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Research Report

The role of *Mycobacterium Tuberculosis* detection on oral mucosa in diagnosis of pulmonary tuberculosis

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ABSTRACT

Background: Tuberculosis is one of the world's health problems, with larger mortality rate than HIV/AIDS. In order to control this disease, a new and inexpensive diagnostic method is needed. Oral samples, such as buccal mucosa epithelium, can be an alternative specimen, other than sputum, in the diagnosis of tuberculosis. **Purpose:** This study aimed to analyze the presence of Mycobacterium tuberculosis on buccal swabbing of tuberculosis patients. **Methods:** Study was conducted on 18 new pulmonary tuberculosis patients in TB DOTS unit of Dr. Soetomo General Hospital. Each subject was swabbed on the buccal mucosa of the oral cavity for subsequent examination of PCR, AFB, and culture to detect Mycobacterium tuberculosis in the oral cavity. **Results:** The results showed that Mycobacterium tuberculosis was detected from 2 study subjects (11%) using PCR, whereas in AFB and culture examination methods no Mycobacterium tuberculosis was detected on buccal mucosa of the oral cavity. **Conclusion:** This study showed that Mycobacterium tuberculosis was detected on buccal mucosa swab of tuberculosis patients based on PCR technique, but this method is less suitable in diagnosis of pulmonary tuberculosis.

Keywords: Oral; Mycobacterium tuberculosis; examination; swab

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INTRODUCTION

Tuberculosis (TB) is an infectious disease that is one of the world's health problems with large effects on mortality. The COVID-19 pandemic has a notable impact on access to TB diagnosis and treatment and the burden of TB disease. Large global drops in newly diagnosed TB patients were reported in 2020, from 7.1 million in 2019 became 5.8 million., then its number was partially recovered became 6.4 million in 2021. The reduction number of people diagnosed with TB was correlated with undiagnosed and untreated TB which has grown then simultaneously increased the number of TB deaths and infection transmission. India, Indonesia, and Philipines were three countries with the most reduction[1]. TB control measures in the form of the directly observed treatment short course (DOTS) strategy have been implemented in many countries, also Indonesia, since 1995. One of the main pillars of the strategy is integrating patient-centered TB services and prevention efforts, including early diagnosis[2].

The tuberculosis diagnostic method currently used for 15 years of implementation and expansion of the DOTS strategy program is acid-fast bacilli (AFB) in direct smear sputum. This bacteriological examination identifies the presence of *Mycobacterium tuberculosis*, but this test only has a sensitivity ranging from 20 to 65%[3–5]. It shows that a new, simple, inexpensive diagnostic method for tuberculosis case detection is still needed. Increased early detection of TB will reduce morbidity, mortality, and infection transmission risk.

Several studies have been conducted to obtain alternative specimens other than sputum in diagnosing TB since high-quality sputum as a primary specimen is not easily collected, especially from children and HIV/AIDS patients. Cultural factors could affect the ability to collect good sputum sample. Its viscosity also affects the sensitivity, by increasing the heterogenity of sample, cost, and worker in examination[6]. Those samples tried as alternative specimens in TB diagnosis include exhaled breath condensate (EBC), blood, urine, and oral samples, including mouth rinse, saliva, dental plaque, and oral buccal mucosal epithelium[7]. The buccal mucosal swab tends to have uniform volume and composition, allowing a simpler and cheaper diagnostic approach, particularly as a point-of-care check application.

Detection of *M. tuberculosis* in the oral cavity is associated with TB characteristics that may also lead to manifestations in the oral cavity, either as primary or

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secondary tuberculosis, commonly called oral tuberculosis, ranging from 0.05 to 5% of total cases of patients tuberculosis[8]. This study aimed to analyse the presence of *Mycobacterium tuberculosis* in swabbing oral mucosa of the oral cavity of tuberculosis patients based on polymerase chain reaction (PCR) technique.

MATERIALS AND METHODS

This study was held in TB DOTS outpatient unit of Dr. Soetomo General Hospital on July 2017– January 2018. Ethical clearance of this study was issued by Dr. Soetomo General Hospital Ethical Committee No. 446/Panke.KKE/VII/2017. A total 18 subjects consented to participate in this study, and their age ranged between 18 – 66 years old. The participants consisted of 10 males and 8 females. TB patients were diagnosed by pulmonologist at the pulmonary outpatient unit of Dr. Soetomo General Hospital using clinical symptoms assessment and supporting examination, including bacteriological and radiographic examination. All subjects were patients who just had a positive pulmonary tuberculosis diagnosis (no more than 7 days of diagnosis) and haven't received any antituberculosis treatment. Each subject received sampling on the oral mucosa of the oral cavity for subsequent examination of PCR, AFB, and culture, to prove detection of *Mycobacterium tuberculosis* in the oral cavity.

