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Research Report

Acid fast bacilli detected in the oral swab sample of a pulmonary tuberculosis patient

Reiska Kumala Bakti,[1] **Ni Made Mertaniasih,**[2] **Diah Savitri Ernawati,**[1] **Bagus Soebadi,**[1] and **Priyo Hadi**[1]
[1]Department of Oral Medicine, Faculty of Dental Medicine, Universitas Airlangga
[2]Department of Clinical Microbiology, Faculty of Medicine, Universitas Airlangga
Surabaya - Indonesia

ABSTRACT

Background: *Tuberculosis (TB) is an infectious disease that persists as a health problem worldwide. Mycobacterium tuberculosis, as an etiological agent, is transmitted from infected to uninfected individuals via airborne droplet nuclei. Oral health care workers or dental practitioners may be at high risk of TB infection because of their close proximity to infected individuals during treatment procedures. Simple and rapid screening of Mycobacterium tuberculosis in the oral cavity is necessary in order to prevent transmission of infection.* ***Purpose:*** *To investigate the presence of acid-fast bacilli in the buccal mucosa of pulmonary TB patients.* ***Methods:*** *Nineteen pulmonary TB patients of both sexes, ranging in age from 19 to 74 years old participated in this study. The diagnosis of tuberculosis was performed by clinical symptom assessment and supporting examination, including acid-fast bacilli on sputum examination. Two buccal mucosa swabs taken from pulmonary TB patients were collected for acid fast bacilli direct smear by Ziehl Neelsen staining.* ***Results:*** *With regard to Mycobacterium tuberculosis, acid-fast bacilli presented in 10.5% of the oral buccal mucosa swabs of subjects, whereas in the sputum specimens, bacilli were found in 52.6% of subjects.* ***Conclusion:*** *Acid-fast bacilli can be found in the buccal epithelial mucosa of pulmonary tuberculosis patients, although its presence was very limited.*

Keywords: *tuberculosis (TB); oral; buccal mucosa; acid fast bacilli; Mycobacterium tuberculosis*

Correspondence: Reiska Kumala Bakti, Department of Oral Medicine, Faculty of Dental Medicine, Universitas Airlangga, Jl. Mayjend. Prof. Dr. Moestopo no. 47, Surabaya 60132, Indonesia. E-mail: reiska91@gmail.com

INTRODUCTION

Tuberculosis (TB) is a disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) that remains a global health problem. WHO estimates *M. tubercolusis* in 2015, there were 10.4 million new cases of TB worldwide, resulting in the deaths of 1.8 million people. Indonesia, together with India and China, is one of three countries with the highest incidence rate of TB since 2014 and one of six countries accounting for 60% of new TB cases. Of these, China, India and Indonesia together accounted for 45% of global cases in 2015.[1,2]

Transmission of tuberculosis occurs from an infectious patient to other individuals via droplet nuclei that contain *Mycobacterium tuberculosis*.[3] Droplet nuclei are small particles, approximately 1–5 micrometers in diameter that can remain airborne for minutes to hours after expectoration through coughing, sneezing or talking by carriers of pulmonary TB.[4,5] In dental clinics, oral health care workers and dental practitioners may be at high risk of *Mycobacterium tuberculosis* infection because of their close proximity to patients producing infectious secretions and exposure to the aerosol used during the dental treatment process. The potential for transmission of *Mycobacterium tuberculosis* cannot be discounted since patients and oral health care workers share common air space. The probable transmission of *Mycobacterium tuberculosis* from infected patients to two oral health care workers has been documented, while evidence exists of TB transmission from an oral surgeon with active bilateral pulmonary tuberculosis to 15 patients post-extraction.[6]

Patients suffering from TB may visit dental clinics because of oral problems necessitating prevention of the transmission of infection. Simple and rapid screening for *Mycobacterium tuberculosis* in oral samples is required, given that this microorganism can be transmitted to others,

