Prospective study to find out the role of gastric aspirate examination by Ziehl-Neelsen staining (ZN staining) and cartridge based nucleic acid amplification test (CB-NAAT) as a diagnostic method in childhood tuberculosis

Akansha Arora*, Anil Jain, B. S. Karnawat, Rakesh Kumawat

Department of Paediatrics, Jawaharlal Nehru Medical College, Ajmer, Rajasthan, India

Received: 07 May 2018
Accepted: 31 May 2018

*Correspondence:
Dr. Akansha Arora,
E-mail: akankshamydreams@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: Tuberculosis in children has been relatively neglected mainly because clinical diagnosis has low specificity, radiological interpretation is subject to inter-observer variability and the tuberculin skin test is a marker of exposure, not disease. The recent introduction of Cartridge based nucleic acid amplification test has significantly transformed the diagnostics of tuberculosis in adults but its application for Paediatric Tuberculosis is under evaluation. Therefore, authors conducted a study on role of gastric aspirate examination by ZN stain and Cartridge based nucleic acid amplification test in the diagnosis of childhood Tuberculosis.

Methods: Authors did a prospective hospital-based study from Nov 2016 to Nov 2017 consisting of 100 randomly selected patients suspected of tuberculosis who had their gastric aspirate tested for CBNAAT and ZN stain for acid fast bacilli (AFB) along with Mantoux test and other routine investigations. Chi square test was used.

Results: Culture positive tuberculosis was found in 21 out of 100 children. The sensitivity, specificity, positive predictive value and negative predictive value for CBNAAT were 76.1%, 98.7%, 94.1% and 93.9% and for ZN stain were 47.6%, 98.7%, 90.9% and 87.6% respectively. Positive history of contact (p value 0.0217), reactive Mantoux test (p value < 0.001) and low socioeconomic status were independently associated with a positive CBNAAT result.

Conclusions: Analysis of gastric aspirate samples with CBNAAT is a sensitive and specific method for rapid diagnosis of pulmonary tuberculosis in children who cannot produce sputum. Compared with microscopy, CBNAAT offers better sensitivity and its scale up will improve access to tuberculosis diagnostics in children.

Keywords: AFB, CBNAAT, ZN staining

INTRODUCTION

World Health Organization (WHO) estimated the global burden of tuberculosis (TB) at 9 million new cases and 1.5 million deaths in 2013.1 This included up to 15% burden of paediatric cases. About 74000 children die of TB every year and there are around half a million new cases annually.2 Tuberculosis in children has been relatively neglected, mainly due to challenges in the availability of effective diagnostic tools.3 India is 17th among 22 high burden countries in terms of overall TB incidence rate.4 In most settings the diagnosis in children is traditionally based on the basis of contact tracing and very few attempts have been made for active case detection. This is mainly due to lack of pathognomonic clinical presentation in paediatric Tuberculosis and lack of sensitive diagnostic tools.3,6 Microbiological confirmation with identification of drug resistance is
increasingly important in the context of an emerging drug resistant tuberculosis epidemic. Infants and young children do not expectorate but instead swallow their sputum. Aspiration or lavage of gastric content is best procedure in them for obtaining specimen from which to culture *Mycobacterium tuberculosis*. Cartridge based nucleic acid amplification test is a nucleic acid amplification test which simultaneously detects DNA of Mycobacterium tuberculosis complex (MTBC) and resistance to rifampicin (RIF) in less than 2 hours. This system integrates and automates sample processing, nucleic acid amplification and detection of the target sequences.

Therefore, authors have conducted a study on role of gastric aspirate in detection of acid fast bacilli by Ziehl-Neelsen stain and Cartridge based nucleic acid amplification test in the diagnosis of childhood Tuberculosis.

**METHODS**

The study was a prospective hospital-based study conducted from November 2016 to November 2017 in the Department of Paediatrics, JLN Medical College and Associated group of Hospitals, Ajmer. Study group comprised of 100 randomly selected patients ≤12 years of age of either sex who met the following criteria.

**Patient with clinical/investigational suspicion of tuberculosis who met any of the following**

- Fever and or cough for > 2 weeks
- History of weight loss or no weight gain in past 3 months (loss of weight defined as loss of more than 5% body weight as compared to highest weight recorded in last 3 months)
- Hilar lymphadenopathy on chest skiagram
- Tuberculin skin test positivity
- Coexistence of precipitating illness
- Abdominal USG suggestive of tuberculosis
- ADA positivity in pleural fluid
- CSF findings consistent with tubercular meningitis
- Cranial CT suggestive of tuberculoma.

**Patient who has not taken anti tuberculosis drugs.**

All the selected patients were subjected to detailed history, general physical examination, systemic examination, haematological investigations, tuberculin sensitivity test and gastric aspiration for Ziehl-Neelsen staining and cartridge based nucleic acid amplification test. Other relevant Investigations were done where ever necessary to support the diagnosis.

