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A clinicopathological profile and role of newer methods in diagnosis of paediatric tubercular lymphadenopathy

Abstract

Objectives: Lymphadenopathy is a common finding in children and poses a diagnostic dilemma. There are various conventional methods for the diagnosis of tubercular lymphadenopathy with some newer methods like GeneXpert Assay recently introduced. This study emphasizes the role of newer diagnostic methods and a detailed clinicopathological profile of paediatric patients.

Methods: This was a four-year prospective study including a total of 512 children under 13 years who were clinically suspected of tubercular lymphadenopathy were included. FNAC followed by ZN staining, Modified bleach method, MTP 64 Antigen testing, GeneXpert MTB/RIF Assay analysis and Culture was performed on the adequate samples and studies of comparison were done.

Results: Sensitivity, specificity and diagnostic accuracy of different methods were compared and GeneXpert outshined the other method of diagnosis with a sensitivity of 93.1%, specificity of 100 % and diagnostic accuracy of 97.5%.

Interpretation & conclusions: GeneXpert essay should be included in early in the diagnostic workflow of paediatric lymphadenopathy which will potentially improve the overall detection and reduce the turn-around time, thus preventing the unnecessary delay in treatment thus reducing the overall burden of TB.

Keywords: paediatric lymphadenopathy, tuberculosis, FNAC, MTB64 antigen, modified bleach technique, geneXpert MTB/RIF assay

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Introduction

Lymphadenopathy due to various causes is a commonly encountered problem in the paediatric population.[1] A proper understanding of these causes of lymphadenopathy is mandatory in determining the most appropriate diagnostic workup and management.[2] A thorough history and physical examination along with diagnostic studies play a vital role in narrowing the differential diagnosis of lymphadenopathy.[1] In India, reactive hyperplasia is the most common type of lymphadenitis followed by granulomatous lymphadenopathy in children.[3] Children are at a greater risk of developing extrapulmonary tuberculosis, the most common manifestation being superficial lymphadenitis.[4] Diagnosis of TB in children is based on cumulative data like clinical history, investigations along with Fine needle aspiration, histopathology, Ziehl Nelson Staining for Acid Fast bacilli along with other tests like culture, GeneXpert *Mycobacterium Rifampicin* (MTB/RIF Assay). On FNAC granuloma with necrosis is the hallmark of tuberculous lymphadenitis. Smear from Tuberculous lymph nodes may sometime show only polymorphs and necrotic debris without histiocytes, particularly in immunocompromised patients.[5] A positive result is highly reliable whereas a negative result has to be confirmed by other ancillary tests. Inconclusive reports pose a predicament as performing a repeat FNAC is quite problematic in the case of the pediatric population. Culture confirms the diagnosis but is time-consuming, compromising patient care and outcome. Ziehl Nelson staining can be used as a supplementary test in the detection of acid-fast bacilli has been limited by its low sensitivity. Even when mycobacteria are seen, it is impossible to distinguish Mycobacterium tuberculosis from nontuberculous mycobacterial disease.[6] The modified Bleach method is a simple, inexpensive method that involves liquefaction by sodium hypochlorite (NaOCl, bleach) and concentration of bacilli through centrifugation improves the sensitivity of detection of acid-fast bacilli and also acts as a potent disinfectant thus limiting the risk of laboratory-acquired infections.[7] MTP 64 Antigen rapid immunochromatographic assay is a simple reliable identification kit useful in resource-poor laboratories which detects the Rv1980c protein which is specific for mycobacterium tuberculosis complex and reduces the overall turnaround time and has high sensitivity and specificity.[8] Newer diagnostic tests including Nucleic acid amplification tests for rapid tuberculosis diagnosis are increasingly being used. WHO recommends the use of GeneXpert MTB/RIF assay (Cepheid Inc., CA, USA) which is a multifunctional diagnostic automated closed system based on real-time PCR and can be used with minimal technical expertise, enabling diagnosis of TB and simultaneous assessment of rifampicin resistance to be completed within 2 h.[9] Thus, this study was done to study the clinicopathological profile of the paediatric patient and compare the role of various methods including some newer methods in the diagnosis of paediatric tubercular lymphadenopathy.[10]

