ORIGINAL ARTICLE

Evaluation of Fine Needle Aspiration for diagnosis of Tuberculous Lymphadenitis in Children using the Xpert MTB/RIF Ultra at the University Teaching Hospitals Children's Hospital, Lusaka, Zambia

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ABSTRACT

Background: Tuberculous Lymphadenitis (TBL) is the commonest form of Extra Pulmonary TB and poses diagnostic challenges in setting with no access to Histopathology. Our goal was to evaluate the diagnostic yield of Xpert MTB Ultra performed on lymph node aspirate obtained using Fine Needle Aspiration (FNA) in children with suspected TBL.

Methods: This was a cross-sectional study conducted at a tertiary and main referral hospital in Zambia. Children aged 6months to 15 years with lymphadenopathy and presumed TBL were enrolled. FNA was performed and lymph node aspirate samples were analysed using XPERT MTB/RIF ULTRA. Data was collected and analysed as frequencies and percentages. Logistic regression was used to measure the odds of testing positive for MTB.

Results: Sixty one children were enrolled in the study, 34(55.7%) were males and a median age of 6 years (IQR: 3-11 years). Sixteen (26%) were diagnosed with TB, 7 on clinical basis while, one was positive on gastric lavage, five (31%) were positive for TB on lymph node FNA using Xpert MTB/RIF Ultra. Then, 3 were positive Histopathology, with one also positive on XPERT MTB/RIF Ultra. Alternative diagnosis included malignancies like lymphomas and acute Leukemias. While non malignant lymphadenopathy included, benign reactive lymphadenopathy and HIV associated lymphadenopathy. The prevalence of HIV among the children diagnosed with TB was 37.5% (6/16). Thirty one percent (5/16) of children with TB had a close contact with TB, OR=9.0 (95% CI: 1.3-77, p-value (<0.05).

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Conclusion: TBL is not uncommon in children presenting with Lymphadenopathy. FNA is a less invasive, inexpensive technique and coupled with use of Xpert MTB/RIF Ultra has potential for early diagnosis of TBL, in a low resource setting with limited Histopathology services.

INTRODUCTION

According to the World Health Organization (WHO), Tuberculosis (TB) remains one of the leading causes of morbidity and mortality especially in low income countries, with about 10 million infections Worldwide. Children account for about a tenth of cases and yet almost 15% of the deaths.[1,2] Data for children in Zambia remains scanty, with low notifications and limited reports on actual incidence and proportion especially of Extra Pulmonary Tuberculosis (EPTB).[3] Bacteriological confirmation of tuberculosis in children is challenging and mainly utilizes clinical and radiological findings; both neither specific nor sensitive. TB in children is paucibacillary disease resulting in low sensitivity of standard diagnostic tests for TB.[4,5]

TB lymphadenopathy (TBL) is the commonest manifestation of EPTB, commonly affecting children below the age of 10 years, with cervical lymphadenopathy being the predominant site involved.[6,7] In endemic areas, TBL can account for as much as 60% of all forms of EPTB, with risk higher in children who are immunocompromised.[7]

In low resource settings, definitive diagnosis of TBL in children is often difficult because of limited availability of surgical and histopathology facilities when a lymph node biopsy is required. Fine needle aspirate (FNA) has demonstrated to be a key diagnostic tool in establishing a diagnosis

The introduction of Xpert MTB/RIF(Gene Xpert) as a cartridge based Nucleic Acid Amplification Automated Test (NAAT) revolutionized the diagnosis of TB for both pulmonary and extra-pulmonary samples including FNAs, as it could process samples, detect MTB DNA and rifampicin resistance in less than two hours.[11] As systemic review of the performance of Xpert MTB/RIF on extra pulmonary samples, showed a pooled sensitivity of 96% (95%, CI). However, it had limited sensitivity in individuals with paucibacillary disease and those living with HIV, this necessitated the introduction of Xpert/MTB/RIF Ultra (Xpert Ultra) which has a higher sensitivity that approximates that of the gold standard, culture, with much less turnover time.[12]