Swab specimens were collected from the buccal mucosa of subjects using three different cytobrush sticks immediately after a positive diagnosis, and under standardised conditions, at least after 1-hour eating and oral hygiene procedures. The swabs were brushed along the inside of the subject's cheek for about 10 seconds (7–8 times) to collect the specimen in uniform volume and composition. Each swab was then distributed to glass slides for direct smear, and put into two conical tubes, each tube containing Middlebrook 7H9 (Fluka, Sigma-aldrich, Swiss) and phosphate saline buffer.

Swabs of buccal mucosa were obtained and then smeared onto the glass objects that had been labelled. The slides were fixed by heating above flame from a Bunsen burner three to five times for 4 seconds. The slides were poured Carbol fuchsin 3% on the entire surface, then heated on the flame until the smoke appeared but not boiling or drying, for 5 minutes. The slides were allowed to cool for 5 to 7 minutes and longer. The slides were flooded with 3% alcoholic acid solution (hydrochloric acid - ethanol), and left for 2 to 4 minutes. The acid-alcohol was washed off with distilled water and the slides were tilted to drain. The slides were flooded with methylene blue, stand for 1-2 minute. The methylene blue was washed off with distilled water and the slides tilted to drain[9]. The slides were then examined under microscope with 1000x for the presence of AFB.

Deep swabbing that was put in a sterile conical tube containing approximately 3 mL of melting liquid Media Middlebrook 7H9 then incubated in a CO_2 incubator for 1 week. The samples were then decontaminated prior to culture examination. The initial phase of decontamination was centrifugating samples at 4000 rpm for 15 minutes. The supernatant was then discarded, and 4% NaOH was added at a ratio of 1: 2. The sample was then vortexed back and left in room temperature for 10 minutes. Samples were recentrifugated at 4000 rpm for 15 minutes. The sample was left for 10 minutes and the supernatant was discarded. The washing was then done twice, by adding 10 ml of distilled water. Centrifugation at 4000 rpm for 15 minutes was performed once more, and the sample was left at room temperature for 10 minutes to wait for the aerosol to fall. Once the decontamination was done, supernatant was discarded, and the samples could be inoculated onto Middlebrok 7H10 agar (Fluka, Sigma-aldrich, Swiss), then incubated at 37° C, 10% CO_2 dan 90% air atmosphere. Bacterial colony was observed once a week in 3 – 5 weeks. Identification was done by Ziehl Neelsen (Karya Husada, BBLK Surabaya, Indonesia) staining and niacin test [9].

Polymerase chain reaction technique was initiated by DNA isolation using DNeasy kit (QIAGEN, Germany). Amplification DNA region of *gyrB* gene *Mycobacterium tuberculosis* was conducted for gene target 500-bp of *gyrB*. The suspension of PCR mix Dream Taq Green PCR Master Mix (Fermentas, ThermoFisher, California) was added by primer target 500-bp region as is MTUB-f and MTUB-r each 1 1µl, and DNA template 5µl, reaction volume 25µl. Amplification reaction is 94° C 2 minutes; 94° C 20 seconds; 54.3° C for 10 seconds; 72° C for 30 seconds; and 72° C for 5 minutes; and the reaction 35 cycles. *Mycobacterium tuberculosis* H37Rv was used as a positive control, whereas aqua dest was used as a negative control. Examination results in data of the buccal swab were compared to the sputum smear examination data from the medical record. Measuring agreement of new technique with the established diagnostic method performed by calculating concordance, subsequently analysed using Cohen's Kappa with value reference in Table 1.