Materials and methods

A four-year prospective study was conducted in the Department of Pathology and Microbiology, Jawaharlal Nehru Medical College and Hospital, AMU, Aligarh. A total of 512 children under 13 years who were clinically suspected of tubercular lymphadenopathy were included. Informed consent was taken and FNAC was carried out on the enlarged, palpable lymph nodes under aseptic conditions using a 23-gauge needle. Subsequently, smears were made for further cytopathological examination. From the adequate aspirated sample (at least 2ml), Ziehl Nelson Staining for Acid Fast Bacilli, modified bleach method was done. Aspirated material was mixed with buffer

and inoculated in immunochromatographic test kits for MTB 64 Antigen testing and the Residual sample was sent for GeneXpert MTB/RIF Assay analysis and culture in the Department of Microbiology. Culture on LJ media was taken as the gold standard and statistical comparison was done by MedCalc Software.[11]

Observations

The present study was conducted on 512 patients of the Paediatric age group presenting with lymphadenopathy. Out of which 237 patients had adequate samples for testing by different methods. Clinicopathological profiles and studies of comparison were done on these samples. Out of the total of 237 cases, 135(56.9%) were found to be reactive lymphadenopathy, 91 cases of tubercular lymphadenopathy(38.4%), 7 (2.9%) cases were of abscess and 4 (1.6%) were a lymphoproliferative disease.

Results

In the present study maximum cases (27.2%) of tubercular lymphadenopathy occurred in the age group of 5-10 years (Figure 1–3). Female children were more affected (56%) as compared to males (44%) with a Female to male ratio of 1.3:1 (Figure 4). Out of 237 children, 70.4 % had a negative history of contact while 67% had previous BCG vaccination (Figure 5,6). When the clinical profile of these children was studied, cough (67%) followed by fever (57.8%) was the most common presenting complaint (Table 1). Local examination of the swelling revealed that maximum cases were >2x2 cm in size, 79.7% were non-tender swelling,62.8% had a soft consistency and 66.2% had free mobility (Table 2). Haematological parameters of these patients were also analysed under which 43% had a haemoglobin range between 10-12gm/dl, 49.3% had TLC >1500/ml and 61.6% of the patients had lymphocytosis with 71.3% having a platelet count within normal range. 75.1% of the children had elevated ESR and 67% with elevated C Reactive Protein (Table 3). Five different cytomorphological patterns were observed most common pattern observed was well-formed epithelioid granuloma with necrosis (36.2%). Necrosis with degenerated lymphocytes, macrophages and polymorphs was the least common pattern observed (9.9%) (Table 4). Detection of TB was done by different methods, from a total of 91 cases, 88 cases were detected for mycobacterium by the culture which was taken as the gold standard. GeneXpert detected 82 cases, MPT 64 Antigen kit detected 73, modified bleach technique detected 60 cases, ZN stain detected 44 cases only. Further, the sensitivity specificity and diagnostic accuracy of different methods were compared and GeneXpert outshined the other method of diagnosis with a sensitivity of 93.1%, specificity of 100 % and diagnostic accuracy of 97.5%.

