ORIGINAL RESEARCH PAPER

Clinical Microbiology

COMPARISON OF THE UTILITY OF CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST (CBNAAT) AND ZIEHL NEELSEN STAINING IN CULTURE POSITIVE GA SAMPLES IN DIAGNOSING PULMONARY TB IN PAEDIATRIC POPULATION.

KEY WORDS: Paediatric Tuberculosis, Gastric aspirate, MGIT, CBNAAT, ZN staining

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ABSTRACT

Tuberculosis is caused by the slow - growing bacteria Mycobacterium tuberculosis (MTB) and can manifest as pulmonary or extrapulmonary disease. The real extent of childhood TB in India is unknown because of it's variable presentation often making it difficult to diagnose clinically compared to adult TB. Children, typically, are unable to expectorate sputum therefore alternate samples like bronchoalveolar lavage fluid, gastric aspirate, laryngeal swab and stool samples are collected to diagnose TB. A total of 407 gastric aspirate samples, of children aged between 6 months to 12 years, presenting to the paediatric out-patient department, from March 2024 to February 2025 with strong clinical suspicion of pulmonary TB were included in the study. These samples were processed for detection of MTB by liquid culture method. Further, Cartridge based nucleic acid amplification test and Ziehl Neelsen staining was performed on the MGIT positive samples to evaluate these diagnostic parameters. Thus, our study was carried out to compare the utility of Cartridge based nucleic acid amplification test (CBNAAT) and ZN staining in culture positive GA samples in diagnosing pulmonary TB in children under 12 years of age.

INTRODUCTION:

Tuberculosis (TB) is caused by the slow - growing bacteria Mycobacterium tuberculosis (MTB) and can manifest as pulmonary or extrapulmonary disease. TB continues to infect millions of people and is one of the topmost infectious agent leading to death worldwide if left untreated. The total number of deaths caused by TB between 2015 and 2023 worldwide was 23%, with the global incidence being 134 per 100,000 population as per the Global TB WHO report 2024. In the same year around 1.09 million people including 200,000 children lost their lives to TB with most of them being under the age of 5 years.[1] The real extent of childhood TB in India is unknown because of diagnostic challenges, since paediatric TB has a variable presentation and is often difficult to diagnose clinically as compared to adult TB.[2] Children, typically, are unable to expectorate sputum therefore alternate samples such as bronchoalveolar lavage, gastric aspirate (GA), laryngeal swab and stool samples are used for diagnosing tuberculosis. According to a study conducted by Sun L, Zhu Y, Fang M, et al, GA samples appear to generate a higher detection rate, as compared to sputum specimen, 10.4% versus 5.7 % by Ziehl Neelsen (ZN) stain and 32.5% versus 17.9% by culture methods. Hence, GA samples provide a superior diagnostic yield in non-sputum producers like in younger age group.[3]

Method:

A total of 407 gastric aspirate of non - expectorating children, aged between 6 months to 12 years, presenting to the paediatric out-patient department (OPD), from March 2024 to February 2025 with strong clinical suspicion of pulmonary TB were included in the study. Early morning, empty stomach GA was collected and then processed in the Department of Microbiology. The sample was divided into two parts - the first part was processed using the N-acetyl-L cysteine-sodium hydroxide (NALC-NaOH) method as per the manufacturer's instructions, cultured on Mycobacterium growth indicator tube (MGIT) ,7H9 media and incubated in MGIT BACTEC 960 liquid culture system for a period of 42 days until they were flagged negative by the system. Tubes that were positive by culture for MTb complex were further subjected to ZN staining & CBNAAT. The positive samples were centrifuged and approximately 200 1 was taken for smear preparation and was stained by ZN method as per the National Tuberculosis Elimination Program (NTEP) guidelines. The slides were scanned by light microscopy for positive AFB. For GeneXpert assay processing, 0.5 ml of the remaining sample deposit was processed according to the manufacturer's standard operating procedure (SOP) (Cepheid, USA) in Cartridge based nucleic acid amplification test (CBNAAT) machine and the results were obtained in 2 hours. Since the samples were collected with the aim of diagnosis, ethical approval was not required for this study. The identity or information related to identity was not disclosed at any point during the study.

