Association of Cartridge Based Nucleic Acid Amplification Test (CBNAAT) Grading on the Basis of Cycle Threshold Value with Conventional Microbiological Diagnosis in Pediatric Tuberculosis

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Abstract

**Background:** Microbiological confirmation of tuberculosis disease in children remains difficult due to paucibacillary disease and inability to obtain optimal samples. Recently introduced Cartridge based nucleic acid amplification test (CBNAAT) has improved microbiological diagnosis in pediatric tuberculosis.

**Objectives:** We aimed to study association of CBNAAT grading based on cycle threshold value with conventional microbiological diagnosis. **Methodology:** This prospective study was conducted over a period from November 2016 to October 2017 in the Departments of Microbiology and Pediatrics, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi. CBNAAT positive pediatric TB cases ≤12 years were recruited and subjected to Ziehl-Neelsen staining for acid fast bacilli (AFB) & culture on Lowenstein Jensen medium. CBNAAT positivity was graded based on cycle threshold value: very low, low, medium and high. **Results:** Smear and culture positivity was highest (100%) among specimens with high positive CBNAAT result based on CT value. Time to culture positivity was inversely related to CBNAAT grading (p=0.000). **Conclusion:** CBNAAT grading has significant positive association with smear and culture positivity. [Bangladesh Journal of Infectious Diseases, December 2020;7(2):72-77]

**Keywords:** CBNAAT; CT value; pediatric; tuberculosis

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Introduction

Despite the discovery of effective and affordable anti tubercular therapy more than fifty years back, TB remains one of the top ten causes of mortality worldwide. Children constitute approximately 10.0 to 20.0% of the total TB cases in developing economies like India. According to the WHO Global Tuberculosis Report 2018, the total incidence of TB in India in 2017 was 27.4 lakhs of which 2.24 lakhs cases were contributed by the pediatric age group.

Children may present with vague symptoms mimicking other common childhood diseases. Moreover, microbiological diagnosis also remains difficult in children due to paucibacillary disease and suboptimal specimens leading to diagnostic dilemma. The sensitivity of culture for Mycobacterium tuberculosis, considered as gold standard in adult cases, remains less than 30 to 40% in pediatric cases. These factors lead to diagnostic delays and hence, pediatric TB remains underreported. There is no gold standard for the diagnosis of TB in children. Therefore, in most cases, the clinician has to rely on clinical diagnosis based on history, tuberculin skin test, contact tracing, radiology and lack of response to antibiotics.

Cartridge based nucleic acid amplification test (CBNAAT) which was recently introduced and is now recommended by WHO and Revised National Tuberculosis Control Program (RNTCP) as a preliminary diagnostic tool among children, has proved to be a breakthrough in the diagnosis of TB in pediatric age group. It provides rapid identification and rifampicin resistance from direct specimen within 2 hours. In this heminested rt-PCR assay Mycobacterium tuberculosis is detected by five overlapping molecular probes complementary to the entire 81bp rpoB core region. Mycobacterium tuberculosis is detected when at least 2 of the five probes give positive signals with a cycle threshold (CT) of ≤38 cycles. A semi quantitative estimate of the concentration of bacilli can be defined by CT range (>28=very low, 22-28=low, 16-22=medium, <16= high). Rifampicin resistance is reported when difference between the first (early CT) and the last (late CT) M. tuberculosis specific beacon was >3.5 cycles and was reported sensitive if ≤3.5 cycles. Smear microscopy is usually the first test available for suspected cases of TB. Cultures take a long time to show growth. The use of CBNAAT has reduced the median time to start treatment for AFB smear negative TB from 56 days to 5 days. Few studies have been done to compare conventional microbiological techniques with CBNAAT.