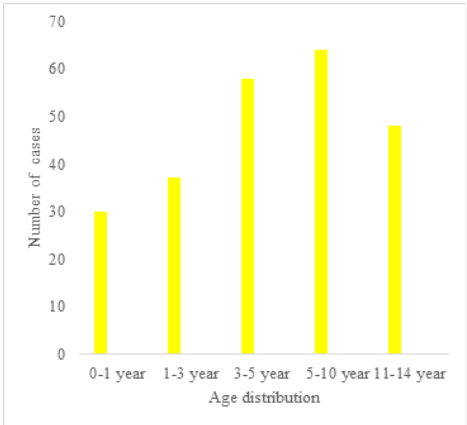

Figure 1 Distribution of total cases according to age

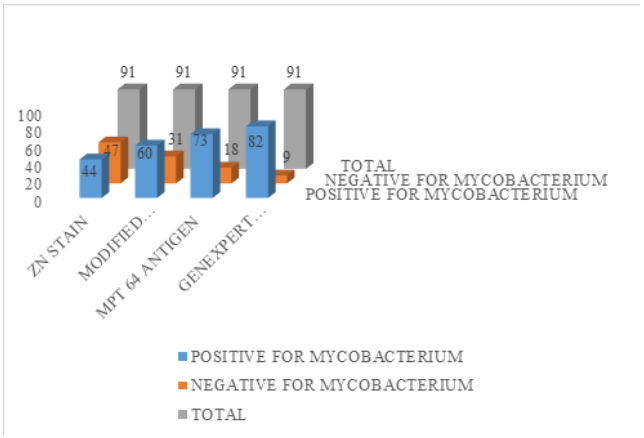

Figure 2 Comparison of detection of *Mycobacterium Tuberculosis* by different methods

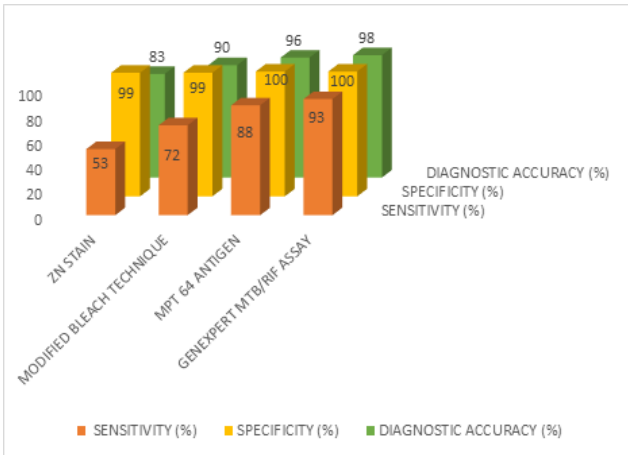

Figure 3 Comparison of statistical parameters of different methods in diagnosis of tubercular lymphadenopathy

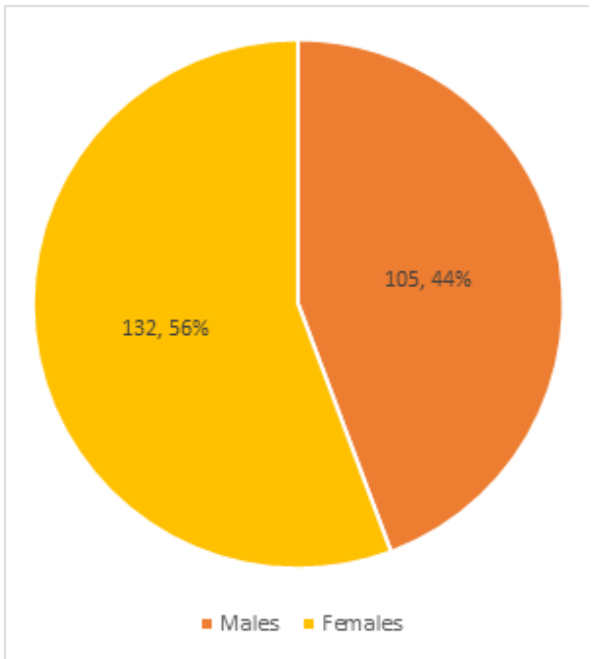

Figure 4 Distribution of total cases according to gender

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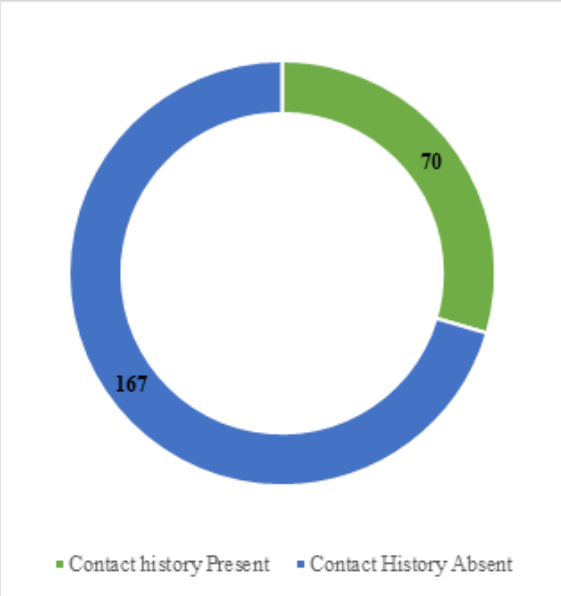

