

# Detection of *Mycobacterium tuberculosis* in Buccal Swab Specimens in Children with Pulmonary Tuberculosis Using Cartridge-based Nucleic Acid Amplification Test

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## ABSTRACT

**Aim and objective:** The study was designed to detect *Mycobacterium tuberculosis* (*M.tb*) in buccal swab specimens using cartridge-based nucleic acid amplification test (CBNAAT) in children suffering from pulmonary tuberculosis (TB) and to compare with CBNAAT results with gastric aspirate (GA) and sputum specimen.

**Materials and methods:** This observational study included children  $\leq 15$  years of age attending Department of Pediatrics of a tertiary care hospital diagnosed as presumptive pulmonary TB. Gastric aspirate/induced sputum (IS) sample and buccal swab were collected from all the study subjects and subjected to CBNAAT. Acid-fast bacilli (AFB) microscopy was also performed on GA/IS samples.

**Results:** Fifty presumptive cases of pulmonary TB were enrolled in the study. Fifteen (30%) buccal swab samples and 41 (82%) GA/IS samples were positive for CBNAAT. Gastric aspirate was positive in 23/24 (98%) subjects which was significantly higher as compared to buccal swab results ( $p = 0.0001$ ). Induced sputum was positive in 18/26 (69.2%) samples which was comparable to buccal swab results ( $p < 0.092$ ). AFB microscopy was positive in only 10 (5%) subjects. Rifampicin resistance was demonstrated in 9 (18%) subjects on GA/IS and 4 (8%) cases on buccal swab detected by CBNAAT.

**Conclusion:** Buccal swabs can be used to detect *M.tb* in children with pulmonary TB. The results were statistically comparable to IS but inferior to GA specimen. It can serve as simple and convenient alternative method.

**Keywords:** Buccal swabs, Cartridge-based nucleic acid amplification test, Diagnostic test, Pediatrics, Pulmonary tuberculosis.

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## INTRODUCTION

Tuberculosis (TB) remains a major global health problem. According to the World Health Organization (WHO), there are an estimated 10.4 million people with TB disease and 1.7 million TB deaths in 2015.<sup>1</sup> Children  $< 14$  years account for about 1 million of TB disease cases worldwide.<sup>1</sup>

The diagnosis of pulmonary TB in children is a challenge due to paucibacillary nature of the disease and difficulty in getting appropriate samples. Most frequently used samples for the diagnosis of pulmonary TB in children include sputum or induced sputum (IS) and gastric aspirate (GA). Sputum is difficult to obtain in children as they fail to expectorate. Gastric aspirate has the disadvantage of being invasive, uncomfortable, and time-consuming.<sup>2</sup> An alternative, less invasive sample matrix could greatly simplify TB diagnosis. There has been a constant search for alternative clinical specimen for years but with limited success. A recent study applied cartridge-based nucleic acid amplification test (CBNAAT) to a variety of non-traditional samples (exhaled breath concentrate, saliva, blood, and urine) obtained from culture confirmed, pulmonary TB patients which demonstrated sensitivity of 0, 38.5, 8.3, and 3.8%, respectively, showing saliva to have highest diagnostic yield as compared to other non-traditional methods.<sup>3</sup>

*Mycobacterium tuberculosis* (*M.tb*) or its DNA has been demonstrated in buccal swabs of pulmonary TB adult patients.<sup>4,5</sup> The concept appears feasible in part because *M.tb* like most bacteria have evolved mechanism to adhere to surfaces including mammalian cells.<sup>6-9</sup> Wilbur et al. first demonstrated *M.tb* in the mouths of macaques using PCRIS6110.<sup>10</sup> These studies have shown

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the great potential of buccal swab as a diagnostic tool. In this study, we used buccal swab specimens using CBNAAT for the diagnosis of TB in children with pulmonary TB and compared its results with CBNAAT on IS and GA.

