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INVESTIGATING PEDIATRIC TUBERCULAR LYMPHADENOPATHY: CLINICOPATHOLOGICAL INSIGHTS AND ADVANCED DIAGNOSTIC STRATEGIES

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ABSTRACT

Objective: Lymphadenopathy is a common occurrence among children and poses significant challenges in diagnosis. While traditional diagnostic methods exist for identifying tubercular lymphadenopathy, recent advancements, such as the GeneXpert Assay, offer new avenues for investigations. This study seeks to highlight the importance of these innovative diagnostic approaches while also providing a comprehensive clinicopathological overview of pediatric patients.

Materials and Methods: Over a one-year period, this prospective study included 112 children below 13 years of age having suspected tubercular lymphadenopathy based on clinical symptoms. Various diagnostic techniques were employed, including Fine Needle Aspiration Cytology (FNAC,Ziehl-Neelsen (ZN) staining, culture, Modified bleach method, GeneXpert MTB/RIF Assay, andMTP 64 Antigen testing. Adequate sample sizes were utilized, and comparative analyses were conducted. The obtained data was analyzed using SPSS 24.0.

Results: Comparison of diagnostic accuracy, sensitivity, and specificity across different diagnostic methodologies revealed that GeneXpert Assay as the most reliable, demonstrating a diagnostic accuracy of 97.2%, sensitivity of 92.8%, and specificity of 100%.

Conclusion: Currentinvestigation advocates for the early integration of the GeneXpert Assay into diagnostic protocols for pediatric lymphadenopathy. Its incorporation holds the promise of improving detection rates and expediting diagnostic timelines, thereby minimizing unnecessary treatment delays and minimizing the burden of tuberculosis.

Keywords: Tuberculosis, lymphadenopathy, pediatrics, GeneXpert, Diagnosis, Accuracy.

Introduction

Lymphadenopathy poses a frequent challenge among pediatric patients, stemming from diverse causes. Understanding these underlying causes is crucial for crucial for directing appropriate diagnostic investigations and subsequent management strategies. The integration of comprehensive patient history, thorough physical examination, and diagnostic assessments is pivotal in refining the lymphadenopathy and its differential diagnosis[1]. In Asian countries, reactive hyperplasia ranks as primary lymphadenitis form in children, followed closely by granulomatous lymphadenopathy. Notably, children have an increased risk to developextrapulmonary tuberculosis, with superficial lymphadenitis emerging as the most common presentation[2].

Diagnosing tuberculosis in pediatric patients relies on a comprehensive evaluation encompassing clinical history, investigative procedures, and ancillary tests such as Ziehl-Neelsen staining for Acid Fast bacilli, histopathology, and Fine Needle Aspiration Cytology (FNAC)[3]. FNAC often reveals granulomas with necrosis, characteristic of tuberculous lymphadenitis. However, interpreting FNAC results in children, especially in cases of inconclusive reports, presents challenges due to practical limitations associated with repeated procedures[4]. While culture remains the diagnostic gold standard, its prolonged processing time compromises patient care[5]. Similarly, the utility of Ziehl-Neelsen staining is hindered by its low sensitivity and inability to differentiate *Mycobacterium tuberculosis* from non-tuberculous mycobacterial infections[6].

Modified Bleach method offers a cost-effective solution, utilizing sodium hypochlorite for liquefaction and centrifugation for bacilli concentration, thus improving sensitivity while also serving as a potent disinfectant[7]. Additionally, the MTP 64 Antigen rapid immunochromatographic assay emerges as a promising tool, detecting the specific Rv1980c protein characteristic of the *Mycobacterium tuberculosis* complex. Its simplicity and reliability render it particularly suitable for resource-limited settings, facilitating expedited turnaround times with high specificity and sensitivity[8]. Moreover, the introduction of newer diagnostic modalities, including Nucleic Acid Amplification Tests, represents a significant advancements in tuberculosis diagnosis[9]. Notably, the World Health Organization recommends the GeneXpert MTB/RIF assay, an automated system based on the real-time PCR, capable of diagnosing tuberculosis and assessing rifampicin resistance in a remarkably short timeframe[10]. Against this backdrop, this study aims to the clinicopathological profile of pediatric patients

and evaluate the comparative effectiveness of various diagnostic methodologies, including newer approaches, in diagnosing pediatric tubercular lymphadenopathy.

Materials and Methods

A prospective study spanning one year was conducted at a tertiary care hospital. The study cohort comprised 112 children under the age of 13 with clinical suspicion of having tubercular lymphoadenopathy. Prior informed consent was obtained, following with Fine Needle Aspiration Cytology (FNAC) was performed on palpable, enlarged lymph nodes with aseptic measures, employing a needle of 23-gauge. Successively, the smears were prepared for subsequent examination of cytopathology. From sufficient aspirated samples (minimum 2mL), the Ziehl-Neelsen stain for Acid Fast Bacilli and the modified bleach method were conducted .The aspirated contentswere then combined with the buffer and utilized for MTB 64 Antigen testing usingimmunochromatographic kits. The remainingmaterial was forwarded to the Department of Microbiology for assay of GeneXpert MTB/RIF analysis and culture. JL media culture served as the reference standard, with statistical association conducted using SPSS 24.0.