Tabel 1. Categorization of Cohen's Kappa value[10]

K value	Quality of agreement
<0.20	Poor
0.21 – 0.40	Fairy
0.41 – 0.60	Moderate
0.61 – 0.80	Good
0.81 – 1.0	Very good

RESULTS

Based on medical record, among 18 subjects, there were 6 patients with a negative result of AFB sputum smear examination. Twelve subjects showed positive result of AFB presence on sputum specimens. Thorax radiography results were available for 9 of 18 subjects (50%), of which 6 (67%) results showed lungs fibroinfiltrate and one (11%) showed pulmonary TB with tuberculoma. Four patients had type 2 Diabetes Mellitus as comorbid disease, but

Table 2. Concordance of three methods of buccal swabs relative to sputum smear examination

Swab Result	No. of patients with AFB on sputum result		Concordance (%) = True Positive + True Negative	
	Positive	Negative	True Positive	True Negative
AFB			0%	33.3%
Positive	0	0		
Negative	12	6		
Culture			0%	33.3%
Positive	0	0		
Negative	12	6		
PCR			5.5%	27.8%
Positive	1	1		
Negative	11	5		

the HIV status of all subjects was unknown. The results showed no *Mycobacterium tuberculosis* was detected on buccal mucosa using Ziehl neelsen staining, but 2 samples (11%) showed 500bp white band as positive control, considered as *Mycobacterium tuberculosis* DNA detection on buccal swab using PCR. Culture examination did not show any *Mycobacterium tuberculosis* growth either. The concordance percentage of three examination methods of buccal swabs relative to sputum smear examination valued same, that was 33% (Table 2). Cohen's Kappa calculation resulted 0, 0, and -0.06 for each AFB, culture, and PCR examination.

DISCUSSION

TB, as an infectious disease, is transmitted by droplet nuclei. TB primarily occurs on the lungs, but it also affects other organs, such as joints, meninges, bones, and oral cavity, and is called as extrapulmonary tuberculosis. Tuberculosis in the oral cavity is known as oral tuberculosis, but its occurrence is infrequent, about 0.5 – 1% worldwide [11].

Kakisi suggested that oral tuberculosis lesions were mostly found on the buccal mucosa or vestibulum oris of head and neck tuberculosis patients from 1991 to 2007. Primary oral tuberculosis could be developed, although less common, and predominantly occurs on gingiva or buccal mucosa[12]. Other intraoral sites that could be affected include salivary glands, tonsils, uvulae, and mandibular ridges[13].

Naturally, infectious agents in sputum are carried away into the oral cavity while coughing. The bacilli then deposited on saliva, lip, buccal, and gingival mucosa.[14] They could also reside on gingival pockets since it is a favourable site for microorganism growth and has low oxygen pressure[12]. Deposited *Mycobacterium tuberculosis* on the mucosal surface then multiplies and result destruction of the mucosa as oral tuberculosis development [15].

Some previous studies suggested that *Mycobacterium tuberculosis* had been detected in oral cavity of pulmonary tuberculosis patients. A study that was performed by Eguchi (2003) showed that *Mycobacterium tuberculosis* DNA had presented on caries lesions, dental plaque, saliva, and denture plaque, with sensitivity ranging from 89% - 100%, whereas the culture of these samples showed a sensitivity of about 0 – 17.3%[16]. Davis (2009) suggested that among 37 patients with negative sputum smears, the sensitivity of Mtb DNA detection of oral wash sample was 63%, and the sensitivity of DNA detection of sputum sample was 100%. At the same time, among 63 smear-positive results, the sensitivity of *Mycobacterium tuberculosis* DNA detection of oral wash sample was 80%, and the sensitivity of DNA detection was 98%[17]. Another study stated that *Mycobacterium tuberculosis* DNA was detected on 92% and 68% of saliva and dental plaque samples[18]. Other study conducted by Wood suggested that *Mycobacterium tuberculosis* DNA was detected on buccal swabs of 18 from 20 pulmonary tuberculosis patients[19].

Oral samples, such as saliva and swabs, are considered a sample which has an easy, simple and non-invasive procedure to collect with minimal discomfort. They also could be obtained in smaller and more uniform volumes and compositions. Consideration of oral mucosa swab selection was because naturally, *Mycobacterium* species tends to be associated with surfaces than fluid matrices [19,20].

In this study, there were two subjects with *Mycobacterium tuberculosis* findings on buccal mucosal swabs based on the PCR method. On the other hand, both direct smear staining and culture examination could not detect any AFB on the buccal mucosa swabs of 18 subjects. This indicates that *Mycobacterium tuberculosis* can be found in the oral cavity of tuberculosis patients, but the adequate local defence of the oral cavity results in the rare occurrence of oral tuberculosis. They include antibacterial function against *Mycobacterium tuberculosis* of oral normal flora growth and components of saliva. On the other hand, continuous salivary flows mechanically prevent attachment of both living and fragments of bacilli on the oral mucosa. Besides, continuous oral mucosa epithelial regeneration prevents these microorganisms either[21].