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both oral health care workers and fellow dental patients. Various oral samples could be used, such as mixed saliva, dental plaque, caries lesions, denture plaque, oral wash and buccal swabs. *Mycobacterium tuberculosis* might be found in the oral cavity since the bacilli is passed from the lungs and airways to the oral cavity of pulmonary TB patients and then accumulated on the buccal mucosa.[7–9] This study was aimed to investigate acid-fast bacilli detection on the buccal mucosa epithelium of pulmonary TB patients. An oral mucosa swab was selected because, naturally, *Mycobacterium* species are more commonly associated with surfaces than with fluid matrices.[8]

MATERIALS AND METHODS

This study was conducted at Universitas Airlangga Hospital from September to October 2016 and at Dr. Soetomo General Hospital between July and October 2017. A total of 19 subjects participated, ranging in age between 19 and 74 years old. The participants consisted of ten males and nine females. Tuberculosis in the patients were diagnosed by pulmonologists at the pulmonary outpatient unit of Universitas Airlangga Hospital and the tuberculosis outpatient unit of Dr. Soetomo General Hospital. A diagnosis of tuberculosis was established by clinical symptom assessment and supporting examination, both bacteriological and radiographic, while confirmation of diagnosis was provided by an outpatient unit pulmonologist. Bacteriological examination was performed three times on three sputum specimens: 'spot', 'morning', and 'spot'. One of the TB patients was HIV-positive, but tuberculosis constituted the only other systemic disease then affecting the other subjects.

A swab specimen was collected from the buccal mucosa of subjects immediately after a positive diagnosis. This involved the use of a cotton swab stick under standardized conditions at least one hour after eating and the completion of oral hygiene procedures. The swabs were brushed along the inside of each subject's cheek 7–8 times for about 10 seconds on each occasion in order to collect specimens uniform in both volume and composition. The swabs were then applied to glass slides for direct smear. The slides were processed and stained with Ziehl Neelsen acid-fast stain and examined under a microscope at 1000× to establish the presence, or otherwise, of acid-fast bacilli. The acid-fast bacilli on the slides were counted and subsequently evaluated using the International Union Against Tuberculosis and Lung Diseases (IUATLD) scale (Table 1). The amount of acid-fast bacilli present on the buccal mucosa of TB patients was compared with the previous acid-fast bacilli contained in the sputum.

RESULTS

According to the IUATLD scale, there were five levels of bacilli number (Table 1). Based on their medical records, nine of the 19 subjects (47.4%) presented negative results in the acid-fast bacilli of sputum specimens. One patient presented large numbers of acid-fast bacilli (3+ on all three sputum specimens), while others presented varied levels of bacilli number. On the other hand, most subjects (89.5%) presented negative acid-fast bacilli of buccal mucosa swab specimens, with the exception of two subjects with weak results. The presence of 2–9 bacilli in 100 fields was observed in both the sputum and buccal mucosa swab specimens and considered slightly positive. The systematic result could be seen in Table 2.

Compared to the sputum specimens, the identification of acid fast bacilli in oral swab specimens occurred in those

Table 2. Acid fast bacilli presence on sputum and oral swabs

No. of subjects (Sex)	Acid Fast Bacilli	
	Sputum	Oral swabs
1 (M)	(–)	(–)
2 (F)	(–)	(–)
3 (F)	(–)	(–)
4 (M)	(–)	(–)
5 (M)	(2+)	(3)
6 (F)	(–)	(–)
7 (F)	(3+)	(4)
8 (M)	(1+)	(–)
9 (M)	(5)	(–)
10 (M)	(1+)	(–)
11 (F)	(–)	(–)
12 (F)	(–)	(–)
13(F)	(1+)	(–)
14 (F)	(1+)	(–)
15 (M)	(2+)	(–)
16 (M)	(–)	(–)
17 (M)	(1+)	(–)
18 (M)	(–)	(–)
19 (F)	(7)	(–)

Table 1. International union against tuberculosis and lung disease scale[10]

