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Clinical Research

DETECTION OF PULMONARY TUBERCULOSIS BY USING ORAL SWAB AS AN ALTERNATIVE TO SPUTUM SAMPLE

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ABSTRACT Tuberculosis(TB), caused by Mycobacterium Tuberculosis(MTB), represents a significant global health issue. While sputum is the gold standard for pulmonary TB diagnosis, its collection can be difficult in some patients. This study evaluated oral swabs as a non-invasive alternative. Among 745 sputum samples, 104 were MTB-positive. Oral swabs from 37 MTB-positive and 37 MTB-negative patients were tested using Nucleic acid amplification test (NAAT)-based diagnostics. Sensitivity of oral swabs was 37%, and specificity was 100%. Although oral swabs showed high specificity, low sensitivity limits their standalone use. They may serve as an adjunct tool, with further research needed to improve diagnostic accuracy.

KEYWORDS : Mycobacterium Tuberculosis, NAAT, Oral Swab, Point-Of-Care, Sputum, Sensitivity, Specificity

INTRODUCTION

Tuberculosis(TB) is a chronic bacterial infection caused by MTB that primarily affects the lungs, but can also impact other parts of the body. According to the World Health Organization (WHO), millions of new cases are reported annually, resulting in considerable morbidity and mortality worldwide [1]. TB transmission typically occurs through airborne droplets when an infected individual coughs or sneezes, and certain populations, such as those with compromised immune systems or malnutrition, are more susceptible to developing active TB disease [2]. Early diagnosis and effective treatment are crucial for controlling TB transmission and improving patient outcomes. However, challenges such as limited access to healthcare services, and social determinants of health continue to hinder efforts to eliminate TB as a public health threat. Addressing these challenges requires a comprehensive approach that includes strengthening healthcare systems, enhancing awareness and education, and promoting research and innovation in TB prevention, diagnosis, and treatment. By working together, it is possible to accelerate progress toward TB elimination and reduce the burden of this disease on individuals and communities worldwide.

TB remains a significant global health concern, particularly in low- and middle-income countries [3]. Accurate diagnosis is crucial for effective disease management and control. Sputum samples are traditionally used for diagnosing pulmonary TB. However, collecting sputum can be challenging, especially among children, elderly patients, and individuals with difficulty expectorating[4]. In such cases, alternative sample collection methods are essential to improve TB diagnosis and treatment outcomes. Sputum-based diagnosis of MTB has limitations, including difficulty in collecting sputum samples from certain patient populations having expectoratory challenges, inadequate sample quality, leading to false-negative results and risk of transmission during sample collection.

Oral swabs can offer a promising alternative, with potential benefits as it is non-invasive and easy to collect, secondly it has reduced risk of transmission, Oral swabs also offers improved patient compliance[5].

This study seeks to assess the diagnostic accuracy of oral swabs compared to sputum samples for MTB detection, exploring their potential as a reliable alternative for TB diagnosis, particularly in challenging patient populations.

Materials and Method

Study Design: We performed a cross-sectional study to evaluate the potential of oral swab as an alternative to Sputum samples for detection of Mycobacterium Tuberculosis.

Study Population and Study Site: The study included suspected Tuberculosis patients attending Molecular Biology Laboratory of ESI Hospital, Manicktala, which is considered as a NAAT center approved by National Tuberculosis Elimination Program(NTEP). The laboratory is involved in routine diagnosis of Tuberculosis with the facility for molecular testing. All cases willing to provide consent were enrolled in the study.

Inclusion Criteria: The following criteria were recommended for choosing the study participants:

- Presumptive Pulmonary Tuberculosis
- Patient with positive Sputum Acid Fast Bacilli (AFB)test /Chest X-Radiation (Xray)/High-Resolution Computed Tomography (HRCT)thorax positive/suggestive of Tuberculosis.
- Age group ≥18 years
- Individuals consenting for study participation
- Individuals willing to provide both early morning sputum and early morning oral swab samples

Exclusion Criteria: The following exclusion criteria were considered for the study

- Patients unable to produce sputum (e.g., children or individuals with non-productive coughs)
- Individuals on Anti-Tubercular Drugs
- PLHIV

Sample Size: A total number of 745 sputum samples from suspected Tuberculosis patients were tested for the presence of MTB. 37 oral swab samples collected from patients fulfilling inclusion criteria and having their sputum result positive for MTB were subjected to molecular analysis for the detection of MTB..

Sample collection, processing and storage: Since early morning

samples carries higher bacterial load, hence early morning Sputum samples were collected. Similarly, early morning oral swab samples high in biomass were collected from the buccal mucosa, gingival surfaces, and the dorsum of the tongue. Patients were instructed to give their oral swab samples and sputum samples early morning prior to any intake of food or drink, smoking, rinsing mouth and brushing teeth. The morning sample helps reduce potential biases in test results. Sample were stored at 2-8 degree Celsius until processing. The extracted and purified DNA obtained was then subjected to POC thermo-cycler, based on NAAT for the detection of MTB. All samples were processed under standard biosafety conditions to minimize exposure to potentially infectious materials

Diagnostics Test

All sputum and oral swab samples were subsequently analysed using a POC thermo-cycler, based on NAAT, targeting insertion sequence(IS), IS6110 and IS1081, which is unique to the MTB complex.

Statistical Analysis

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of oral swabs were calculated against the sputum RT-PCR result as the gold standard. A Receiver Operating Characteristic (ROC) curve was generated to visualize diagnostic performance.

