International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Utility of Oral Swab in the Diagnosis of Pulmonary Tuberculosis

Garima Kalla[1], Swapana Kanade[2], Gita Nataraj[3]

[1]Department of Microbiology, 3rd Floor, Sahyadri Hospitals Corporate Office, Pune 411038. Maharashtra, India
Email: *garimakalla2[at]gmail.com*

[2] Department of Microbiology, Seth G. S. Medical College and KEM Hospital, Mumbai 400012. Maharashtra, India
Email: *swapnakanade71[at]gmail.com*

[3]Department of Microbiology, Seth G. S. Medical College and KEM Hospital, Mumbai 400012. Maharashtra, India
Email: *gitanataraj[at]gmail.com*

Abstract: ***Design and setting: Results of MGIT performed on oral swab within one year were compared with results of Xpert MTB/RIF assay done on sputum in Department of Microbiology in a tertiary care centre. Objective: To determine usefulness of oral swab as a specimen in diagnosis of pulmonary tuberculosis. Results: Of the total 110 patients tested for tuberculosis, MTB was detected in 31 specimen (28.1%) by any one or more tests. Sputum Xpert assay was positive in 30 patients and oral swab culture was positive in 21. Oral swab microscopy was positive in 11. Concordant results between all the above tests were observed in 85 specimens, i. e., six specimens were positive by all tests and 79 were negative. Sensitivity and specificity of oral swab Amicroscopy and culture in comparison to Xpert assay was 36.66% and 100% and 66.66% and 98.75% respectively. (P = 0.0117). Oral swab culture detected MTB in one additional patient whose sputum Xpert assay was negative. Median time for culture positivity was 24 days. Conclusion: Patients with active pulmonary TB has viable M. tuberculosis organisms on oral mucosa which can be detected by liquid culture. Oral swab culture may be helpful in patients who are not able to produce appropriate sputum sample.***

Keywords: Oral swab, Pulmonary TB, MGIT

1. Introduction

Tuberculosis (TB) is one of the top 10 causes of death worldwide and the leading cause of death from a single infectious agent (ranking above HIV/AIDS) (1). About a quarter of the world's population is infected with *M. tuberculosis* and thus at risk of developing TB disease (1). India has the world's highest incidence of TB, with 2.8 million cases annually, and accounts for more than a quarter of the global TB burden. India also has the largest burden of multi - drug - resistant TB (MDR - TB) among all countries, with almost 150, 000 cases every year (2) .

M. tuberculosis primarily affects the lungs (pulmonary TB) but can also affect other sites (extra pulmonary TB). With a timely diagnosis and appropriate treatment, most people who develop TB can be cured and onward transmission of infection curtailed.

Various laboratory tests available for diagnosis of tuberculosis is microscopy, culture and Xpert assay. For PTB microscopy has a sensitivity of 40 - 80% and specificity of 100% in diagnosis of tuberculosis. Xpert assay (Cepheid, Sunnyvale, CA, USA) is a cartridge based nucleic acid amplification [CBNAAT] test using automated hemi - nested real time PCR and molecular beacon technology (8). It simultaneously detects presence of MTB as well as rifampicin resistance (8) . It is not standardized for oral swab and saliva. Although mycobacterial culture is the gold standard and is the most specific test for diagnosis, it can be done on any specimen and provide the results in 4 - 8 weeks. Also, it detects the presence of only viable bacilli while the above mentioned tests are unable to differentiate between viable and nonviable bacilli (8).

Hence this study was undertaken to determine usefulness of oral swab as a specimen in diagnosis of pulmonary tuberculosis and to determine sensitivity and specificity of microscopy and culture for oral swabs in comparison with sputum Xpert assay.

2. Literature Survey

Previous studies have shown that M. tuberculosis DNA can be detected in oral swabs from human and non - human primates (4, 5) . Mycobacterium cells, like most bacteria, have evolved mechanisms to adhere to surfaces, including mammalian cells (6) . Some bacilli that pass through the oral cavity with sputum of TB patients might accumulate on the oral epithelium and be detectable by analysis of oral swab samples (7) . This property of mycobacterium can be utilized by detecting their presence in oral swab for early detection of PTB.

Problem definition:
Collection of sputum specimen is difficult in a number of patients like pediatric and geriatric population, unconscious patients and others who are unable to produce sputum. In such patients, early detection gets compromised and more invasive procedures are required for specimen collection, leading to increased risk of transmission and delayed treatment initiation. Sputum as a diagnostic sample has some limiting factors like the viscosity of the sputum restricts test sensitivity, increases sample - to - sample hetero - genesity, and increases costs and labor associated with testing (3) . Moreover, sputum production (which requires coughing) in a healthcare setting maybe an occupational hazard for healthcare workers (3) . A sample that is easier, safer, and

more uniform to collect and handle would simplify TB diagnosis.