Studies involving the performance of Xpert Ultra in both adult and paediatric populations have focused on respiratory samples with paucity of data on lymph node aspirates in children with suspected TBL.[13,14,15] FNA provides a less invasive way of obtaining samples for analysis making it suitable for a possible effective tool in diagnosing EPTB, specifically TB adenitis. FNAs analysed using a tool like Xpert Ultra with established good sensitivity in low bacillary disease provides a possibility of quick diagnosis of TB and early institution of treatment.[12,16,17]

METHODS

Study setting and recruitment

This was a cross sectional study of children with suspected TBL at the University Teaching Hospitals, Children's Hospital Lusaka, Zambia a tertiary referral hospital and included children reviewed in the inpatient and outpatient departments. The study was conducted between August, 2020 and March, 2022. Children presenting with lymphadenopathy (one or more lymph nodes greater or equal to 1cm)[18] between the ages of 6 months and 15 years were referred for suspected TBL or for Lymph node biopsy were recruited. However, children with lymphadenopathy but on already on Anti tuberculous treatment or confirmed

TB in other sites were excluded. HIV counseling and testing was done as part of routine care. Physical examination was done including anthropometry. Then FNA was done and sample was analysed with Xpert Ultra. Children found positive for TB were treated as per national guidelines.[6] Children negative for TB were then recommended for possible biopsy by paediatric surgeons through their managing units. Biopsies were further sent for histopathology. Further, respiratory samples collected and X-rays done by managing units were tracked and results recorded.

Fine needle Aspirate procedure

Under aseptic conditions, a 21-G needle attached to a 10cc syringe was used by the study physician. The enlarged lymph node was then fixed in position and needle inserted and obtained the aspirate. Aspirate was washed into a labeled specimen bottle with 1ml of normal saline and was sent to the TB laboratory for immediate analysis with Xpert Ultra.

Histology examination of lymph node Biopsies

Standard histopathology preparation was done as follows: specimen reception, gross examination, tissue processing, tissue embedding, microtomy, tissue staining, slide mounting, microscopic examination and report generation. Following gross examination of specimen sections of the tissue samples were taken and placed in cassette for processing with formalin, alcohol and xylene and Paraffin wax. The tissue was then embedded and placed on a mold to form a paraffin wax tissue block called formalin fixed paraffin embedded (FFPE) tissue block. Three micrometer slices of tissue were made, stained, dried and later examined by pathologists. Caseating granulomatous inflammation was suggestive of histomorphological manifestations of Mycobacterial tuberculosis. An alternative diagnosis to TB was reported as such.

Statistical analysis

Data was collected on the study questionnaire and entered into an electronic data base (Excel Sheet). Double data entry on excel sheet was then analysed using R4.1.3 software for windows.

Goal of analysis	Non-parametric test
To describe each group categorical variable	Fishers exact test
To compare continuous variables	Mood's Median test
To make an association between clinical characteristics	Fisher exact test
To rule out confounders	Multiple logistic Regression

RESULTS

We screened a total of 70 Children of which 61 met the inclusion criteria and were enrolled in the study. FNAs were done on all the enrolled children and specimen samples were subjected to Xpert MTB/RIF/Ultra. Lymph node biopsy was recommended to participants who were negative for MTB on Xpert/ ultra, only 23 consented to Biopsy and had final report ready by the end of the study. When indicated, Bone marrow biopsy was done by the managing unit as part of the investigation for Lymphadenopathy. Table 1 highlights the characteristics of the children enrolled in the study.