Methodology

This prospective study was conducted over a period from November 2016 to October 2017 in the Departments of Microbiology and Pediatrics, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, India after approval by the institutional research ethics committee. Written consent and assent were taken wherever applicable. All clinically suspected pediatric TB cases ≤12 years of age were recruited in the study based on presence of clinical features, suggestive radiography findings and/or history of exposure to an infectious case of TB and/or reactive tuberculin skin test (TST). Pulmonary TB cases: Either clinically diagnosed or microbiologically confirmed cases with involvement of lung parenchyma, tracheobronchial tree, miliary TB and cases with both pulmonary as well as extrapulmonary features. Patients presented with features like fever and cough for ≥2 weeks, unexplained significant weight loss, loss of appetite, history of contact with infectious case, suggestive chest radiography and reactive TST. Extrapulmonary TB cases: Either clinically diagnosed or microbiologically confirmed cases with involvement of organs other than lungs. Patients presented with features like fever, loss of weight, anorexia and specific symptoms as per the site of involvement with/without supportive radiographic evidence and/ or reactive TST. Immunocompromised patients and previous history of TB were excluded from this study. Relevant samples from suspected pediatric TB cases were collected in sterile, leak-proof, disposable and appropriately labeled containers in 2 aliquots. One aliquot was subjected to CBNAAT at DOTS centre of the hospital as per the standard protocol. A semiquantitative grading of CBNAAT positive specimen indicating bacillary load was reported on the basis of cycle threshold (CT) value as very low (CT>28), low (CT 22-28), medium (CT 16-22) and high (CT<16). The other aliquot was subjected to Ziehl-Neelsen staining for acid fast bacilli (AFB) and culture on Lowenstein Jensen (LJ) medium. All specimens were handled and processed in biosafety cabinet type II B2. Non sterile specimens were initially processed using N-acetyl L-cysteine (NALC) - 4% Sodium Hydroxide (NaOH) method.
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and then inoculated onto LJ slants. Cultures were incubated at 37°C and were observed weekly for up to 8 weeks before reporting as negative. Cultures were identified by characteristic rough, tough and buff colored colonies and isolates were further confirmed as Mycobacterium tuberculosis complex by MPT64 antigen detection test and no growth on media containing Para nitro benzoic acid14. First 50 CBNAAT positive specimens were included in the study. Data was analyzed using SPSS 20.0 software. Non-parametric tests were applied to report significance.

Results

The age of the patients in this study ranged from 2 months to 12 years (mean±7.34±3.93 years). Of total 50 cases, 27(54%) were females and 23 were males. Pulmonary specimens constituted a majority of 35/50 (70%) while only 15 (30%) were extrapulmonary. AFB smear were positive for 17 (34%) while culture showed growth of Mycobacterium tuberculosis for 30 (60%) specimens.

Table 1: Conventional Microbiological Diagnosis of Various Specimens in Pediatric TB

| Specimen                  | Positive by Smear Microscopy, n=17 (%) | Positive by Culture for M. tuberculosis, n=30 (%) |
|---------------------------|----------------------------------------|-----------------------------------------------|
| Pulmonary (n=35)          | 15/35 (43%)                            | 24/35 (69%)                                    |
| Extrapulmonary (n=15)     | 2/15 (13%)                             | 6/15 (40%)                                     |
| Types of extrapulmonary specimens |                          |                                                |
| CSF (n=8)                 | 0                                      | 2 (25%)                                       |
| Pus (n=3)                 | 1 (33%)                                | 3 (100%)                                      |
| Pleural fluid (n=1)       | 0                                      | 1 (100%)                                      |
| Empyema pus (n=1)         | 0                                      | 0                                             |
| Wound aspirate (n=1)      | 1 (100%)                               | 0                                             |
| Liver aspirate (n=1)      | 0                                      | 0                                             |
| Total (n=50)              | 17 (34%)                               | 30 (60%)                                      |

The conventional microbiological diagnosis of various specimens in pediatric TB was recorded. Smear positivity and culture positivity was much higher among pulmonary specimens in 43.0% and 69% respectively as compared to extrapulmonary in 13.0% and 40.0% respectively (Table 1).

Maximum number of samples was reported to be “low” positive (48%) by CBNAAT while minimum number was reported to be “high” positive (12%). Only pulmonary specimens that are sputum and gastric aspirate were reported as “high” or “medium” positive. All extrapulmonary specimens were very low or low positive except 1 cerebrospinal fluid (CSF) specimen which was reported to be medium positive (Table 2).

Figure I shows status of AFB smear and result of culture on Lowenstein Jensen media in relation to CBNAAT grading. 100% of the specimens reported as “very low” positive did not show growth on culture while 100% of those reported as “high” or “medium” positive showed growth of Mycobacterium tuberculosis in culture. Among specimens reported as “low” positive, 58.0% specimens showed growth on culture. All specimens in “very low” category were smear and culture negative while all in “high” category were positive for AFB on smear and showed growth of Mycobacterium tuberculosis in culture.