## MATERIALS AND METHODS

This observational study included children  $\leq 15$  years of age attending Department of Pediatrics of a tertiary care hospital

diagnosed as presumptive pulmonary TB. The diagnosis was made in accordance with the National Guidelines for Pediatric TB.<sup>11</sup> The patients with persistent fever and/or cough >2 weeks with/without loss of weight, history of contact, and chest X-ray suggestive of TB were diagnosed as presumptive pulmonary TB. Patients with HIV infection and prior history of antitubercular drug intake were excluded from the study. The study commenced after obtaining ethical clearance from institutional ethical committee. Study subjects were enrolled between March 2017 and October 2018 after obtaining written informed consent from their guardian.

The subjects underwent detailed clinical examination including anthropometric measurements. Investigations included tuberculin skin test (TST) and chest radiograph. Gastric aspirate or IS and buccal swabs were collected from each subject. These clinical samples were subjected to the following tests: Acid-fast bacilli (AFB) microscopy using Ziehl–Neelsen staining and CBNAAT.

### Procedure for Collecting Gastric Aspirate

The subjects were kept overnight fasting (at least for 6 hours). Measured length of nasogastric tube was gently inserted through the nose and advanced into the stomach. A syringe (usually 10 mL) was attached to the nasogastric tube and the gastric contents were withdrawn. If no fluid was aspirated, 5–10 mL of sterile water or normal saline was flushed, and aspiration was repeated. Gastric fluid was transferred from the syringe into a falcon collection tubes.

### Procedure for Collecting IS Sample

In children who could expectorate, early morning sputum sample was collected in sterile container by asking the child to cough. In younger children who were not be able to expectorate, IS was collected. The patients were nebulized with bronchodilators followed by saline nebulization. Chest physiotherapy was performed and sputum was collected from the throat or nasopharynx using a collector attached to a suction at one end and a catheter or tube to the other end. The specimen was collected in falcon tube.

### Procedure for Collecting Buccal Swabs

Buccal swabs were collected using sterile cotton swabs (Hi-Media). After ensuring that subjects' mouth was empty, buccal swabs were brushed along the inside of the subject's cheek and posterior pharynx for about 10 seconds (7–8 times) ensuring that entire swab tip made contact with the cheek to collect cells and saliva. The head of each swab was ejected in 2 mL of sterile buffer (phosphate-buffered saline). The swab was capped properly, put in a zip plastic bag, and labeled.

The clinical samples were transported to the Reference Laboratory, State TB Demonstration Centre for CBNAAT testing and AFB microscopy. When there was delay in transportation, these samples were stored at 2–8°C till the transport of the sample.

### Statistical Analysis

The results obtained were tabulated in excel sheet and statistically analyzed using SPSS version 20. Chi-square test was used to compare diagnostic yield of CBNAAT on buccal swab with GA and IS. *p* value < 0.05 was taken as significant.

## RESULTS

A total of 50 subjects with presumptive pulmonary TB were included in this study. Clinical and demographic details are given in Table 1. Majority of the patients (70%) belonged to the age-group

**Table 1:** Clinicodemographic profile of study subjects (n = 50)

Variable		n (%)
Age (mean)		11.18 ± 3.08 years
Gender	Male	21 (42)
	Female	29 (58)
Body mass index	<3rd centile	14 (28)
	2nd–3rd centile	20 (40)
	Normal	16 (32)
Hemoglobin, g (%)		10.48 ± 1.84
Socioeconomic status	Middle	32 (64)
	Low	18 (36)