Figure 5 Contact history of TB among patients

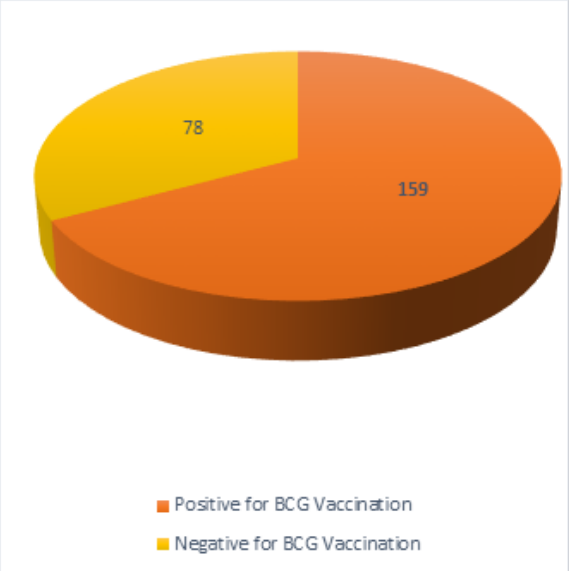

Figure 6 Distribution of total cases according to the history of BCG Immunization

Table 1 Clinical Profile of total Paediatric cases

Symptoms/sign	Number of patients	Percentage
Fever	137	57.8
Cough	159	67
Fever with cough	101	42.6
Weight loss with loss of appetite	96	40.5
Ear discharge	28	11.8
Dental pain	11	4.6

Table 2 Local examination of cervical lymph node

Features	Criteria	N=237	Percentage
Size	>2x2cm	80	33.7
Non-tender	Present	189	79.7
Consistency	Soft	149	62.8
	Firm and matted	67	28.2
	Hard	21	8.8
Mobility	Freely Mobile	157	66.2
	Restricted mobility	80	33.7
Sinus	Present	11	4.6

Table 3 Haematological parameters in patients

Haematological Parameters	Value	Number of patients	Percentage
Haemoglobin	13-15gm/dl	87	36.7
	10-12gm/dl	102	43
	<10gm/dl	48	20.2
Total leucocyte count (TLC)	>15000/ml	117	49.3
	5000-14000/ml	108	45.5
	<5000/ml	12	5
Differential leucocyte count (DLC)	neutrophilia	125	52.7
	lymphocytosis	146	61.6
	eosinophilia	74	31.2
	Monocytosis	86	36.2
	Basophilia	11	4.6
Platelet count	Normal	169	71.3
	Thrombocytosis	40	16.8
	Thrombocytopenia	28	11.8
Erythrocyte Sedimentation Rate	Elevated in	178	75.1
C reactive protein (CRP)	Elevated in	159	67

Table 4 Cytomorphological Patterns observed in Fine Needle Aspiration Cytology

Cytomorphological Pattern	Numbers	Percentage
Well-formed epithelioid granuloma with necrosis	33	36.2
Epithelioid Granuloma without necrosis	24	26.4
Early epithelioid granuloma with reactive lymphadenopathy	14	15.3
Necrosis with epithelioid cells and degenerated lymphocytes	11	12.1
Necrosis with degenerated lymphocytes, macrophages and polymorphs	9	9.9

