GeneXpert MTB/RIF in diagnosis of tuberculous meningitis (TBM) in paediatric patients at a tertiary care children's hospital in Pakistan over a period of 4 years

Nadia Majeed[1], Naima Mehdi[2], Anum Tahir[3], Iqra Aroob[4], Shameeqa Omer[5], Farwa Ali[6]

Abstract

The current study was planned to report the frequency of tuberculosis meningitis in the paediatric population of Pakistan using the GeneXpert Mycobacterium tuberculosis/Rifampicin (MTB/RIF) assay. Data of 569 cerebrospinal fluid samples collected between October 2019 and October 2023 at a tertiary care children's hospital was retrospectively analysed. Mycobacterium tuberculosis was detected in 101(17.7%) samples, with 57(56%) of them being from male patients. The highest tuberculous meningitis incidence 55(54.5%) was found in children aged 0-4 years, while those aged 10-14 years showed the lowest incidence 13(12.9%). Rifampicin resistance was present in 3(2.9%) cases. The findings highlighted the importance of incorporating GeneXpert MTB/RIF testing in clinical evaluation for suspected tuberculous meningitis cases in paediatric patients.

Keywords: Tuberculosis meningitis, GeneXpert MTB/RIF assay, Rifampicin resistance, Paediatric TBM, *Mycobacterium tuberculosis.*

Introduction

Worldwide, tuberculosis (TB) remains the most important infectious disease in causing morbidity and death.[1] About one-third of the global population has currently contracted TB infection through Mycobacterium TB (MTB). Pakistan remains fifth among countries most affected by TB.[1] According to the roadmap towards ending TB in children and adolescents, persistent gaps in data and shortcomings in research and development targetting TB in children should be addressed on a priority basis and reported through national TB control programmes (NTPs)[2] but due to difficulties in diagnosis, non-specific symptoms and poor recording and reporting practices, childhood TB has faced a significant neglect, and treatment outcomes in childhood TB are often not evaluated by NTPs.

There are two major classifications of TB, pulmonary and extra-pulmonary. Pulmonary TB is when the causative agent, MTB, affects the lungs, while extra-pulmonary TB is characterised and named after the disease site; pleural, lymphatic intra-thoracic, lymphatic extra-thoracic, osteoarticular spine and osteoarticular other than the spine, the central nervous system (CNS), including meninges, abdominal, including peritoneal, and disseminated, including military, TB. Tuberculous meningitis (TBM) is a form of TB characterised by inflammation of the membranes (meninges) around the brain or spinal cord caused by MTB. Of all the extra-pulmonary variants, TBM shows the highest mortality rate.[3]

Laboratory diagnosis of TBM is a difficult step.[4] Cerebrospinal fluid (CSF) smear has low sensitivity in routine diagnostic laboratories.[5] Cultural technique takes 4-8 weeks and needs special requirements. Interferon gamma (γ) release assays are not recommended for the diagnosis of active TB disease. Line probe assay is expensive and not easily available. Alternatively, GeneXpert Ultra is a system that is highly advanced and recommended technique for the diagnosis of TB that has been endorsed by the World Health Organisation (WHO) since 2013.[1] It works on the basis of real-time polymerase chain reaction (PCR). The current study was planned to estimate the frequency of TBM using GeneXpert among children aged up to 14 years.

Methods and Results

The retrospective study was conducted at the Department of Microbiology, University of Child Health Sciences (UCHS), The Children's Hospital (TCH), Lahore, Pakistan, and comprised data from October 2019 to October 2023 collected using consecutive sampling approach. The paediatric patients aged up to 14 years whose CSF samples (n=569) were submitted for MTB GeneXpert testing because of TBM suspicion were selected. Data was retrieved after approval from the institutional ethics review board.

[1]Department of Infectious Diseases, University of Child Health Sciences, The Children's Hospital, Lahore, Pakistan; [2,6]Department of Microbiology, University of Child Health Sciences, The Children's Hospital, Lahore, Pakistan; [3]University College of Medicine and Dentistry, The University of Lahore, Lahore, Pakistan [4,5]School of Allied Health Sciences, University of Child Health Sciences, The Children's Hospital, Lahore, Pakistan.

Correspondence: Iqra Aroob. e-mail: iqra.aroob@chich.edu.pk
ORCID ID: 0000-0002-2430-8646

GeneXpert MTB/RIF in diagnosis of tuberculous meningitis (TBM) in paediatric patients ......