DISCUSSION AND RESULT:

In our study out of the 407 samples processed by MGIT, a total of 35 (8.6%) samples detected Mycobacterium tuberculosis complex (MTBc) by MGIT, out of the 35 MGIT positive samples, 33 (94.28%) samples tested positive for MTBc by CBNAAT and ZN stain was able to detect the mycobacterium bacilli in 11 (31.4%) of the total samples. The 2 samples which were MGIT positive but not detected by CBNAAT belonged to the Nontuberculous Mycobacteria (NTM) species. Infection by NTM species though rare are very challenging due to various issues ranging from intrinsic antibiotic resistance, complex drug regimens, poor tolerability to significant side effects associated with therapy and poor response rate.[4] In a study conducted by Agarwal M, Pandey D, Tiwari P, Dhawan A, among the 144 GA samples tested, 9 (6.25%) were positive for MTB using CBNAAT, 5 were culture-positive by liquid culture and 2 tested positive for acid-fast bacilli by microscopy.[5] Similarly, in a study conducted by Sharma S et al. there was a total of 210 GA samples tested, out of which 34 (16.19%) were positive by CBNAAT, 15 (7.14%) were positive for acid-fast bacilli in smear microscopy, and 35 (16.66%) were found to be culture positive by MGIT.[2] All these studies help us understand that the detection of MTB by CBNAAT on GA samples is an effective diagnostic tool for MTB, particularly in

children and patients unable to produce sputum since diagnosing pulmonary TB in the paediatric population is still a challenge because of its elusive presentation. Majority of paediatric TB cases are smear-negative due to two important factors which are - the typical paucibacillary nature of presentation in children and also their inability to produce sputum on demand. Another contributing factor is that a bacterial load of 10^4/ml organisms is required for smear microscopy, hence, ZN staining may not be the ideal method for detection of MTB in sputum samples as compared to GA sample since the number of bacilli is very small in respiratory secretions. Meanwhile, culture and CBNAAT helps in enhancing the bacillary count substantially making it easier to detect MTB [6] with the limit of detection by MGIT culture being 5×10^5 CFU/mL and 131CFU/ml of bacilli or 5 genome copies of DNA being the requirement by CBNAAT to flag the sample as positive.[7] For the diagnosis of tuberculosis, speed is of the essence and a positive test can result in faster initiation of therapy where perhaps CBNAAT plays a pivotal role in obtaining results faster as compared to culture especially in regards to rifampicin resistance as well as in smear negative cases.[8] Thus, the laboratory confirmation of TB is of utmost importance for the management of the disease, which further helps in curtailing the transmission of infection.

CONCLUSION:

In this study we have taken culture as the gold standard and performed ZN staining and CBNAAT and compared them as the two other diagnostic modalities. Although smear microscopy by ZN staining for acid-fast bacilli is a rapid, inexpensive method and does not require laboratory set up for diagnosis of TB but it lacks sensitivity due to subjectivity, whereas, CBNAAT is an automated nucleic acid amplification test which has a very low chance of transmission to the sample handlers and along with the detection of MTB it can also detect rifampicin resistance gene directly from clinical samples - the results being available by 2 hours. Therefore, it is simple, less time consuming, a test that does not require special technical expertise and biosafety requirements and provides quick and accurate results which eventually helps with providing accurate treatment especially in the paediatric age group where the diagnosis of pulmonary tuberculosis is elusive clinically. GeneXpert and smear microscopy are comparable as far as specificity is concerned but GeneXpert is a much more sensitive assay for rapid and accurate diagnosis of TB. This study therefore advocates the use of CBNAAT in addition to ZN staining for diagnosing additional cases of pulmonary TB missed by ZN stain alone in GA samples in non - expectorating paediatric patients.

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