Regarding to normal oral flora, the presence of *Mycobacterium tuberculosis* in oral cavity also could be associated with the oral hygiene status of the subjects. A study suggested that there were microbiome differences between the saliva of individuals with active caries and without caries. The microbiome in the oral cavity of an individual with active caries is more varied than in a healthy one. However, there was no specific microorganism in the oral cavity of active caries individuals that would not be able to detect in healthy individuals, and vice versa. *Prevotella* spp is mostly found in both groups, but there was

a difference between its species distribution [3]. Some bacteria such as *A. naeslundii*, *P. gingivalis*, and *F. nucleatum* suggested inhibiting *M. tuberculosis in* the oral cavity [16].

The supporting examination performed to determine tuberculosis diagnosis in this study was a thorax radiography examination. One subject with a positive result on Mtb DNA detection showed tuberculoma, multiple non-suspicious lymph nodes supraclavicular, paraaortic, prevascular, upper paratracheal, lower para trachea, subcarinal, peribronchial and paracardial fibro infiltrate. Tuberkuloma is a rare manifestation of tuberculosis. Determining the diagnosis of tuberculoma is challenging since the invasive examination is necessary and sometimes accompanied by malignancies in some cases. Tuberculoma tends to be stable, but once there is impaired body resistance, it could be damaged, resulting in tuberculosis dissemination.[22]

Another subject showed paracardial fibro infiltrate, which is located in the lower lungs. The subject was a diabetes mellitus patient, and Singh (2011) stated that the thorax radiography of a pulmonary tuberculosis patient was affected by some factors, such as disease duration and immunity status. Pulmonary tuberculosis with diabetes mellitus tends to affect the lower lung than those without diabetes mellitus which affect the upper lung, and diabetes mellitus is considered to be susceptibility to tuberculosis infection[23].

Mycobacterium tuberculosis detection role was assessed by concordance analysis relative to sputum direct smear examination. This diagnostic method currently used for 15 years of implementation and expansion of the DOTS TB strategy program, but this test has a sensitivity ranging from 20 to 65% [3,4]. Three examination methods of buccal swab samples resulted in same concordance value, which was 33%. Concordance analysis was performed by Cohen's Kappa calculation relative to sputum direct smear examination, Cohen's Kappa is representative to quality of agreement of two compared different method, ranged from -1 to 1, and resulted -0.06, 0, and 0 for each PCR, AFB, and culture examination. In this study, Cohen's Kappa value of these three methods against the sputum direct smear examination results are in the poor category, so it can be mentioned that the role of *Mycobacterium tuberculosis* detection in the buccal mucosal swab of tuberculosis patients is a less suitable method as the diagnostic approach of pulmonary tuberculosis. This result was supported by Caulfield (2016) that swab tend to give poor result because only transfer sample in small volume.[24] Other study focused on oral tuberculosis lesion suggested that histopathology examination is necessary in definitive diagnosis determination since there might be bias result if the microorganism is not originate from the lesion.[11]

However, this study showed that the PCR method could detect *Mycobacterium tuberculosis* in the oral cavity, although rarely. Some patients infected with tuberculosis may come to the dentist for oral health care but do not know the signs and symptoms of tuberculosis in the individual patient, so there is the possibility of transmission of *Mycobacterium tuberculosis* infection through aerosols produced by dental instruments. This method might be used to screen microorganisms in the oral cavity as part of the prophylactic action of transmission of tuberculosis infection in dental and oral health services. On the other hand, oral health care workers should still be careful and always use standard precautions in performing dental and oral health care.