Again, 100% of the specimens reported as “very low” positive did not show growth on culture while 100% of those reported as “high” or “medium” positive showed growth of Mycobacterium tuberculosis in culture. Among specimens reported as “low” positive, 58.0% specimens showed growth on culture. All specimens in “very low” category were smear and culture negative while all in “high” category were positive for AFB on smear and showed growth of Mycobacterium tuberculosis in culture.

Figure I: AFB Smear Status and Culture Result in Relation to CBNAAT grading
Table 2: Distribution of Specimens According to CBNAAT Results

| Specimens          | CBNAAT Positive Results on The Basis of Cycle Threshold Value | Total | P value |
|--------------------|-------------------------------------------------------------|-------|---------|
|                    | Very Low | Low | Medium | High |                   |       |         |
| GA                 | 5        | 13  | 6      | 4    | 28                |       | >0.05   |
| CSF                | 3        | 4   | 1      | 0    | 8                 |       |         |
| Pleural fluid      | 0        | 1   | 0      | 0    | 1                 |       |         |
| Pus                | 0        | 3   | 0      | 0    | 3                 |       |         |
| Sputum             | 1        | 1   | 3      | 2    | 7                 |       |         |
| Empyema pus        | 1        | 0   | 0      | 0    | 1                 |       |         |
| Liver aspirate     | 0        | 1   | 0      | 0    | 1                 |       |         |
| Wound biopsy       | 0        | 1   | 0      | 0    | 1                 |       |         |
| **Total**          | 10       | 24  | 10     | 6    | 50                |       |         |

Table 3 shows distribution of specimens reported as “medium” positive and “low” positive on CBNAAT along with their smear and culture status. Pulmonary specimens (GA & sputum) showed maximum smear positivity and 100.0% culture positivity among medium positive specimens while among low positive specimens, 5/24 (21.0%) specimens were smear positive and 14/24 (58.0%) showed growth of *Mycobacterium tuberculosis* on culture.

Figure II shows time to culture positivity of the specimens and also correlation of specimens according to CBNAAT grading with time to positivity of cultures. Of 30 positive cultures, maximum number of specimens (40%) showed growth during 4th-5th weeks of incubation while 30% showed growth from 2nd to 3rd weeks and the remaining 30.0% showed growth from 6th to 8th weeks of incubation. Specimens reported to be “high” positive by CBNAAT showed growth early and latest by 5th week while “medium” positive specimens showed growth over a wide range of incubation period from 2nd week to 8 weeks. No specimen reported as “low” positive showed growth before 4th week. Hence, time to culture positivity was found to be inversely related to CBNAAT grading (Spearman’s rho= -0.644) and the correlation was statistically significant (p=0.000).

Table 3: Smear and Culture Status of Specimens with Medium and Low Positive Result on CBNAAT

| Sample          | Medium Positive Specimens | Low Positive Specimens |
|-----------------|---------------------------|------------------------|
|                 | Total | Smear Positive | Culture Positive | Total | Smear Positive | Culture Positive |
| GA              | 6     | 3 (50%)        | 6 (100%)             | 13    | 3 (23%)        | 8 (61.5%)       |
| Sputum          | 3     | 3 (100%)       | 3 (100%)             | 1     | 0              | 1 (100%)        |
| CSF             | 1     | 0              | 1 (100%)             | 4     | 0              | 1 (25%)         |
| Pus             | 0     | 0              | 0                    | 3     | 1 (33.33%)     | 3 (100%)        |
| Pleural fluid   | 0     | 0              | 0                    | 1     | 0              | 1 (100%)        |
| Wound aspirate  | 0     | 0              | 0                    | 1     | 1 (100%)       | 0               |
| Liver aspirate  | 0     | 0              | 0                    | 1     | 0              | 0               |
| **Total**       | 10    | 6 (60%)        | 10 (100%)            | 24    | 5 (21%)        | 14 (58%)        |

Chi-square test was applied. CBNAAT grading was found to have a significant positive association with smear (p=0.000) and culture (p=0.000) positivity.