**Table 2:** Chest X-ray finding in study subjects (buccal swab CBNAAT positive vs negative)

Chest X-ray findings	Buccal swab CBNAAT+, (n = 15) (%)	Buccal swab CBNAAT–, (n = 35) (%)	Total (n = 50) (%)
Consolidation	7 (46.6)	11 (31.4)	18 (36)
Hilar adenopathy	2 (13.4)	13 (37.2)	15 (30)
Miliary pattern	6 (40)	6 (17.2)	12 (24)
Hilar adenopathy and consolidation	–	3 (8.6)	3 (6)
Cavity	–	1 (2.8)	1 (2)
Collapse	–	1 (2.8)	1 (2)

of 11–15 years with the mean age of 11.18 ± 3.08 years. Female to male ratio was 1.4:1. Twenty-nine (58%) patients were found to be underweight (≤2 SD). History of contact with a TB case was present in 23 (46%) cases. Bacillus Calmette Guerin (BCG) immunization was present in 32 (64%) patients. Twenty-five (61%) patients had positive TST. Majority of the study group 18 (36%) belonged to lower socioeconomic class.

The most common finding on chest X-ray was consolidation in 18 (36%) cases followed by hilar lymphadenopathy in 15 (30%), miliary shadows in 12 (24%) cases. It was observed that miliary pattern was seen more commonly in buccal swab positive cases (40%) as compared to buccal swab negative cases (17.2%) (Table 2).

Out of 50 presumptive cases of pulmonary TB, 41 (82%) were positive for CBNAAT on GA/IS. Fifteen (30%) patients were positive for CBNAAT taken from buccal swab. Induced sputum was taken in 26 cases, of which 18 (69.2%) were positive for CBNAAT. Of 24 GA samples, 23 (95.8%) were found to be positive by CBNAAT (Table 3). Positivity of CBNAAT on GA was significantly more than CBNAAT positivity on buccal swab (*p* ≤ 0.0001), whereas positivity of CBNAAT on IS was statistically comparable to positivity on buccal swab (*p* = 0.092). Cartridge-based nucleic acid amplification test on GA and IS could detect 19 (35%) and 7 (14%) additional cases which were negative on buccal swab. Acid-fast bacilli microscopy was positive in only 5 (10%) of subjects.

Cartridge-based nucleic acid amplification test on GA/IS showed 9 (18%) subjects to be rifampicin resistant. Among buccal swab positive for CBNAAT, 4 (8%) were resistant to rifampicin.

## DISCUSSION

Diagnosis of pulmonary TB in children has revolutionized with the use of CBNAAT as the diagnostic test. Cartridge-based nucleic acid amplification test has been recommended by WHO to be used

**Table 3:** Comparison of CBNAAT on buccal swab with CBNAAT on gastric aspirate (GA), induced sputum (IS), and acid-fast bacilli (AFB) microscopy

Diagnostic test		Buccal swab		p value*
		CBNAAT+, 15 (30%)	CBNAAT–, 35 (70%)	
CBNAAT Gas- trich aspirate	Positive 23 (95.8)	4 (17.4)	19 (82.6)	0.001
	Negative 1 (4.2)	–	1 (100)	
CBNAAT- induced sputum	Positive 18 (69.2)	11 (61.1)	7 (38.9)	0.092
	Negative 8 (30.8)	–	8 (100)	
AFB microscopy	Positive 5 (10)	3 (60)	2 (40)	0.123
	Negative 45 (90)	12 (26.6)	33 (73.4)	

\*p value—Buccal swab CBNAAT vs gastric aspirate CBNAAT, induced sputum CBNAAT and AFB microscopy

for the diagnosis of TB in children.<sup>12</sup> It is automated nucleic acid amplification molecular assay that detects *M.tb* and rifampicin resistance simultaneously. Sputum and GA are the conventional samples used for the diagnosis of pulmonary TB in children. Gastric aspirate has the disadvantage of being invasive and stressful for children. Moreover, it often needs hospital admission and overnight fasting.<sup>2</sup> This has led to the search of alternative sampling method, which is easier, quick, noninvasive for the diagnosis of pulmonary TB in children. The buccal swab samples are easy to collect, have smaller volumes, are less viscous, and more uniform in composition as compared to sputum. It can be used for active case findings where the children are unable to expectorate sputum. Moreover, buccal swab does not pose a risk of TB infection to health workers since it does not involve aerosol production as in sputum production. To the best of our knowledge, this is the first study on children with pulmonary TB to show detection of *M.tb* using CBNAAT with buccal swabs.