Discussion

Tubercular lymphadenopathy in the pediatric population is quite a frequent complaint encountered and is the most common form of extrapulmonary TB in India.[10] The mainstay to reach the diagnosis of tubercular lymphadenitis includes clinical history, routine investigations along with an ancillary diagnostic test for TB. Conventionally ZN staining is the most widely used technique for the

detection of Acid-fast Bacilli but has a low sensitivity ranging from 9 to 46%.[11,12] The sensitivity of Detection of AFB by ZN staining was 53.1% in the present study, similar findings were noted by Tansuphasiri.[11] The variable sensitivities in various studies can be attributed to many factors like prevalence and severity of disease, quality of the specimen, method of processing, the staining technique and quality of examination therefore to augment the sensitivity of ZN staining, concentration methods like modified bleach technique are being used to improve the diagnosis.[13] With the modified bleach technique the sensitivity increased to 72.2%, which was very similar to Gangane et al.[13] (72%) while Patel et al.[14] showed a sensitivity of 61.7%. This could be attributed to the presence of abundant neutrophils and debris which probably obscured the visibility of AFB in the routine ZN smear. Therefore, the digestion of neutrophils and cellular debris by the bleach method resulted in a clear background with easy visibility of AFB. Similar observations were noted by others.[15] Our results showed that the majority of the positive cases on routine ZN staining had few AFB and searching for them was time-consuming and tedious. Whereas, the bleach concentration method increased the number of bacilli per field and also gave a clean background making the bacilli easily visible thereby reducing the screening time. The morphology of AFB also appeared to be better preserved, and they were thicker and longer than the routine ZN smears. This could probably be due to the swelling of bacilli in the solution. The above-mentioned observations were noted in other studies as well.[7,16] Detection of Mycobacterium by MTB64 Antigen Test which is an immunochromatographic test helps in differentiating between mycobacterium tuberculosis complex and non-tuberculous mycobacteria which helps in early and accurate diagnosis.[17] Sensitivity by MTB 64 was found to be 87.9% Shenoy et al.[18] Maurya et al.[19] and Kanade et al.[20] found the sensitivity to be 100%,99.1%,99.2% and specificity of about 100%. MTP 64 is a reliable immunochromatographic test that is simple, has a rapid turnaround time, is cost-effective and with high sensitivity and specificity. GeneXpert MTB/RIF (Cepheid, Sunnyvale, USA) is an automated, cartridge-based nucleic acid amplification test (NAAT) for the detection of Mycobacterium tuberculosis complex and rifampicin drug resistance with a turnaround time of fewer than two hours (20). Recent WHO policy recommends testing for pulmonary and extrapulmonary tuberculosis in both children and adults. Though limited studies are done so far, GeneXpert has been increasingly recommended as a replacement test for conventional diagnostic techniques.[21] The sensitivity of Xpert is found to be 87.8% and specificity to be 91.1% in cases of extrapulmonary TB.[22]

Conclusion

The GeneXpert MTB/RIF assay is a revolutionary diagnostic method and provides a cutting edge over other conventional diagnostic methods as it has a high sensitivity, and specificity compared to other conventional diagnostic methods, minimum turnaround time and in addition, identifies rifampicin resistance. This helps to prevent delay in initiation and choosing of an appropriate regimen of anti-tubercular therapy. Thus, we conclude that the GeneXpert assay is a simple and reliable technique and should be included in early in the diagnostic workflow of paediatric lymphadenopathy which will potentially improve the overall detection and reduce the turn-around time, thus preventing the unnecessary delay in treatment thus reducing the overall burden of TB, especially in high burden TB countries like India.

Acknowledgments

None.