Discussion

Pediatric TB remains difficult to confirm microbiologically by conventional techniques. WHO recommended upfront CBNAAT for diagnosing TB in presumptive pulmonary and extra-pulmonary pediatric TB cases\(^{15}\). This assay may prove to be a reliable solution to achieve the objective of early and accurate diagnosis of TB and rifampicin resistance which is crucial in pediatric population for early initiation of accurate treatment\(^{16}\).
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Figure II: Correlation of CBNAAT Grading and Time To Positivity

Since, there is a lack of studies on pediatric population comparing CT values of CBNAAT and conventional microbiological techniques, most of the studies referred to were conducted on adult population. Smear and culture positivity were much higher among pulmonary specimens (43.0% and 69.0% respectively) as compared to extrapulmonary (13% and 40% respectively) specimens in this study. 68.0% of the specimens in this study tested “very low” or “low” positive by CBNAAT. Above findings clearly indicate that in most of the cases, the bacillary load in children remains low which leads to difficulty in diagnosis by conventional methods. Microbiological diagnosis is even more difficult to be established in extrapulmonary TB due to paucibacillary nature of specimens.

In this study, 100% of the specimens with CT value more than 28 (very low positive) were smear negative; 21.0% with CT 22 to 28 and 60.0% with CT 16 to 22 were smear positive while 100.0% of those with CT value less than 16 (high positive) were positive for AFB on microscopy. Chi-square test was applied which showed that CBNAAT grading had a significant positive association with smear positivity that is higher the grading, higher is the chance of smear being positive. A recent study conducted in Uganda also found a decreasing trend in CT values with increasing grades of smear. About 75.0% specimens with CT value less than 22 were detected to have AFB on direct microscopy. Similar results were obtained in a recent study on patients with pulmonary TB, where 81.0% of the cases with cut off value of CT as 21.1 were identified as smear positive. Hence, low CT value can identify even smear negative specimens with high bacillary load. Since smear negative cases are a possible source of transmission, grading or quantification in CBNAAT could be utilized for identifying such potentially infectious cases. Other studies have also reported strong association of CBNAAT CT values and smear positivity with cut off values ranging from 27.7 to 31.8 with variable sensitivities and specificities.

About 100.0% specimens in this study with CT value up to 22 that is “medium” and “high” positive showed growth of Mycobacterium tuberculosis on LJ media while 100% of the specimens with CT value >28 “very low” positive were culture negative. Among specimens with CT value 22-28 (“low” positive), 58% showed growth on culture. Hence, higher grade of CBNAAT is associated with significantly increased chances of culture being positive. Such positive correlation between growth on culture and CT value has been seen in previous studies too. In one of those, there was also a decreasing pattern of median CT values associated with increasing categories of culture grade from scanty to +3 on LJ media.

Time to culture positivity had a significant inverse relation with CBNAAT grading in our study which is also supported by another recent Indian study. Other studies have also suggested strong correlation of time to culture positivity and CT values; and that CT values or CBNAAT grading may be used as a surrogate marker for mycobacterial load and response to treatment in both pulmonary and extrapulmonary TB.

Conclusion

CBNAAT grading has significant positive association with smear and culture positivity. Paucibacillary disease in children is indicated by high percentage of low or very low CBNAAT grading. High and medium CBNAAT grading may be more reliable for initiation of ATT. Wider availability and easy accessibility of CBNAAT might be helpful in diagnosis of at least some cases negative by conventional microbiological techniques. Since culture positivity and time to positivity can act as an indirect measure of bacillary load, cases with “high” and “medium” positive specimens have higher culture positivity rates. These patients need to be put on treatment immediately.

For cases with “very low” positive samples, physician might wait for the culture reports before intervening unless there is a very high suspicion of TB. A strong clinical correlation is advisable to aid decision making in “low” positive cases. Newer diagnostic techniques are needed to be developed which can detect even a very low bacillary load in children.
References

1. Glaziou P, Sismanidis C, Floyd K, Raviglione M. Global epidemiology of tuberculosis. Cold Spring Harb Perspect Med. 2015;5:a017798

2. Gupta N, Kashyap B, Dewan P, Hyanki P, Singh NP. Clinical spectrum of pediatric tuberculosis: a microbiological correlation from a tertiary care center. J Trop Pediatr. 2018. https://doi.org/10.1093/jtpe/jmy026.

3. World Health Organization. Global tuberculosis report 2018. Geneva: World Health Organization; 2018. Available from: https://www.who.int/tb/publications/global_report/gtbr2018_an nex2.pdf?ua=1.