Results

The study enrolled 112 pediatric patients presenting with lymphadenopathy of whom 112 (56%) were diagnosed with tubercular lymphadenopathy. Clinicopathological profiles and comparative analysis were conducted on the samples. The highest proportion (43%) of the tubercular lymphadenopathy cases were observed in the 5-10 years group, yielding a male-to-female ratio of 1:1.4 (Figure 1). Among 112 children, 74.3% had no contact history, while 63.5% had received BCG vaccination previously. The commonest presenting complaints were toothache (66.07%), followed by cough (46.42%), fever (38.39%), weight loss (34.82%), and ear discharge (13.39%). On local examination, the majority of swellings were >2x2 cm in size (Table 1).

Cytomorphological analysis revealed five distinct patterns with most common being well-formed epithelioid granuloma along with necrosis (36.6%), while necrosis along with degenerated lymphocytes, polymorphs, and macrophages were least common (8.03%) (Table 2). Tuberculosis detection utilized various methods (Figure 2-3).

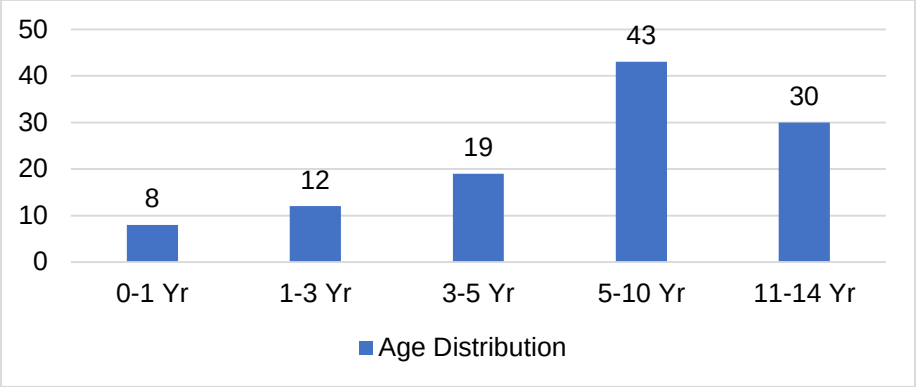

Figure 1: Age distribution among pediatric patients (n=112)

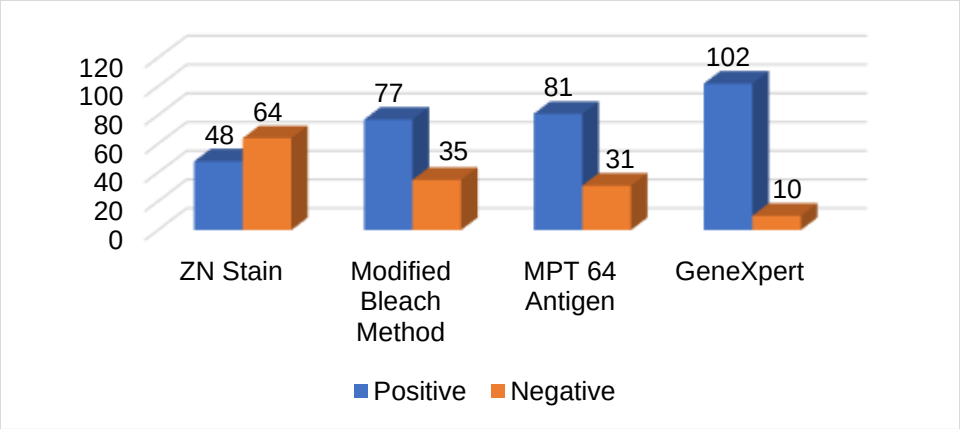

Figure 2: Tuberculosis detection by various methods among pediatric patients (n=112)

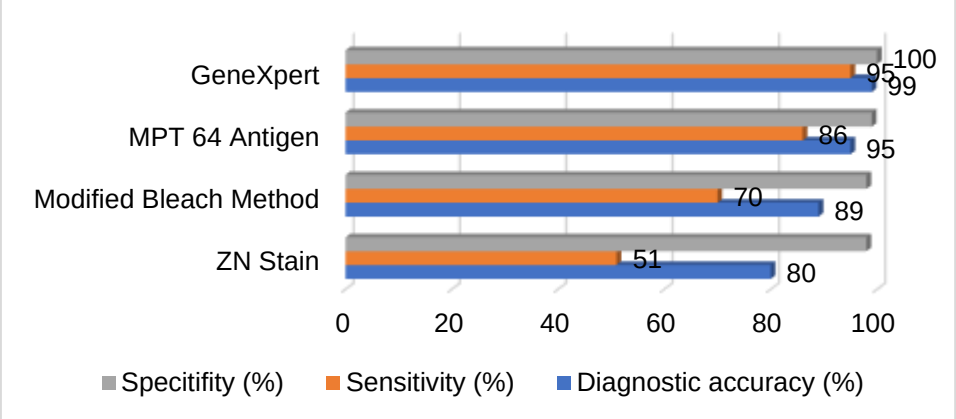

Figure 3: Diagnostic accuracy, sensitivity, and specificity of tuberculosis among pediatric patients (n=112)