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

1. Citak EC, Koku N, Demirci M, et al. A retrospective chart review of evaluation of the cervical lymphadenopathies in children. *Auris Nasus Larynx*. 2011;38(5):618–621.
2. Rajasekaran K, Krakovitz P. Enlarged neck lymph nodes in children. *Pediatr Clin North Am*. 2013;60(4):923–936.
3. Yaris N, Cakir M, Sözen E, Cobanoglu U. Analysis of children with peripheral lymphadenopathy. *Clin Pediatr (Phila)*. 2006;45(6):544–549.
4. Wallis RS, Pai M, Menzies D, et al. Biomarkers and diagnostics for tuberculosis: progress, needs and translation into practice. *Lancet*. 2010;375(9729):1920–1937.
5. Milauskas J. Lymph nodes. Orell and Sterret's Fine Needle Aspiration Cytology. 5th edn; 2012:Elsevier. Pp. 86.
6. Selvakumar N, Gomathi Sekar M, Kumar V, et al. Sensitivity of Ziehl-Neelsen method for centrifuged deposit smears of sputum samples transported in cetyl-pyridinium chloride. *Indian J Med Res*. 2006;124(4):439–442.
7. Annam V, Karigoudar MH, Yelikar BR. Improved microscopical detection of acid-fast bacilli by the modified bleach method in lymph node aspirates. *Indian J Pathol. Microbiol*. 2009;52(3):349–352.
8. Kumar VG, Urs TA, Ranganath RR. MPT 64 Antigen detection for Rapid confirmation of *M.tuberculosis* isolates. *BMC Res Notes*. 2011;4:79.
9. Lawn SD, Zumla AI. Diagnosis of extrapulmonary tuberculosis using the Xpert(®) MTB/RIF assay. *Expert Rev Anti Infect Ther*. 2012;10(6):631–635.
10. Singh SK, Tiwari KK. Tuberculous lymphadenopathy: Experience from the referral centre of Northern India. *Niger Med J*. 2016;57(2):134–138.
11. Tansuphasiri U, Kladphuang B. Evaluation of sputum staining by modified cold method and comparison with Ziehl-Neelsen and fluorochrome methods for the primary diagnosis of tuberculosis. *Southeast Asian J Trop Med Public Health.* 2002;33(1):128–135.
12. Balows A, Hausler WJ, Herrmann KL. Manual of clinical microbiology. 5th ed. Washington, D.C: *American Society for Microbiology*. 1991;33(6):308–311.
13. Gangane N, Anshu, Singh R. Role of modified bleach method in staining of acid-fast bacilli in lymph node aspiration. *Acta Cytol*. 2008; 52: 325–328.
14. Patel MM, Patel KK, Italiya SL. Improved diagnosis of tuberculosis in lymph node cytology by bleach method for detection of acid-fast bacilli in comparison to conventional with Nielsen staining method. *Int J Med Sci Public Health*. 2013;2(4):935–939.
15. Bandopadhyay SN, Roy KK, Dasgupta A, Ghosh RN: Role of fine-needle aspiration cytology in the diagnosis of cervical lymphadenopathy. *IJO & HNS*. 1996;48:289–294.
16. Bharadwaj S, Kashyap I, Kumari R. Comparative evaluation of two staining techniques for detection of Tubercular bacilli in lymph nodal aspirates. *Int J Health Sci Res*. 2015;5(7):115–121.
17. Chandrasekhar B, Prayaga AK. Utility of concentration method by modified bleach technique for the demonstration of acid-fast bacilli in the diagnosis of tuberculous lymphadenopathy. *J Cytol*. 2012;29(3):165–168.
18. Shenoy VP, Mukhopadhyay C. Rapid Immunochromatographic Test for the Identification and Discrimination of Mycobacterium tuberculosis Complex Isolates from Non-tuberculous *Mycobacteria*. *J Clin Diagn Res*. 2014;8(4):13–15.

19. Maurya AK, Nag VL, Kant S, et al.Evaluation of an immunochromatographic test for discrimination between *Mycobacterium tuberculosis* complex & non-tuberculous mycobacteria in clinical isolates from extra-pulmonary tuberculosis. *Indian J Med Res*. 2012;135:901–906.

20. Kanade S, Nataraj G, Suryawanshi R, Mehta P. Utility of MPT 64 antigen detection assay for rapid characterization of mycobacteria in a resource-constrained setting. *Indian J Tuberc.* 2012;59(2):92–96.

21. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med*. 2010;363(11):1005–1015.

22. Tadesse M, Abebe G, Abdissa K, et al. GeneXpert MTB/RIF Assay for the Diagnosis of Tuberculous Lymphadenitis on Concentrated Fine Needle Aspirates in High Tuberculosis Burden Settings. *PLoS One*. 2015;10(9):e0137471.

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