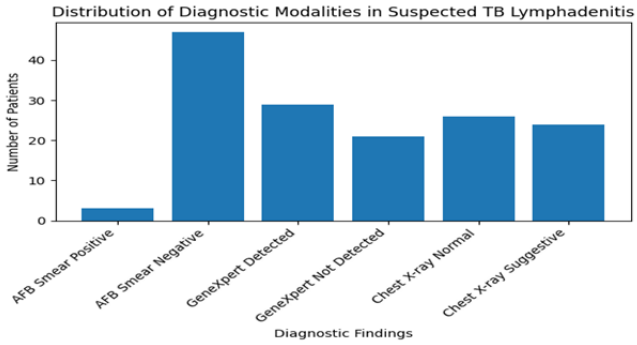
Table 3: Histopathological Findings on FNAC/Biopsy

Histopathological diagnosis	Frequency	Percentage
Granulomatous inflammation with caseous necrosis	13	26.0
Granulomatous inflammation without necrosis	12	24.0
Reactive lymphoid hyperplasia	13	26.0
Chronic non-specific lymphadenitis	6	12.0
Suggestive of malignancy	6	12.0
Total	50	100.0

Table 4: Association of Clinical Variables with Diagnostic Modalities (Chi-square Test)

Variable	FNAC/Biopsy (p-value)	GeneXpert (p-value)	Microbiological findings (p-value)
Age group	0.000	0.000	0.031
Fever	0.005	0.047	0.021
Lymph node enlargement	0.026	0.024	0.001

Figure 1: Distribution of Diagnostic Modalities in Suspected Tuberculous Lymphadenitis



Statistical means showed that the age, clinical features, and diagnostic modalities were significantly associated with each other. Important statistically significant associations between age groups, fever and lymph node enlargement and FNAC/biopsy, GeneXpert and microbiological findings outcomes were identified using chi-square and Fisher exact tests. Additional tests such as independent sample t-tests supported the finding that there are significant differences in microbiological results, GeneXpert results, and FNAC/biopsy results.

A bar chart demonstrating the proportion of positive and negative results for AFB smear microscopy, GeneXpert MTB/RIF assay, and chest X-ray findings among the study population. The graph highlights the higher detection rate of GeneXpert compared to smear microscopy.

DISCUSSION

Tuberculous lymphadenitis has remained a leading presentation of extrapulmonary tuberculosis in children and has been a problematic diagnostic entity because it is a paucibacillary disease which has similar clinical manifestation to other benign and malignant diseases. Early and proper diagnosis is needed in order to avoid the needless exposure to anti-tuberculosis medication, as well as, to avoid the missed alternative diagnoses. This paper assessed the diagnostic correlation between GeneXpert MTB/RIF, the examination findings, and microbiological outcomes of children with suspected tuberculous lymphadenitis.

In the current study, most of the patients were of the elderly pediatric group, with most of the cases falling within the[10-13] years. It is the age trend that is in keeping with the earlier studies that indicated high incidence of extrapulmonary tuberculosis among older children because of the high level of exposure to the environment and the developing immunity[9,10]. The most common clinical characteristics were fever, and the enlargement of lymph nodes, which underlines their low specificity and the necessity of laboratory testing[11].

Microbiological profile showed that acid-fast bacilli smear microscopy was very low in positivity. It has been established that this finding is well described in extrapulmonary and pediatric tuberculosis where the bacillary load is usually low and hence, the smear sensitivity is poor[12]. The small diagnostic value of the smear microscopy justifies its poor clinical value as a diagnostic method in the diagnosis of tuberculous lymphadenitis and the necessity of the adjunctive diagnostic methods.

In this study, the rate of detection by GeneXpert MTB/RIF assay was significantly higher than that of the smear microscopy. This is in the line with the existing evidence that molecular testing is quite beneficial in enhancing the detection of the case in extrapulmonary tuberculosis especially in lymph node samples[13,14]. The rapid turnaround time and the possibility of detecting rifampicin resistance also increase its clinical utility particularly in the setting where delay in diagnosis may result in the development of the disease and transmission[15].

The histopathological studies showed various results such as granulomatous inflammation with or without caseous necrosis, reactive lymphoid hyperplasia, chronic non-specific lymphadenitis and malignancy. Although granulomatous inflammation with caseation is a characteristic that indicates tuberculosis, its absence does not rule out the diagnosis, with similar appearance histological patterns can be found in other infectious and inflammatory diseases[16]. The risk of empirical treatment without the confirmation of the tissues is emphasized by the presence of non-tuberculous and malignant diagnoses in a significant percentage of cases.