The sensitivity, specificity, positive predictive value and negative predictive value were calculated for both cartridge based nucleic acid amplification test and Ziehl-Neelsen staining using culture as the gold standard. Chi square test was used to correlate the various findings with the test results.

**RESULTS**

Majority of the cases included in present study were in the age group of 0-5 years (57%) followed by age group of 5-12 years and least were 10-12 years (16%). 55% males and 45% females were included in this study. Maximum no. of cases was of lower middle class (class III) with 42% and upper lower (class IV) with 23% according to kuppuswamy scale for socioeconomic status. Progressive pulmonary tuberculosis was the most common type of tuberculosis included in this study. Fever, cough of more than 2 weeks, weight loss/no weight gain and anorexia were the most common presenting complaints.

The sensitivity, specificity, positive predictive value and negative predictive value of Ziehl-Neelsen staining for acid fast bacilli were 47.6%, 98.7%, 90.9% and 87.6% respectively as compared to culture as gold standard. The sensitivity, specificity, positive predictive value and negative predictive value of Cartridge based nucleic acid amplification test were 76.1%, 98.7%, 94.1% and 93.9% respectively. There was no association for absence of BCG scar and positivity of Cartridge based nucleic acid amplification test. Statistical significance was recorded for positive Cartridge based nucleic acid amplification test and presence of history of contact with tuberculosis.

There was a positive association found between reactivity of Mantoux test and positive Cartridge based nucleic acid amplification test result. Cartridge based nucleic acid amplification test was found positive maximum in class III and class IV socioeconomic groups according to kuppuswamy classification. Statistical significance was recorded for positive Ziehl-Neelsen staining and presence of history of contact with tuberculosis There was a positive association found between reactivity of Mantoux test and positive Ziehl-Neelsen stain for acid fast bacilli.

**Table 1: Association of cartridge based nucleic acid amplification test and Mantoux test.**

<table>
<thead>
<tr>
<th></th>
<th>Mantoux test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>CBNAAT Detected</td>
<td>5</td>
</tr>
<tr>
<td>CBNAAT Not detected</td>
<td>61</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>66</strong></td>
</tr>
<tr>
<td><strong>P Value</strong></td>
<td>&lt;0.001 (S)</td>
</tr>
</tbody>
</table>

Table 1 shows total no. of children with positive Mantoux test were 34 while with negative Mantoux test were 66. rpoB gene was detected by Cartridge based nucleic acid amplification test in 12 children with positive Mantoux test and 5 children with negative Mantoux test with a p-value of <0.001 which is statistically significant.
Table 2 shows total no. of cases in class I were 4, class II were 22, class III were 42, class IV were 23 and class V were 9 rpoB gene was detected by Cartridge based nucleic acid amplification test maximum in both class III and class IV children with a percentage of 29.4%.

Table 2: Distribution of cartridge based nucleic acid amplification test and socioeconomic status.

| Socioeconomic status (Kuppuswamy scale) | CBNAAT | | |
|----------------------------------------|--------|--------|--------|--------|--------|--------|
| Total                                  | Not detected | Detected | Class I | Class II | Class III | Class IV | Class V |
| N                                      | 3       | 22     | 42     | 23     | 9       | 100     |
| %                                      | 3.6%    | 22.9%  | 44.6%  | 21.7%  | 7.2%    | 100.0%  |

Table 3 shows percentage of children in present study with positive history of contact were 33% while 67% had no history of contact rpoB gene was detected by cartridge based nucleic acid amplification test in 41.1% cases with absent history of contact and 58.8% cases with positive history of contact with a p-value of 0.0217 which is statistically significant.

In Table 4, the sensitivity, specificity, positive predictive value and negative predictive value for Ziehl-Neelsen staining were 47.6%, 98.7%, 90.9% and 87.6% respectively taking culture as the gold standard.

Table 4: Statistical measure of accuracy of Ziehl-Neelsen staining.

<table>
<thead>
<tr>
<th>Culture*</th>
<th>Total</th>
<th>Statistic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ziehl-Neelsen staining</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>11</td>
<td>Sensitivity</td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td>Specificity</td>
</tr>
<tr>
<td>11</td>
<td>78</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>Positive predictive value</td>
</tr>
<tr>
<td>21</td>
<td>79</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Negative predictive value</td>
</tr>
</tbody>
</table>

Table 5: Statistical measure of accuracy of CBNAAT staining.