Microscope examination	Smear result	Smear interpretation	Infectiousness of patient
No acid fast bacilli was found in 100 fields	Negative	Negative	Probably not infectious
1–9 acid fast bacilli in 100 fields	Number of acid fast bacilli	Moderately positive	Probably infectious
10–99 acid fast bacilli in 100 fields	1+	Moderately positive	Probably infectious
1–10 acid fast bacilli 1 fields	2+	Strongly positive	Probably very infectious
>10 acid fast bacilli 1 fields	3+	Strongly positive	Probably very infectious

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patients with a significant number of acid-fast bacilli in their sputum. As described above,[10] the interpretation of the results of these patients with regard to their sputum could be classified as strongly positive, while the patients themselves were probably highly infectious. Seven patients with moderately positive results in their sputum examination presented negative results in a buccal swab examination, whereas subjects with negative sputum examination results presented the same results in buccal swab specimens.

DISCUSSION

Tuberculosis is an infectious disease caused by a pathogenic agent, *Mycobacterium tuberculosis* complex. This complex includes *M. tuberculosis*, *M. bovis*, *M. caprae*, *M. africanum*, *M. microti*, *M. pinnipedii*, *M. mungi*, *M. orygis* and *M. canetti,* with the species most commonly affecting humans being *M. tuberculosis.*[3] This bacteria is bacillus shaped, approximately 0.4–0.5 × 3 µm in size and aerobic. *Mycobacterium tuberculosis* could not be classified as a positive or negative gram bacteria, due to the difficulty of identifying it in gram staining.[3,11,12]

Not all individuals with TB present symptoms, and as a result, diagnosis of the disease can be delayed with the result that the patient may remain ill and possibly infectious to others for a prolonged period.[10] A diagnosis of tuberculosis can be confirmed by means of microscopic examination with Ziehl Neelsen staining, culture, radiographic examination and a tuberculin test.[13] Bacteriological examination which identifies the presence of *Mycobacterium tuberculosis* is crucial to diagnosis. Specimens used for such examinations include: sputum, pleural fluid, cerebro-spinal fluid, joint fluid, gastric washings, blood and other tissues.[11] A variety of clinical specimens other than sputum may be submitted for examination when extrapulmonary TB disease is suspected.[10] Based on its natural history, tuberculosis is transmitted by air-borne droplet nuclei containing bacteria from an infected person while coughing, sneezing, speaking and singing.[6] The TB bacilli in sputum from the lungs may be deposited passively in the oral cavity during expectoration. Consequently, self-inoculation of the oral mucosa may occur and oral lesions as a manifestation of tuberculosis would be present.[14,15]

Table 3. Comparation of acid fast bacilli on sputum and buccal swab specimens

Sputum specimens	Oral buccal swab specimens		
	Strongly positive	Moderately positive	Negative
Strongly Positive	0	2	1
Moderately Positive	0	0	7
Negative	0	0	9
Total	0 (0%)	2 (10.5%)	17 (89.5%)

The diagnostic method used for the last 15 years as the implementation and expansion of a DOTS strategy program is the examination of direct smear of acid fast bacilli on sputum.[13,16] At least three consecutive sputum specimens are required, each collected at 8 to 24-hour intervals, with at least one being an early morning specimen.[10] The specimens are then referred to as 'spot', 'morning', and 'spot' specimens.[17] The identification of acid-fast bacilli in sputum underpins a presumptive diagnosis of tuberculosis and indicates that the patient is capable of transmitting the disease. Conversely, the absence of acid-fast bacilli in sputum smears has been taken as an indication that such patients are relatively less infectious, although they should not be regarded as immune to TB.[18] Smear results show a number of acid-fast bacilli observed at 1000× magnification that are categorized into five levels as described in the IUATLD scale (Table 1). In short, the greater the number, the more infectious the patient.[10] In this study, the number of bacteria in sputum and buccal swabs were compared. It was observed that acid-fast bacilli were present on the buccal swab of subjects with 2+ and 3+ direct smear test results of sputum. The acid-fast bacillus smear status of the source case provides a strong indication of which patients are the most contagious. As an airborne transmitted disease, it has been estimated that 10 secondary infections arise annually from untreated smear-positive cases of tuberculosis.[19]