A statistical analysis revealed that there was a significant correlation between the GeneXpert results, microbiological results and the FNAC or biopsy results. The strong association of GeneXpert and histopathology indicates the possibility to use molecular testing to supplement cytological and histological analysis especially when the traditional microbiological analysis is negative[17]. These results justify the incorporation of GeneXpert into the daily diagnostic algorithms of suspected lymphadenitis tuberculosis in children.

The results of chest radiographs consisted almost equally of normal and suggestive ones. This highlights the lack of sensitivity of chest X-ray in extrapulmonary tuberculosis and reinforces the earlier findings that pulmonary disease can be absent not only in a significant proportion of cases of lymph node tuberculosis[18]. Thus, the use of chest radiography can lead to the underdiagnosis or late detection of extrapulmonary disease.

The results of the conducted research indicate the significance of a multimodal diagnostic method comprising of the clinical examination, histopathological examination, and microbiological examination and molecular diagnostics. GeneXpert seems to be a useful complementary method that increases the precision of diagnosis and can minimize unproductive empirical treatment among children with possible tuberculous lymphadenitis[19,20].

Limitations: This research was limited in a number of ways. The study sample was quite small and was performed in one center, which can restrict the extrapolation of the results. The mycobacterial culture which is the diagnostic gold standard was not performed regularly because of the logistical reasons, and it could not be directly compared with the molecular and histopathological ones. Also, the type of design was cross-sectional, which did not permit evaluation of the outcomes of treatments or follow-up.

CONCLUSION

This work illustrates that tuberculous lymphadenitis in children is a diagnostically difficult disease because of the low microbiological results and non-specific clinical and radiological manifestations. GeneXpert MTB/RIF demonstrated a better level of detection than the conventional smear microscopy and had a significant relationship with histopathological results, which are in favor of the technique as a useful addition to the diagnostic assessment of suspected cases. Histopathology is crucial in the detection of alternative diagnoses such as malignancy thus avoiding the use of empirical treatment. The integrated diagnostic methodology that involves the use of molecular, histopathological, and microbiological diagnosis can enhance the accuracy of the diagnosis, enable the correct therapeutic response to be started as soon as possible, and help to eliminate unnecessary exposure of anti-tuberculosis treatment to pediatric patients with lymphadenopathy.

REFERENCES

1. Sarfaraz S, Iftikhar S, Memon Y, Zahir N, Hereker FF, Salahuddin N. Histopathological and microbiological findings and diagnostic performance of GeneXpert in clinically suspected tuberculous lymphadenitis. International Journal of Infectious Diseases. 2018 Nov 1;76:73-81.
2. Sharif N, Ahmed D, Mahmood RT, Qasim Z, Khan SN, Jabbar A, Khattak AA, Asad MJ, Ahmed W, Khan MM, Awan UA. Comparison of different diagnostic modalities for isolation of Mycobacterium Tuberculosis among suspected tuberculous lymphadenitis patients. Brazilian Journal of Biology. 2021 Aug 20;83:e244311.
3. Onkari Amruta S, Iravane JA. Comparative utility of GeneXpert assay over fine needle aspiration cytology in early diagnosis of tuberculous lymphadenitis. IOSR J Dent Med Sci. 2021;20:23-30.
4. Raja R, Sreeramulu PN, Dave P, Srinivasan D. GeneXpert assay–A cutting-edge tool for rapid tissue diagnosis of tuberculous lymphadenitis. Journal of Clinical Tuberculosis and Other Mycobacterial Diseases. 2020 Dec 1;21:100204.
5. Nur TE, Akther S, Kamal M, Shomik M, Mondal D, Raza M. Diagnosis of Tuberculous Lymphadenitis using fine needle aspiration cytology: a comparison between Cytomorphology and GeneXpert Mycobacterium Tuberculosis resistant to rifampicin (MTB/RIF) test. Clinical Infection and Immunity. 2019 Jan 1;1(01):4.
6. Ahmed S, Ahmed S, Tayyaba U. A clinicopathological profile and role of newer methods in diagnosis of paediatric tubercular lymphadenopathy. Int Clin Pathol J. 2022;9(1):9-13.
7. Ahmed S, Ansari HA, Fatima N. A comparative study of the role of cytopathology, staining for acid-fast bacilli and genexpert MTB/RIF