1439

All the collected CSF samples, referred from the wards or from the out-patient department (OPD), were processed using the relevant standard operating procedures (SOPs), and positive TBM cases were confirmed using the GeneXpert system (Cephid®).

The assay procedure involved centrifugation of CSF samples for 15 minutes followed by removal of the supernatant, and resuspension of the deposit in a final volume of 2ml using the Xpert MTB/RIF sample reagent. The sample-reagent mixture was allowed to stand for 5 minutes. The Xpert MTB/RIF cartridge, labelled with the laboratory number, was utilised. The sample was drawn into the transfer pipette, and transferred into the sample column of the cartridge, which was then installed in the MTB/RIF assay machine. Results were displayed on the accompanying software after 1 h and 45 min.

The WHO has identified three age-specific categories for paediatric and adolescent TB patients for research purposes: 0-4 years, 5-9 years, 10-14 years and 15-19 years.[2] The current study adopted the same pattern, excluding all patients beyond the age limit.

Data was analysed using SPSS 16. Continuous variables were presented as mean±standard deviation, while categorical variables were expressed as frequencies and percentages. Chi-square test was utilised to assess associations between age and disease prevalence. P<0.05 was taken as statistically significant.

Table: Frequency of tuberculosis meningitis (TBM) in different paediatric age groups.

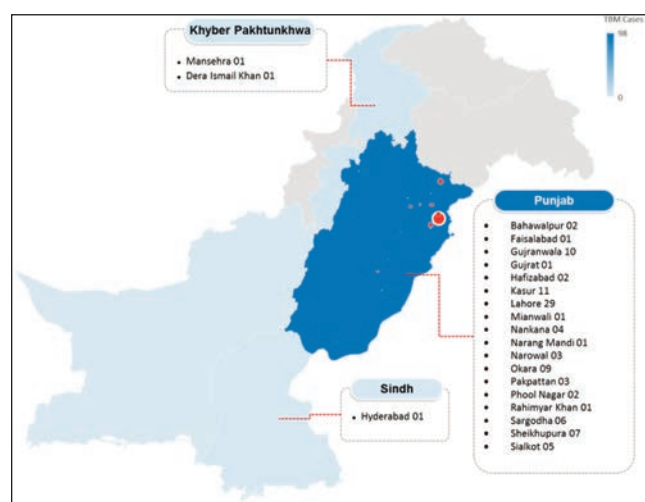| Age (years) group	TBM [n (%)]
0-4	55 (54.5)
5-9	33 (32.6)
10-14	13 (12.9)

(p<0.05)

Figure: Geographical distribution of tuberculous meningitis (TBM) in paediatric patients admitted at The Children's Hospital, Lahore, between 2019 and 2023.

Of the 569 patients, 101(17.7%) were positive for TBM. The mean age of TBM-positive patients was 4.66±3.83 years. There were 55(54.5%) patients aged 0-4 years, 33(32.6%) aged 5-9 years, and 13(12.9%) aged 10-14 years (p<0.05) (Table). There were 98(97%) hailing from Punjab, 2(2%) from Khyber Pakhtunkhwa, and 1(1%) from Sindh (Figure). The high percentage of patients from Punjab was due to the convenient access of patients from the region. Rifampicin resistance was present in 3(2.9%) cases.

Discussion

Globally, TB remains a major cause of mortality, with particular challenges in diagnosing TBM due to delayed clinical presentation and resource constraints.[6] As the largest tertiary paediatric care hospital in Punjab, The Children's Hospital receives referrals for complex cases, such as meningitis. GeneXpert MTB/RIF, a rapid diagnostic tool, has proven essential for early diagnosis and timely treatment initiation, which are critical for reducing TBM-related morbidity and mortality. Previous studies on TBM in Pakistan have focussed on the adult population, with an average age of 24, and have reported 4.6% positivity,[7] considerably lower than the 17.7% TBM cases in the current study. Consistent with prior studies,[8] the current study showed a male predominance (56%). The observed male dominance in TBM infections in developing countries like Pakistan may be influenced by social and cultural factors. It is plausible that male children receive priority in being taken to the hospital for treatment compared to females.