3. Methodology Approach

It is a prospective cross - sectional study done in a tertiary care hospital during a period of 1 year (Jan to Dec 2020) after Institutional ethics committee permission (approval no: EC (I) /4093/18) was obtained. Clinically suspected cases of pulmonary tuberculosis of any age and gender, who visit the lab to submit sputum specimen for Xpert assay and provided informed consent were included. Patients already on anti - tuberculosis treatment were excluded from the study.

Consecutive 110 patients fulfilling the inclusion criteria were included in the study. A sterile flocked swab was collected by brushing along the inside of the subject's oral cavity starting from right cheek, tongue and left cheek for about 10 seconds to collect cellular material. Same procedure was repeated to collect a second swab. Each swab was put into a tube containing 2 ml of sterile saline and labeled as A and B. Swab 'B' was used for microscopy and swab 'A' was used for culture after digestion and decontamination. Patient were also asked to submit approximately 1 ml of saliva in each of the two sterile containers which were used for microscopy and culture after digestion and decontamination.

All processing was carried out in Biological Safety Cabinet (BSC) Type II. Biosafety level II practices were followed. Each patient submitted sputum, oral swab and saliva specimen. Xpert assay was performed on sputum specimen. Direct smear was prepared from oral swab B and saliva and stained by fluorescent method as per NTEP guidelines (9) . Liquid culture using MGIT 960 was performed on oral swab B and saliva (10–12) . Growth of MTBC was confirmed by microscopy and MPT64 detection test. Results of sputum Xpert assay was considered as gold standard for the present study. Xpert assay was performed only on sputum (8, 13) .

4. Results

Of the 110 patients enrolled, 65 (59%) were male and 45 (41%) were female. Age of the enrolled patients ranged from 10 to 62 years [Mean= 35.4 years]. Of the total 110 sputum specimens tested by Xpert assay, four showed 'ERROR' result. The assay was repeated on leftover specimen, which gave a valid result. Xpert assay results of all 110 specimens were considered for analysis. Liquid culture using MGIT was done on 110 oral swabs and 110 saliva specimens. Out of these 15 specimens (6.8%) showed contamination. These were re - decontaminated and inoculated into MGIT again, yielding either positive or negative valid results. Fluorescent microscopy was performed on each of the 110 sputum, oral swabs and saliva specimens. Of the total 110 patients tested for tuberculosis, MTB was detected in 31 specimen (28.1%) by any one or more tests. Sputum Xpert assay was positive in 30 patients, oral swab culture was positive in 21 and saliva culture was positive in 6 patients. Oral swab microscopy was positive in 11 and saliva microscopy was positive in 4 patients. Concordant results between all the above tests were observed in 85 specimens, i. e., six specimens were positive by all tests and 79 were negative. Sputum Xpert assay was considered as gold standard and result of microscopy / culture of oral swab/ saliva was compared with it. Sensitivity and specificity of oral swab and saliva microscopy and culture in comparison to Xpert assay was 36.66% and 100% and 66.66% and 98.75%, 13.33% and 100% and 205 and 100% respectively.

Table 1: Comparison of different test results with sputum Xpert assay

	Sputum Xpert assay - Positive	**Sputum Xpert assay - Negative**	**Total**
Oral swab culture positive	20	1	21
Oral swab culture negative	10	79	89
Total	**30**	**80**	**110**
Saliva culture positive	6	0	6
Saliva culture negative	24	80	104
Total	**30**	**80**	**110**
Oral swab microscopy positive	11	0	11
Oral swab microscopy negative	19	80	99
Total	**30**	**80**	**110**
Saliva microscopy positive	4	0	4
Saliva microscopy negative	26	80	106
Total	**30**	**80**	**110**

Table 2: Comparison of the results of different diagnostic modalities (n=110)

Sputum Xpert Assay	**Oral swab culture**	**Saliva culture**	**Total**
POS	**POS**	**POS**	6
POS	**POS**	NEG	14
POS	NEG	POS	0
POS	NEG	NEG	10
NEG	**POS**	NEG	1
NEG	**POS**	**POS**	0
NEG	NEG	**POS**	0
NEG	NEG	NEG	79
TOTAL			**110**

Oral swab culture detected MTB in one additional patient whose sputum Xpert assay was negative. The sensitivity and specificity of oral swab culture as compared to sputum Xpert assay was 66.66% and 98.75% respectively. (P = 0.0117). Median time for culture positivity was 24 days. The sensitivity and specificity of oral swab microscopy as compared to sputum Xpert assay was 36.66% and 100% respectively (P <0.0001).