assay in the diagnosis of tubercular lymphadenopathy. IP J Diagn Pathol Oncol. 2022;7:22-9.
8. Mansur H, Asif M, Khadim MT, Khan IM, Ahmed R. Concordance of Cytomorphological Features of Cervical Lymphadenitis Suspected for Mycobacterium Tuberculosis on Fine Needle Aspiration Biopsy with GeneXpert for Mycobacterium Tuberculosis on Aspirated Material. J Cytol Histol. 2018;9(526):2.
9. Mayura S, Gaddam P, Cherian S, Chaturvedi U, Naidu R. Tuberculous lymphadenitis: analysis of Cytomorphological features with utility of Genexpert MTB/RIF and other Microbiological Test as an Adjunct to Cytology. SAS J Med. 2023 Aug;8:854-9.
10. Fantahun M, Kebede A, Yenew B, Gemechu T, Mamuye Y, Tadesse M, Brhane B, Jibriel A, Solomon D, Yaregal Z. Diagnostic accuracy of Xpert MTB/RIF assay and non-molecular methods for the diagnosis of tuberculosis lymphadenitis. PLoS One. 2019 Sep 16;14(9):e0222402.
11. Tahseen S, Ambreen A, Ishtiaq S, Khanzada FM, Safdar N, Sviland L, Mustafa T. The value of histological examination in the diagnosis of tuberculous lymphadenitis in the era of rapid molecular diagnosis. Scientific Reports. 2022 May 27;12(1):8949.
12. Puri A, Khairong PD, Singh S. A critical appraisal of clinicopathological, imaging, and genexpert profiles of surgical referrals with pediatric abdominal tuberculosis. Journal of Indian Association of Pediatric Surgeons. 2023 Jan 1;28(1):41-7.
13. Kiruthiga S. Role of Cartridge Based Nucleic Acid Amplification Test (CBNAAT-Genexpert) in the Diagnosis of Pediatric Pulmonary Tuberculosis (Doctoral dissertation, Rajiv Gandhi University of Health Sciences (India)).
14. Jyothy A. The Utility of CSF Genexpert in Diagnosis of Tubercular Meningitis in Children Aged 2 Months to 12 Years (Doctoral dissertation, Rajiv Gandhi University of Health Sciences (India)).
15. Mansur H, Asif M, Khadim MT, Khan IM, Ahmad R, Azhar A, Anwar M. Concordance of Cytomorphological Features of Cervical Lymphadenitis Suspected for Mycobacterium Tuberculosis on Fine Needle Aspiration Biopsy with Genexpert For Mycobacterium Tuberculosis on Aspirated Material. Pakistan Armed Forces Medical Journal. 2019 Dec 1;69(6).
16. Sun W, Gu J, Bi K, Zhang Y, Shen MJ, Wang Y, Fan L. Clinical performance of Xpert MTB/RIF on contrast-enhanced ultrasound-guided core biopsy specimens for rapid diagnosis of superficial tuberculous lymphadenitis in high TB burden settings. Infection. 2021 Aug;49(4):653-60.
17. Shetty D, Vyas D. Combination method for the diagnosis of tuberculous lymphadenitis in high burden settings. Surgical and Experimental Pathology. 2022 May 10;5(1):11.
18. Antel K, Oosthuizen J, Malherbe F, Louw VJ, Nicol MP, Maartens G, Verburgh E. Diagnostic accuracy of the Xpert MTB/Rif Ultra for tuberculosis adenitis. BMC infectious diseases. 2020 Jan 13;20(1):33.
19. Bholla M, Kapalata N, Masika E, Chande H, Jugheli L, Sasamalo M, Glass TR, Beck HP, Reither K. Evaluation of Xpert® MTB/RIF and Ustar EasyNAT™ TB IAD for diagnosis of tuberculous lymphadenitis of children in Tanzania: a prospective descriptive study. BMC infectious diseases. 2016 Jun 6;16(1):246.
20. Iram S. Diagnostic validation of rapid molecular detection of Mycobacterium tuberculosis in pus samples by GeneXpert. JPMA. The Journal of the Pakistan Medical Association. 2018 Jan 1.

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