- Sensitivity =37.8% (14/37)
- Specificity =100% (37/37)
- Positive Predictive Value (PPV) = True Positive(TP)/ True Positive TP+ False Positive(FP)
- Negative Predictive Value (NPV) =True Negative(TN)/False Negative(FN)+True Negative(TN)
- PPV =100% (14/14)
- NPV =61.66% (37/60)

To evaluate the diagnostic concordance between sputum and oral swab samples in detecting MTB, we performed a Chi-square test for independence using Graph Pad Prism. The data included three sample groups: sputum, oral swabs from sputum-positive patients, and oral swabs from sputum-negative patients.

A Chi-square test (χ^2) was applied to assess whether the detection of MTB was significantly different across the three groups.

The overall Chi-square test yielded a χ^2 value of 33.91, with 2 degrees of freedom and a p-value < 0.0001, indicating a statistically significant association between sample type and test positivity.

To further investigate group wise differences, 2×2 post hoc comparisons were performed with Fisher's exact test:

1. Sputum vs. Oral swab (Sputum-positive patients):
   p = 0.0053 (Fisher's exact test)
   Indicates significantly higher positivity in sputum than oral swab in the same group of TB-confirmed patients.
2. Oral swab (Sputum-positive) vs. Oral swab (Sputum-negative):
   p < 0.0001
   Suggests significantly higher positivity among oral swabs from confirmed TB cases compared to non-cases.
3. Sputum vs. Oral swab (Sputum-negative):
   p < 0.0001
   Confirms the absence of false positives in oral swabs among TB-negative individuals.

These results demonstrate a significant difference in test positivity between sample types, with sputum samples showing higher diagnostic yield. However, oral swabs from sputum-positive patients also showed a moderate positivity rate (37.8%), suggesting its moderate ability to detect MTB.

ROC Analysis (Visual Data)

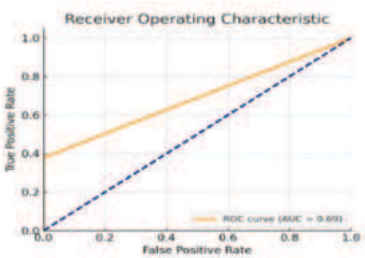

Figure 1: The roc curve yielded an AUC (area under the curve) of 0.69, indicating a moderate diagnostic ability of the oral swabs to detect TB in comparison to sputum

RESULT

Table 01 Comparative Results of Sputum and Oral Swab Samples for Presence of Mycobacterium Tuberculosis

SAMPLE TYPE	TOTAL TESTED	POSITIVE %	NEGATIVE %
Sputum	745	104(14%)	641(86%)
Oral swab (from Sputum positive samples)	37	14(37.8%)	23(62.2%)
Oral swab (from Sputum negative samples)	37	0(0%)	37(100%)

Out of the 37 sputum-positive patients tested with oral swabs:
14 (37.8%) tested positive for MTB on oral swab and 23 (62.2%) tested negative on oral swab.

Out of the 37 sputum-negative patients:
All 37 (100%) tested negative on oral swab.

DISCUSSION

Our study showed 104 sputum-positive patients,37 were selected for oral swab testing based on inclusion criteria. Among these,14 oral swab tested positive for MTB, yielding a sensitivity of (37.8%). This relatively low sensitivity suggests that, while oral swabs may detect MTB in a subset of confirmed TB patients, a significant proportion of infection may go undetected if this method is used in isolation. Factors contributing to the variability in sensitivity include lower bacillary load in oral cavity, quality of swab used, improper sample collection technique, degradation of bacterial DNA prior to testing and molecular testing methods.

To assess potential false negative results, our study included testing 37 oral swab samples randomly selected from sputum negative individuals. All 37 oral swab samples tested negative indicating a specificity of (100%), reinforcing its reliability as a supplemental test with strong negative predictive value when used in sputum-negative cases.

Studies have shown that tongue swabs tend to have higher sensitivity than buccal swabs, and the use of Xpert MTB/RIF Ultra with optimized sample processing methods can enhance diagnostic accuracy[6].

Studies have also shown that tongue swabs yield lower bacterial load for diagnostic testing, in such cases growth supplementation can improve detection rate[7].

Limitations of the study included small sample size for oral swab evaluation, potential sampling errors with oral swabs, single-center study.

Future research should explore ways to improve oral swab sensitivity, such as combining multiple swabs, targeting different oral sites, or integrating with other biomarkers.

However, further research is necessary to standardize oral swab protocols, optimize sample processing, and evaluate the diagnostic performance in diverse populations. The development of standardized guidelines for oral swab testing would facilitate comparisons across studies and promote the adoption of this promising diagnostic approach.

CONCLUSION

Oral swab testing for MTB using POC thermo-cycler, based on NAAT, offered a specific but moderately sensitive alternative to sputum-based diagnostics. Its non-invasive nature makes it an attractive adjunct in TB diagnostics, particularly in resource-limited settings or among patients unable to produce sputum. While not suitable as standalone diagnostic method, it may serve as a viable supplementary approach in specific clinical scenarios where sputum collection is difficult. Further studies involving larger cohorts, standardized swab collection protocols, and comparative study with other non-invasive specimens like saliva or nasopharyngeal aspirated are recommended to further validate and enhance the utility of oral swabs in diagnosis of TB. However, sputum testing remains the gold standard, and further validation is needed before oral swabs can replace sputum as a primary diagnostic specimen.

Ethical Considerations

This study was approved by Institutional Ethics Committee(IEC) of ESI-PGIMSR and hospital, Manicktala, Kolkata, 54, Bagmari Road. Memo No: ESIPGIMSR/MK/DNB/ADN/2023-24/038 dated 12.01.2024. Study participants provided written informed consent. All methods were carried out in accordance with approved guidelines and relevant regulations.

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Conflict of Interest: The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this study.

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