<table>
<thead>
<tr>
<th>Culture*</th>
<th>Total</th>
<th>Statistic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBNAAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>1</td>
<td>11</td>
<td>Sensitivity</td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td>Specificity</td>
</tr>
<tr>
<td>05</td>
<td>78</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>Positive Predictive Value</td>
</tr>
<tr>
<td>21</td>
<td>79</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Negative Predictive Value</td>
</tr>
</tbody>
</table>

In Table 5, sensitivity, specificity, positive predictive value and negative predictive value for cartridge based nucleic acid amplification test were 76.1%, 98.7%, 94.1% and 93.9% respectively considering culture as the gold standard.
DISCUSSION

Present study showed a sensitivity of 76.1% and specificity of 98.7% for Cartridge based nucleic acid amplification test on gastric aspirate samples of children suspected to be suffering from tuberculosis. Positive predictive value and negative predictive value were 94.1% and 93.9% respectively. Sensitivity of Cartridge based nucleic acid amplification test (76.1%) was 30% more than sensitivity of Ziehl-Neelsen staining for Acid Fast bacilli. Bates M et al in their study had a sensitivity of 68.8% and a specificity of 99.3% for gastric lavage samples.7

Detjen AK et al showed that compared with culture, the pooled sensitivity and specificity of Xpert for tuberculosis detection were 66% and 98% respectively with use of samples from gastric lavage. Xpert sensitivity was 36-44% higher than was sensitivity for microscopy.8

Pang et al got a sensitivity of 48.6% and concluded that Xpert is an excellent tool for the diagnosis of smear negative tuberculosis with gastric lavage samples while Singh et al concluded the sensitivity of 62.5% in smear negative and culture positive samples with both gastric lavage and induced sputum samples.9,10

Authors report positive rate of Cartridge based nucleic acid amplification test in patients with history of tuberculosis contact was significantly higher (p value 0.0217) than that with no history of contact. Also, the positivity of Cartridge based nucleic acid amplification test was higher in lower socioeconomic groups (class III and IV). Sekadde et al obtained significant association between positive history of contact and positive Xpert (p value 0.03).11

Qing-Qin Yin et al demonstrated positivity of Xpert associated significantly with history of contact with tuberculosis present in patients (p value 0.010).12 Authors have shown a statistically significant association (p value <0.001) between positive rates of Cartridge based nucleic acid amplification test and reactivity of tuberculin sensitivity test.

Sekadde et al obtained significant association between positive tuberculin sensitivity test and positive Xpert (p value 0.002) which is in accordance to present study.13

The sensitivity of 47.6% for Ziehl-Neelsen staining found in this study, though still low, is an improvement on the finding of 0%, 13%, 29.3% and 43.8% by Abadco and Steiner, Gomez et al, Kalu et al and Elhassan et al respectively.14-17 In present study, the specificity or true-negative rate of Gastric aspirate acid-fast smear for Mycobacterium tuberculosis was found to be 98.7%.

This high specificity finding agrees with the reports of Gomez et al, Bahammam et al and Kalu et al who got specificity values of 96.8%, 100%, 97.6% respectively.14,15,18 Elhassan et al in their comparative study from Sudan showed a specificity of 98.8% for gastric aspirate microscopy by Ziehl-Neelsen stain.16

CONCLUSION

Authors can safely conclude that Cartridge based nucleic acid amplification test on one gastric aspirate sample rapidly and correctly identified the majority of children with culture confirmed tuberculosis with a high specificity.

Positive history of contact, reactive tuberculin sensitivity test and low socioeconomic status were independently associated with a positive Cartridge based nucleic acid amplification test result.

This test is widely anticipated to replace smear microscopy in resource-poor settings where HIV co-infection or drug-resistant tuberculosis are common, and present results suggest that its use is a major improvement over use of smear microscopy. Although time to detection was not a primary outcome for this study, cartridge based nucleic acid amplification test results were available within 1 day, which was substantially faster than for culture results.

Cartridge based nucleic acid amplification test performs well in Gastric aspirate samples for rapid and accurate diagnosis of childhood tuberculosis; the assay is more sensitive than smear microscopy for the diagnosis of childhood tuberculosis with gastric aspirate samples; and the assay detected an extra 6 cases of tuberculosis compared with smear microscopy.

Present study was done in a single, tertiary referral centre with high tuberculosis burden; therefore, it cannot be assumed that the accuracy of the cartridge based nucleic acid amplification test for the detection of childhood pulmonary tuberculosis will be the same in other patient cohorts or settings.

Acid-fast smear of gastric aspirate is a highly specific test for the diagnosis of PTB in children. A positive acid-fast smear should warrant commencement of the full course of anti-TB therapy.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES


Cite this article as: Arora A, Jain A, Karnawat BS, Kumawat R. Prospective study to find out the role of gastric aspirate examination by Ziehl- Neelsen staining (ZN staining) and cartridge based nucleic acid amplification test (CB-NAAT) as a diagnostic method, in childhood tuberculosis. Int J Contemp Pediatr 2018;5:1609-13.