Table 1: Clinical characteristics of Children enrolled

Characteristics	FNAPositive[a] (N=5)	FNANegative (N=56)	TB diagnosis	Overall (N=61)	P-value
Sex-n (%)					
Male	4 (80)	30 (54)	7 (44)	34 (55.7)	0.570*
Female	1 (20)	26 (46)	9 (56)	27(44.3)	
Median Age(years)-(IQR)[b]	6 (2-13)	5 (3-10)	7(2.3-11.3)	6(3-11)	1.000**
HIV-n (%)					
Positive	1 (20)	17 (30)	6 (37)	18 (30)	0.252*
Negative	3 (60)	37 (66)	8 (50)	40 (66)	
Weight Change - n (%)					
Gaining	0 (0)	5 (9)	0 (0)	5 (8)	0.570*
Static	0 (0)	14 (25)	2 (12)	14 (23)	
Cough-n (%)					
Yes	3 (60)	19 (34)	6 (38)	21 (34)	1.000*
Fever-n (%)					
Yes	2 (40)	25 (45)	11 (69)	27 (44)	1.000*
Close TB Contact-n (%)					
Yes	3 (60)	8 (14)	5 (31)	11 (18)	**0.037***
Nutrition status - n (%)					
Good	2 (40)	21 (38)	4 (25)	23 (38)	0.502*
Underweight	0 (0)	2 (4)	0 (0)	2 (3)	
Moderate Wasting	2 (40)	30 (54)	10 (62)	32 (53)	
Lymph node Distribution-n (%)					
Bilateral	3 (60)	38 (68)	2 (12)	41 (67)	1.000*
Lymph node group-n (%)					
Axillary	0 (0)	2 (4)	0 (0)	2 (3)	1.000*
Cervical	5 (100)	49 (88)	14 (88)	54 (88)	
Inguinal	0 (0)	1 (2)	1 (6)	1 (2)	
Parotid	0 (0)	1 (2)	0 (0)	1 (2)	
Submandibular	0 (0)	3 (5)	1 (6)	3 (5)	
Lymph node consistency-n (%)					
Firm	4 (80)	37 (79)	12 (75)	41 (78)	0.517*
Firm and tender	0 (0)	2 (4)	1 (6)	2 (4)	
Fluctuant	1 (20)	3 (6)	1 (6)	4 (8)	
Rubbery	0 (0)	5 (11)	1 (6)	5 (10)	

[a]TB Positive aspirate, Interquartile Range[a], Fishers Exact test* Moods test for equality of the medians**

Laboratory and histology results for Children with Lymphadenopathy

A total 33 respiratory samples were collected from the participants for screening for TB. Of the five participants that were diagnosed with TB by FNA, two had positive respiratory samples for TB as well. Further, of the 16 participants whose final diagnosis was TB, 8 had bacteriological confirmation while 8 were diagnosed clinically as per national guidelines.[6] Rifampicin resistance was detected on one respiratory sample and on one FNA sample processed with XpertMTB/RIF Ultra.

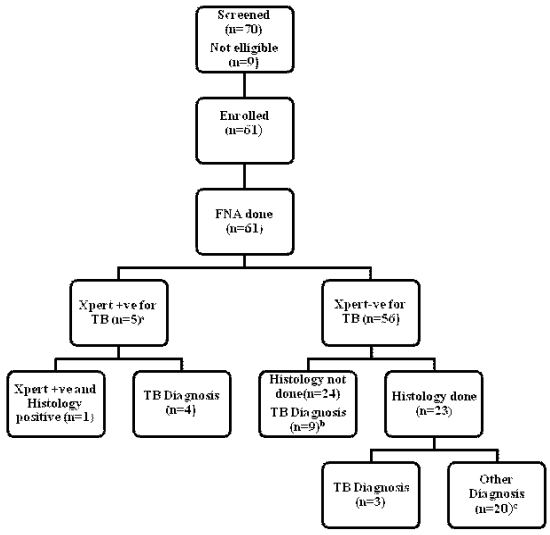

Figure 1 illustrates the findings:Two children with Positive FNAs also had positive respiratory samples on Xpert MTB/Ultra, [b]Eight children were diagnosed clinically, one had a positive respiratory sample for TB, [c]other diagnosis are highlighted in table 2 below.

Table 2: Final diagnosis of children presenting with Lymphadenopathy

Diagnosis	Frequency n (%)
Tuberculosis	16 (26%)
Benign Reactive Lymphadenitis	14 (23%)
HIV associated lymphadenopathy	8 (13%)
Lymphoma	7 (11%)
Acute Leukemia	5 (8%)
Nasopharyngeal carcinoma	2 (3%)
Abscess (staphylococcal infection)	1 (2%)
Bacterial pneumonia	1 (2%)
Kaposi's sarcoma	1 (2%)
Neuroblastoma	1 (2%)
Rhabdomyosarcoma	1 (2%)
Inconclusive	4 (7%)

DISCUSSION

The study showed Xpert Ultra identified one third of children with TBL from samples obtained using FNA out of the total number of children with TB as the final diagnosis. There was no difference in yield between HIV positive children in comparison with those who were HIV negative. Xpert Ultra detected MTB in a third of samples that were positive on histology. There were no clinical features that were unique to children with positive Xpert/ultra-aspirates in comparison to those that that had an Xpert/ultra-negative aspirate but with a final diagnosis of TB.