4. Kumar MK, Kumar P, Singh A. Recent advances in the diagnosis and treatment of childhood tuberculosis. J Nat Sci Biol Med. 2015;6:314–20

5. Singh S, Singh A, Prajapati S, et al. Xpert MTB/RIF assay can be used on archived gastric aspirate and induced sputum samples for sensitive diagnosis of paediatric tuberculosis. J Clin Tuberc Other Mycobact Dis. 2018;13:9–12.

6. Lawn SD, Nicol MP. Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. Future Microbiol. 2011;6:1067–82.

7. Fradejasa I, Ontañón B, Muñoz-Gallego I, Ramírez-Velaa M.J., López-Roa P. The value of xpert MTB/RIF-generated CT values for predicting the smear status of patients with pulmonary tuberculosis. J Clin Tuberc Other Mycobact Dis. 2018;13:9–12.

8. Nurwidya F, Handayani D, Burhan E, Yunus E. Molecular Diagnosis of Tuberculosis. Chonnam Med J. 2018;54:1–9

9. Prakash AK, Datta B, Tripathy JP, Kumar N, Chatterjee P, Jaiswal A. The clinical utility of cycle of threshold value of GeneXpert MTB/RIF (CBNAAT) and its diagnostic accuracy in pulmonary and extra-pulmonary samples at a tertiary care center in India. Indian J Tuberc. 2018;65:296–302.

10. Najiingglo, Muttambara W, Kirenga B, Manyindo S, Nalunjogi J. Bakessima R, Oweny F, et al. Comparison of GeneXpert cycle threshold values with smear microscopy and culture as a threshold for cervical cancer screening in Zambia. J Clin Tuberc Other Mycobact Dis. 2019;6:105. e016901

11. Singh V, Mahto D. Antitubercular drugs and RNTCP guidelines for childhood tuberculosis. In: Gupta P, Menon PSN, Ramji S, Lodha R (eds). PG Textbook of Pediatrics: Infections and Systemic Disorders. 2nd ed. New Delhi: Jaypee Brothers Medical Publishers, 2018; pp1348.

12. Central TB Division, Government of India. Technical and operational guidelines for TB control in India 2016. New Delhi: Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, 2016.

13. Forbes BA, Sahm DF, Weissfeld AS. Mycobacteria and other bacteria with unusual growth requirements. In: Forbes BA, Sahm DF, Weissfeld AS (eds). Bailey & Scott’s Diagnostic Microbiology. 13th edn. Missouri: Mosby Elsevier Inc, 2014. pp 484–512.

14. Central TB Division, Government of India. Revised National TB Control Programme Training Manual for Mycobacterium tuberculosis culture and drug susceptibility testing. New Delhi: Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, 2009. http://www.tbcindia.nic.in/showfile.php?lid=42991. Accessed 3 Oct 2017.

15. World Health Organization. Policy update: automated real time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system for the diagnosis of pulmonary and extrapulmonary TB in adults and children 2013. Geneva: World Health Organization; 2013. Available from: http://apps.who.int/iris/bitstream/10665/112472/1/9789241506335_eng.pdf

16. Raizada N, Khaparde SD, Salhotra VS, Rao R, Kalra A, Swaminathan S, et al. Accelerating access to quality TB care for pediatric TB cases through better diagnostic strategy in four major cities of India. PLOS ONE. 2018;13: e0193194.

17. Theron G, Pinto L, Peter J, Mishra HK, Mishra HK, van Zyl-Smit R, et al. The use of an automated quantitative polymerase chain reaction (Xpert MTB/RIF) to predict the sputum smear status of tuberculosis patients. Clin Infect Dis. 2012;54:384–88.

18. Lange B, Khan P, Kalmambetova G, Al-Darraji HA, Alland D, Antonenko U, et al. Diagnostic accuracy of the Xpert® MTB/RIF cycle threshold level to predict smear positivity: a meta-analysis. Int J Tuberc Lung Dis. 2017;21:493–502.

19. Beynon F, Theron G, Respeito D, Mambuque E, Taavetsein B, Bulo H, et al. Correlation of Xpert MTB/RIF with measures to assess Mycobacterium tuberculosis bacillary burden in high HIV burden areas of Southern Africa. Sci Rep. 2018; 8:5201

20. Theron G, Peter J, Calligaro G, Meldau R, Hanrahan C, Khalfey H, et al. Determinants of PCR performance (Xpert MTB/RIF), including bacterial load and inhibition, for TB diagnosis using specimens from different body compartments. Sci Rep. 2014;4:5658