Table 1: Clinical presentation of pediatric patients (n=112)

Parameter	Number	Percentage (%)
Cough	52	46.42
Toothache	74	66.07
Ear discharge	15	13.39
Fever	43	38.39
Weight loss	39	34.82

Table 2: Cytopathological morphology of pediatric patients with tubercular lymphadenopathy (n=112)

Cytopathology	Number	Percentage
Epithelial granuloma displaying well-defined structure with necrotic tissue	41	36.60
Epithelioid granuloma lacking necrotic features	32	28.57
Reactive lymphadenopathy accompanying early-stage epithelioid granuloma	19	16.96
Necrosis observed alongside epithelioid cells and deteriorated lymphocytes	11	9.82
Necrotic tissue containing deteriorated lymphocytes, polymorphonuclear leukocytes, and macrophages	9	8.03

Discussion

Tubular lymphadenopathy poses a common challenge among pediatric patients and represents the prevalent form of extrapulmonary tuberculosis. The diagnostic journal of tubercular lymphadenitis typically entails a comprehensive review of clinical history, routine investigations, and supplementary tuberculosis tests[11]. Historically, Ziehl-Neelsen (ZN) staining has served as the primary modality for Acid-fast bacilli detection, albeit with a sensitivity ranging from 9 to 46%[12]. In our investigation, ZN staining exhibited a sensitivity of 51% aligning with Tansuphasiri's findings[12]. The variability in sensitivity across studies can be ascribed to diverse factors, including disease prevalence, examiner proficiency, staining methodologies, specimen quantity, and processing techniques. To enhance the sensitivity of ZN staining, concentration techniques such as the modified bleach method have been employed, resulting in an augmented sensitivity of 70%, mirroring outcomes reported by Gangene et al, while Patel et al. recorded slightly decreased sensitivity[13,14]. This enhancement may stem from the occurrence of plentiful neutrophils and debris, potentially obscuring Acid-fast bacilli visibility in routine smears of ZN. The bleach method facilitates

neutrophil and cellular debris digestion, culminating in a clear background and improved AFB detection, as observed in previous studies[7,15]. Our findings suggest that routine ZN staining often yields few AFB-positive cases, necessitating laborious searches. Conversely, the bleach concentration method amplifies bacilli per field and furnishes a clear background, thereby reducing screening time. Additionally, AFB morphology seems better preserved, with thicker and longer bacilli, likely attributed to bacilli swelling in the solution, corroborating observations from previous studies[16–18].

The MTB 64 antigen test plays a pivotal role in distinguishing among *Mycobacterium tuberculosis* complex and the non-tuberculous mycobacteria, enabling prompt and accurate diagnosis[19]. MTB 64 sensitivity was established at 86% with Kanade et al., Shenoy et al., and Maurya et al., reporting sensitivities of 99.2, 99.1, and 100% respectively, alongside an approximate specificity of 100%[20–22]. MTB 64 emerged as a dependable immunochromatographic assay, characterized by its simplicity, swift shift time, cost-effectiveness, and increased specificity and sensitivity.

The GeneXpert MTB/RIF represents an automated and cartridge-dependent nucleic acid amplification investigation for identifying *Mycobacterium tuberculosis* complex as well asresistance against rifampicin, boasting a shiftingperiod of less than 2 hours[10]. Current WHO guidelines advocate both pulmonary and extra-pulmonary tuberculosis testing among adults and children[23]. Despite limited research, GeneXpert is increasingly endorsed as substitute for conventional diagnostic techniques, demonstrating a satisfying sensitivity and specificity.

Conclusion

The GeneExpert MTB/RIF testing introduces a pioneering diagnostic strategy, offering substantial advantages over traditional methodologies owing to its heightened specificity and sensitivity, swift turnaround time, and capability to identify rifampicin resistance. This expedites the commencement of appropriate anti-tubercular treatment, this curtailing treatment delays. Consequently, we suggest the integration of GeneXpert assay at the outset of the diagnostic journey for pediatric lymphadenopathy. This integration holds the promise for elevating overall detection rates and streamlining turnaround time, thereby forestalling unnecessary treatment delays and alleviating the burden of tuberculosis, particularly in regions with high prevalence rates.

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