The sensitivity and specificity of saliva culture as compared to sputum Xpert assay was 20% and 100% respectively. (P < 0.0001). Median time for culture positivity was 28 days. The sensitivity and specificity of saliva microscopy as compared

to sputum Xpert assay was 13.33% and 100% respectively (P < 0.0001).

5. Discussion

Rapid, early and accurate case detection is the cornerstone of tuberculosis control. Microbiological diagnosis provides etiological confirmation as well as gives information about drug resistance. Pulmonary TB, the predominant type of tuberculosis, is diagnosed using expectorated sputum. However, diagnosis remains challenging in pediatric, geriatric and unconscious patients who are unable to expectorate sputum. In such patients an easy and non - invasive method of sample collection would benefit early diagnosis.

In the present study, MTB was detected in 31 (28.1%) patients by any one or more tests/specimen. Maximum numbers of MTB positive cases were detected in the age group 21 - 30 years followed by 31 - 40 years. MTB was detected more in males than in females with prevalence of 33.3% and 20% respectively.

6. Conclusion

Patients with active pulmonary TB has viable M. tuberculosis organisms on oral mucosa which can be detected by liquid culture. Oral swab culture may be helpful in patients who are not able to produce appropriate sputum sample. Role of oral swab in diagnosis of pulmonary tuberculosis by Xpert assay need to be evaluated.

7. Future Scope

To achieve the goal of TB elimination by 2025, there is a need to increase detection of all presumptive TB cases. One of the strategies used is active case finding by doing house to house survey. Many people find it difficult to produce sputum. It is for these situations that easy to collect, non - invasive sputum alternatives are needed (25). Oral swab testing is a very useful tool in such cases. Further development in type and quality of oral swabs may benefit in detection of positive cases. Oral swab has a great benefit in situations that are limited by the drawbacks of sputum collections. As a diagnostic sample, swabs differ from sputum. Compared to sputum swabs have some advantages such as ease of collection and less viscous. A great disadvantage could be that swabs may have fewer bacilli. In addition to passive case finding, oral swab could enable more active TB case finding among individuals who do not produce sputum.

References

[1] Global tuberculosis report 2019 [Internet]. [cited 2020 Sep 25]. Available from: https: //www.who. int/publications - detail - redirect/global - tuberculosis - report - 2019
[2] Tuberculosis in India [Internet].2019 [cited 2020 Sep 25]. Available from: https: //www.usaid. gov/global - health/health - areas/tuberculosis/technical - areas/tuberculosis - india
[3] Collins CH, Grange JM. Tuberculosis acquired in laboratories and necropsy rooms. Commun Dis Public Health.1999 Sep; 2 (3): 161–7.
[4] Wilbur A, Engel G, Rompis A, Putra IA, Lee BYH, Aggimarangsee N, et al. From the mouths of monkeys: Detection of Mycobacterium tuberculosis complex DNA from buccal swabs of synanthropic macaques. Am J Primatol.2012 Jul; 74 (7): 676–86.
[5] Engel GA, Wilbur AK, Westmark A, Horn D, Johnson J, Jones - Engel L. Naturally acquired Mycobacterium tuberculosis complex in laboratory pig - tailed macaques. Emerg Microbes Infect.2012 Oct; 1 (10): e30.
[6] Yamazaki Y, Danelishvili L, Wu M, Hidaka E, Katsuyama T, Stang B, et al. The ability to form biofilm influences Mycobacterium avium invasion and translocation of bronchial epithelial cells. Cell Microbiol.2006 May; 8 (5): 806–14.
[7] Wood RC, Luabeya AK, Weigel KM, Wilbur AK, Jones - Engel L, Hatherill M, et al. Detection of Mycobacterium tuberculosis DNA on the oral mucosa of tuberculosis patients. Sci Rep.2015 Mar 2; 5: 8668.
[8] Cepheid | MTB/RIF Molecular Test - Xpert MTB/RIF Ultra [Internet]. [cited 2020 Nov 23]. Available from: https: //www.cepheid. com/en/tests/Critical - Infectious - Diseases/Xpert - MTB - RIF - Ultra
[9] 7293794058standard operating procedures for C&DST labs. pdf [Internet]. [cited 2020 Oct 31]. Available from: https: //tbcindia. gov. in/WriteReadData/l892s/7293794058standard%20operating%20procedures%20for%20C&DST%20labs. pdf
[10] Koneman's color atlas and textbook of diagnostic microbiology (Book, 2017) [WorldCat. org] [Internet]. [cited 2020 Sep 27]. Available from: https: //www.worldcat. org/title/konemans - color - atlas - and - textbook - of - diagnostic - microbiology/oclc/876002916
[11] tuberculosis2007. pdf [Internet]. [cited 2020 Nov 22]. Available from: http: //pdf. flyingpublisher. com/tuberculosis2007. pdf
[12] Caulfield AJ, Wengenack NL. Diagnosis of active tuberculosis disease: From microscopy to molecular techniques. Journal of Clinical Tuberculosis and Other Mycobacterial Diseases.2016 Aug 1; 4: 33–43.
[13] Inglés_rev_c_version_4_gx_dx_operator_manual_jun12. pdf [Internet]. [cited 2020 Nov 23]. Available from: https: //www.ghdonline. org/uploads/Ingl%C3%A9s_rev_c_version_4_gx_dx_operator_manual_jun12. pdf
[14] Luabeya AK, Wood RC, Shenje J, Filander E, Ontong C, Mabwe S, et al. Noninvasive Detection of Tuberculosis by Oral Swab Analysis. Journal of Clinical Microbiology [Internet].2019 Mar 1 [cited 2020 Sep 9]; 57 (3). Available from: https: //jcm. asm. org/content/57/3/e01847 - 18
[15] Microbiological diagnosis of pulmonary tuberculosis in children by oral swab polymerase chain reaction | Scientific Reports [Internet]. [cited 2021 Mar 10]. Available from: https: //www.nature. com/articles/s41598 - 019 - 47302 - 5
[16] Wood RC, Luabeya AK, Weigel KM, Wilbur AK, Jones - Engel L, Hatherill M, et al. Detection of