The yield of Xpert Ultra on lymph node samples using FNA from our study was similar to a Gambian study involving 131 children with persistent lymphadenopathy, FNA showed 42.7% positivity for TB, though ZN staining and culture were used for diagnosis.[19] A much lower positivity however was noted in a European study with a much larger sample size.[20] Our study findings were comparable with South African study with similar sample size,

though generic Xpert MTB/ RIF was used instead of Xpert Ultra . Similarly, in a Zambian study, Xpert MTB/RIF showed a positivity of 28% on formalin fixed lymph nodes of both adults and children.[22] However, an Indian study demonstrated 10% positivity for TB on lymph node aspirates but this particular study involved both respiratory and non-respiratory samples and was not restricted to children with lymphadenopathy.[23] Two South African studies however,demonstrated a much higher yield with Xpert MTB/RIF with both demonstrating a positivity of greater than 50%. In one of two studies, lymph node aspirates from children, while in the other samples were collected from both children and adults.[21,24] The higher prevalence of TB in South Africa could explain the higher positivity in these two studies.[1]The majority of studies involving Xpert MTB/RIF ultra have been done in adults and involved respiratory samples with paucity of data on extra pulmonary sample especially involving Children.[13,14,15]

Children with HIV especially in sub-Saharan Africa, with a high burden of HIV are at a significantly increased risk of developing TB.[4,25] Six (37.5%) of the children diagnosed with TB were HIV positive. This included one of the 5 children positive on Xpert Ultra was HIV positive. Our findings were consistent with other studies which have demonstrated prevalence of TB in HIV infected children to be around 10-60%.[25,26]

Of the lymph node biopsies done, 3 were positive for TB one of which had a positive lymph node aspirate for TB as well. Studies comparing FNAs with Xpert MTB/RIF and Histopathology have demonstrated a sensitivity varying from 30- 85%.[27,28]

The median age of children with TB was 7years (IQR 2.3-11.3years) with an almost equal sex distribution. A close TB contact was a significant factor with asymmetrical, firm non-tender cervical lymphadenopathy being a common finding. These findings were consistent with other studies in similar setting.[8,19,29]

A systemic review and meta-analysis of causes of Cervical lymphadenopathy in over 2000 children revealed non- specific benign causes were the commonest (68%) followed by viral infections. TB was the commonest cause of granulomatous disease with Lymphomas accounting for half of the malignancies.[18] Our findings were not very different, as benign reactive lymphadenopathy together with HIV associated Lymphadenopathy were the commonest causes of lymphadenopathy in children enrolled in our study. Further Lymphomas were the commonest malignancies found Seconded by Acute Leukemias. And similar results were noted by a study done in Sudan.[30]

Our main limitation was the fact that FNA samples of lymph nodes were not subjected to culture which is the Gold standard in addition to the fact that the study was restricted to the UTHs-CH and was not a multicenter study. Only about two thirds of the desired numbers of children were enrolled, owing to the fact that the study was conducted during Covid-19 waves which led to the suspension of some of the hospital activities resulting in reduced patient in-flow. Further, not all patients being followed up by the managing units had respiratory samples collected or on chest x-ray done as part of their TB workup. This could have had implications in the final diagnosis.

The combination of FNA, which is readily available and inexpensive tool with Xpert MTB/ Ultra was able to detected MTB in a population with paucibacillary disease was a major strength of our study. The implication therefore, being the advocating of FNA to be utilized as an investigating tool for screening children with lymphadenopathy for TB especially in a low resource setting.

CONCLUSION

TBL is not an uncommon finding in children presenting with cervical lymphadenopathy in a TB

endemic setting with FNA providing a non-invasive, non-labour intensive and quick way of obtaining the samples. Xpert Ultra is a useful tool in detecting MTB in paucibacillary disease in a low resource setting with an added advantage of detecting rifampicin resistance and comparable with histology.

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