The WHO has estimated that approximately 12% of TB cases in 2022 occurred in children aged 0-14 years.[1] The available reports likely underestimate the true incidence, as the lack of surveillance testing in most areas of the world limits the ability to assess the true prevalence of the disease, especially in children. Keeping in view the importance of diagnosis and prevalence of TBM in paediatric patients, the current study was conducted on patients aged 0-14 years. The average age of total positive cases in the study was 4.66±3.83 years, with least and highest age as 4 months and 14 years, respectively. Children aged 0-4 years were found to be the most affected by TBM (Table).

Rifampicin resistance is a critical concern in TBM management, given its pivotal role in treatment. Molecular assays, like Xpert MTB/RIF, enable rapid detection of rifampicin resistance, facilitating timely second-line therapy.[9] In the current study, 3(2.9%) cases demonstrated rifampicin resistance, substantially lower than the 39.29% resistance found in adult population in China.[10]

Data on the prevalence of rifampicin resistance in paediatric MTB patients is scarce, and, to the best of our

knowledge, the current study is the first to report on the prevalence of rifampicin resistance in Pakistani paediatric MTB patients.

Limitation of Study: A consecutive sampling approach was employed; therefore, the sample size was not calculated using a specific formula.

Conclusion

MTB was the case in 17.7% CSF samples. The highest incidence of TBM was in children aged 0-4 years, which underscored the need for age-specific public health initiatives and paediatric healthcare strategies. Furthermore, the identification of rifampicin resistance in 2.9% samples raised concerns about drug resistance trends, and emphasised the importance of continuous surveillance, antimicrobial stewardship, and research regarding various therapeutic options.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

References

1. World Health Organization. Global tuberculosis report 2023. [Online] [Cited 2023 November 13]. Available from: URL: https://reliefweb.int/report/world/global-tuberculosis-report-2023

2. World Health Organization. Roadmap towards ending TB in children and adolescents. [Online] [Cited 2023 December 4]. Available from: URL: https://www.who.int/publications-detail-redirect/9789240084254

3. Sudha K, Rao AV, Rao SN, Rao A. Oxidative stress and antioxidants in tubercular meningitis. Indian J Clin Biochem 2002;17:34-6. doi.org/10.1007/BF02867939

4. Daniel BD, Grace GA, Natrajan M. Tuberculous meningitis in children: clinical management & outcome. Indian J Med Res 2019;150:117-30. https://doi.org/10.4103/ijmr.IJMR_786_17

5. Stadelman AM, Ssebambulidde K, Buller A, Tugume L, Yuquimpo K, Bakker CJ, et al. Cerebrospinal fluid AFB smear in adults with tuberculous meningitis: A systematic review and diagnostic test accuracy meta-analysis. Tuberculosis (Edinb). 2022;135:102230. doi: 10.1016/j.tube.2022.102230.

6. Jameel R, Mehdi N, Majeed N, Zafar A, Tahir A, Aroob I. Prevalence and diagnosis of rifampicin-resistant Mycobacterium tuberculosis using the GeneXpert MTB/RIF assay at a tertiary care children's hospital. Pak J Health Sci 2024;5:110-4.doi.org/10.54393/pjhs.v5i07.1764

7. Tahseen S, Khanzada FM, Baloch AQ, Abbas Q, Bhutto MM, Alizai AW, et al. Extrapulmonary tuberculosis in Pakistan: a nationwide multicenter retrospective study. PLoS One 2020;15:e0232134. doi.org/10.1371/journal.pone.0232134

8. Dodd PJ, Osman M, Cresswell FV, Stadelman AM, Lan NH, Thuong NTT, et al. The global burden of tuberculous meningitis in adults: a modelling study. PLoS Glob Public Health 2021;1:e0000069. doi.org/10.1371/journal.pgph.0000069

9. van Kampen SC, Tursynbayeva A, Koptleuova A, Murzabekova Z, Bigalieva L, Aubakirova M, et al. Effect of introducing Xpert MTB/RIF to test and treat individuals at risk of multidrug-resistant tuberculosis in Kazakhstan: a prospective cohort study. PLoS One 2015;10:e0132514. https://doi.org/10.1371/journal.pone.0132514

10. Duo L, Ying B, Song X, Lu X, Ye Y, Fan H, et al. Molecular profile of drug resistance in tuberculous meningitis from Southwest China. Clin Infect Dis 2011;53:1067-73. doi.org/10.1093/cid/cir663

Author Contribution:
NM: Concept and data analysis.
NM: Concept, data analysis and supervision.
AT: Study design.
IA: Data analysis, interpretation, writing, reviewing and drafting.
SO & FA: Data acquisition.