Mycobacterium tuberculosis DNA on the oral mucosa of tuberculosis patients. Sci Rep.2015 Mar 2; 5: 8668.
[17] Silva CAM, Danelishvili L, McNamara M, Berredo - Pinho M, Bildfell R, Biet F, et al. Interaction of Mycobacterium leprae with human airway epithelial cells: adherence, entry, survival, and identification of potential adhesins by surface proteome analysis. Infect Immun.2013 Jul; 81 (7): 2645–59.
[18] Geier H, Mostowy S, Cangelosi GA, Behr MA, Ford TE. Autoinducer - 2 triggers the oxidative stress response in Mycobacterium avium, leading to biofilm formation. Appl Environ Microbiol.2008 Mar; 74 (6): 1798–804.
[19] Freeman R, Geier H, Weigel KM, Do J, Ford TE, Cangelosi GA. Roles for cell wall glycopeptidolipid in surface adherence and planktonic dispersal of Mycobacterium avium. Appl Environ Microbiol.2006 Dec; 72 (12): 7554–8.
[20] Zhang CZ, Cheng XQ, Li JY, Zhang P, Yi P, Xu X, et al. Saliva in the diagnosis of diseases. Int J Oral Sci.2016 Sep 29; 8 (3): 133–7.
[21] Salvatori O, Puri S, Tati S, Edgerton M. Innate Immunity and Saliva in Candida albicans - mediated Oral Diseases. J Dent Res.2016 Apr; 95 (4): 365–71.
[22] Shenai S, Amisano D, Ronacher K, Kriel M, Banada PP, Song T, et al. Exploring Alternative Biomaterials for Diagnosis of Pulmonary Tuberculosis in HIV - Negative Patients by Use of the GeneXpert MTB/RIF Assay. Journal of Clinical Microbiology.2013 Dec 1; 51 (12): 4161–6.
[23] PCR method is essential for detecting Mycobacterium tuberculosis in oral cavity samples - Eguchi - 2003 - Oral Microbiology and Immunology - Wiley Online Library [Internet]. [cited 2021 Mar 12]. Available from: https: //onlinelibrary. wiley. com/doi/abs/10.1034/j.1399 - 302X.2003.00050. x
[24] Kumar P, Pandya D, Singh N, Behera D, Aggarwal P, Singh S. Loop - mediated isothermal amplification assay for rapid and sensitive diagnosis of tuberculosis. J Infect.2014 Dec; 69 (6): 607–15.
[25] Fauci AS, Eisinger RW. Reimagining the Research Approach to Tuberculosis. The American Journal of Tropical Medicine and Hygiene.2018 Jan 22; 98 (3): 650–2.

Author Profile

Dr. Garima Kalla at present Registrar at Department of Microbiology in Sahyadri Specialty Hospitals Pune, Maharashtra, India, did MD Microbiology from KEM hospital and GS Medical college, Mumbai 2018 batch and did MBBS from D. Y Patil Navi Mumbai 2010 batch.