REFERENCES

1. Organization WH. Global Tuberculosis Report 2022. Geneva; 2022.
2. Perhimpunan Dokter Paru Indonesia. Tuberkulosis Pedoman Diagnosis Dan Penatalaksanaan Di Indonesia. Vol 001.; 2021.
3. Yang F, Zeng X, Ning K, et al. Saliva microbiomes distinguish caries-active from healthy human populations. ISME J. 2012;6:1-10. doi:10.1038/ismej.2011.71
4. Leung E, Minion J, Benedetti A, Pai M, Menzies D. Microcolony culture techniques for tuberculosis diagnosis: a systematic review. Int J Tuberc Lung Dis. 2012;16(1):16-23. doi:10.5588/ijtld.10.0065
5. Chang K, Lu W, Wang J, et al. Rapid and effective diagnosis of tuberculosis and rifampicin resistance with Xpert MTB / RIF assay : A meta-analysis. J Infect. 2012;(March):1-9. doi:10.1016/j.jinf.2012.02.012
6. Luabeya AK, Wood RC, Shenje J, et al. Noninvasive detection of tuberculosis by oral swab analysis. J Clin Microbiol. 2019;57(3). doi:10.1128/JCM.01847-18
7. Mesman AW, Calderon R, Soto M, et al. Mycobacterium tuberculosis detection from oral swabs with Xpert MTB/RIF ULTRA: A pilot study. BMC Res Notes. 2019;12(1):10-12. doi:10.1186/s13104-019-4385-y
8. Bayraktar K, Gürer G. Pulmonary tuberculosis presenting with oral aphthae. Eur J Rheumathology. 2015:1-3. doi:10.5152/eurjrheum.2015.0098
9. Stinson KW, Eisenach K, Kayes S, et al. Mycobacteriology Laboratory Manual Editor ,1st Ed. japan: Global Laboratory Initiative; 2014.
10. Kwiecien R, Kopp-schneider A, Blettner M. Concordance Analysis. Dtsch Arztebl Int. 2011;108(30):515-521. doi:10.3238/arztebl.2011.0515
11. Kim SY, Byun JS, Choi JK, Jung JK. A case report of a tongue ulcer presented as the first sign of occult tuberculosis. BMC Oral Health. 2019;19(1):1-5. doi:10.1186/s12903-019-0764-y
12. Krawiecka E, Szponar E. Tuberculosis of the oral cavity: an uncommon but still a live issue. Adv Dermatology Allergol. 2015;32(4):302-306. doi:10.5114/pdia.2014.43284
13. Mishra AK, Kant S, Verma L, et al. Coexistence of carcinoma cheek with tuberculosis : a rarity. Int J Res Med Sci. 2015;3(3):780-782. doi:10.5455/2320-6012.ijrms20150349
14. Porteous NB, Terézhalmy GT. Exposure Control Issues for Oral Healthcare Workers. Contin Educ Course. 2013:1-24.
15. Jain P, Jain I. Oral manifestations of tuberculosis: Step towards early diagnosis. J Clin Diagnostic Res. 2014;8(12):ZE18-ZE21. doi:10.7860/JCDR/2014/10080.5281
16. Eguchi J, Ishihara K, Watanabe A, Fukumoto Y, Okuda K. PCR method is essential for detecting Mycobacterium tuberculosis in oral cavity samples. Oral Microbiol Immunol. 2003;18(25):156-159.
17. Davis JL, Huang L, Kovacs JA, et al. Polymerase Chain

Reaction of secA1 on Sputum or Oral Wash Samples for the Diagnosis of Pulmonary Tuberculosis. Clin Infect Dis. 2009;94110(48):725-732. doi:10.1086/597038
18. Palakuru SK, Lakshman VK, Bhat KG. Microbiological analysis of oral samples for detection of Mycobacterium tuberculosis by nested polymerase chain reaction in tuberculosis patients with periodontitis. Dent Res J (Isfahan). 2012;9(6):688-693.
19. Wood RC, Luabeya AK, Weigel KM, et al. Detection of Mycobacterium tuberculosis DNA on the oral mucosa of tuberculosis patients. Sci Rep. 2015:1-5. doi:10.1038/srep08668
20. Shenai S, Amisano D, Ronacher K, et al. Exploring Alternative Biomaterials for Diagnosis of Pulmonary Tuberculosis in HIV-Negative Patients by Use of the GeneXpert MTB / RIF Assay. J Clin Microbiol. 2013;51(12):4161-4166. doi:10.1128/JCM.01743-13
21. Gill JS, Sandhu S, Gill S. Primary tuberculosis masquerading as gingival enlargement. Nat Publ Gr. 2010;208(8):343-345. doi:10.1038/sj.bdj.2010.344
22. Yang S, Zhan P, Mao H, Shi X, Wang L. Perfusing chemotherapy by percutaneous lung puncture “ holing ” for pulmonary tuberculoma — a ten-year single center experience. J Thorac Dis. 2009;5(4):2013. doi:10.3978/j.issn.2072-1439.2013.07.03
23. Crevel R van, Koesoemadinata R, Hill PC, Harries AD. Clinical management of combined tuberculosis and diabetes. Int J Tuberc Lung Dis. 2018;22(12):1404-1410.
24. Caulfield AJ, Wengenack NL. Journal of Clinical Tuberculosis and Other Mycobacterial Diseases Diagnosis of active tuberculosis disease : From microscopy to molecular techniques. J Clin Tuberc Other Mycobact Dis. 2016;4:33-43. doi:10.1016/j.jctube.2016.05.005

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