The results of our study demonstrate that *M.tb* can be detected in the buccal swab of children suffering from pulmonary TB in 30% cases using CBNAAT. Previous studies have reported detection of *M.tb* DNA in nonhuman primates as well as human adults.<sup>4–6,13</sup> In a study from primates, buccal swabs were collected from 263 macaques representing 11 species in 4 Asian countries and Gibraltar. Following DNA isolation from buccal swabs, the PCR amplified IS6110 from 84 (31.9%) macaques. This was the first demonstration of *M.tb* DNA in mouth of macaques.<sup>6</sup> In another study, oral swabs from laboratory macaques experimentally infected with simian human immunodeficiency virus, 7/7 (100%) of the oral swabs were positive for *M.tb* in the investigation.<sup>13</sup> In a study conducted among native South Americans reported that 37 out of 202 (18%) of the buccal swab samples collected were positive for *M.tb*. Standard PCR protocols using IS6110 element were used for the detection of *M.tb* in buccal swabs.<sup>4</sup> In another study on adult subjects, 3 swabs were collected from 20 cases confirmed TB by CBNAAT and 20 healthy controls were taken. Samples were tested using PCR IS6110 specific to *M.tb* complex. Eighteen out of 20 (90%) yielded at least 2 positive swabs and healthy controls were negative.<sup>5</sup>

In our study, 82% subjects were found to have positive CBNAAT on IS/GA samples. The sensitivity of CBNAAT on GA/IS aspirate

was found to be similar to previous studies.<sup>14,15</sup> In our study, *M.tb* detection by CBNAAT was more on GA samples than IS samples. These results are supported by a previous study which showed that, in children with probable intrathoracic TB, GA identified more cases than IS using smear microscopy, culture, and Xpert *M.tb*/RIF assay.<sup>16</sup> Buccal swab CBNAAT detection was significantly inferior to GA CBNAAT performance. However, *M.tb* detection by buccal swabs was comparable with IS results. This suggests that buccal swabs can be used as an alternative method or as screening method to diagnose pulmonary TB in children.

Our study showed that patients with miliary pattern on chest skiamogram were more likely to have buccal swab positive. The probable reason could be that patients with miliary TB have higher bacillary load in the sputum and the oral cavity leading to higher detection on buccal swabs.

The study shows alarmingly high number of rifampicin-resistant cases in treatment naïve cases as also observed in other studies.<sup>17</sup>

Low yield of buccal swab samples in the study was probably due to small sample size and only one sample of buccal swab taken from each patient. Additional studies with larger sample size may be performed to demonstrate utility of buccal swabs in the diagnosis of pulmonary TB.

## CONCLUSION

The results of this study showed that *M.tb* can be detected on buccal swab in children with pulmonary TB using CBNAAT. Results of CBNAAT on GA were superior to buccal swab results but results on IS were comparable. Buccal swab is a simpler, quick, noninvasive sampling method as compared to GA and IS and can be useful alternative method of *M.tb* detection. It can have wider applicability and can even be used in remote areas where it is difficult to obtain GA or IS due to lack of adequate facilities. However, larger studies are needed to demonstrate utility of buccal swab as a promising alternative to traditional sputum/GA-based testing methods for TB diagnosis in children.

## WHAT THIS STUDY ADDS

This is the first study to show that *M.tb* can be detected on buccal swab using CBNAAT in children with pulmonary TB. Buccal swab can be used as a simpler and quicker alternative sampling method as compared to GA and IS.

## CONTRIBUTION

All the authors were involved in the conception of study, data collection, analysis, preparation of the manuscript. All the authors have approved the study.

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