This study was undertaken to detect the presence of acid-fast bacilli in the oral cavity, and buccal swabs were used as an oral sample, since they were considered as smaller samples, more uniform in volume and composition, and less viscous and heterogenous.[8] Acid-fast bacilli were found in two oral buccal swab samples. This study supports a previous one conducted by Yassen *et al*.,[7] into tuberculosis bacilli detection in the oral cavity which suggested that approximately 60% of patients presented a positive result in acid-fast identification of tuberculosis patient saliva. The study also suggested that there was no acid-fast bacilli present in the parotid saliva of 25 pulmonary tuberculosis patients who had recorded a positive acid-fast bacilli result in their sputum. It was, therefore, suspected there had been contact between infectious sputum and oral epithelial tissue.[7] Shenai suggested that out of 26 cases of culture–positive pulmonary tuberculosis, only 10 subjects provided Mtb positive saliva samples when tested with the Xpert assay.[20] Both samples, saliva and buccal swab, are easily and simply collected with minimal discomfort.[8] Another study suggested that in countries such as India, where tuberculosis, especially active pulmonary tuberculosis is endemic, tuberculosis bacilli deposition in the oral cavity might subsequently contaminate the lips, tongue, gingiva, oral mucosa and saliva during expectoration.[21] Wood also proposed that there might be adherence tuberculosis bacilli on buccal epithelial mucosa, because *Mycobacterium tuberculosis* DNA can be detected in buccal mucosa samples collected from 90% of active pulmonary tuberculosis patient.[8]

Oral cavity sample-based research has also been conducted on dental plaque and caries lesions. Eguchi

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compared the culture examination technique, as the gold standard method, and polymerase chain reaction (PCR) technique on several oral cavity samples. Saliva-based culture examination confirmed a sensitivity of about 17.3%, a figure relatively low when compared to PCR, which reached 98%.[9] Another study comparing salivary and dental plaque samples by the PCR method showed that the sensitivity and specificity of this method in detecting salivary *M. tuberculosis* were 92% and 88% respectively, while the PCR results on *M. tuberculosis* plaques showed lower sensitivity but higher specificity.[21] Eguchi shows that the results of PCR dental plaque are the same at about 92%, but in contrast with the results of a culture (2%). The examination plaque attached to denture showed the results of a sensitivity of 0% on culture and 100% using PCR.[9]

This study showed that, in comparison to the PCR method, the sensitivity of direct smear of buccal mucosa swab test for acid-fast bacilli against sputum was 20%, although the sensitivity in pulmonary tuberculosis patients was only 10.5%. Therefore, molecular-based diagnostic tests were more effective at measuring sensitivity and specificity when detecting *Mycobacterium tuberculosis* in the oral samples. One point to remember here is that the major disadvantage of PCR methods is that they cannot differentiate live from dead cells. PCR technique is based on the bacterium genome, while the staining method is based on bacterial morphology.[22]

Another study suggests that *Mycobacterium tuberculosis* survive less successfully in the oral cavity because of certain inhibitory factors there which have bactericidal features inimical to *M. tuberculosis* growth. This theory is supported by an in vitro study, where *Mycobacterium tuberculosis* isolate was inhibited by *A. naeslundii*, *P. gingivalis*, and *F. nucleatum*, the bacteria commonly found in the oral cavity.[9] Other oral defense systems include: saliva, intact mucosa, enzymes and tissue antibodies that also play a role in resisting the development of tuberculosis. Because of these factors, the incidence rate of oral tuberculosis is extremely rare, approximately 0.05–5% worldwide.[23,24]

In conclusion, the results of this study show that acid-fast bacilli can be detected in a buccal epithelial mucosa sample, although their number was extremely low. The acid-fast bacilli bacterium detected in the oral sample during this study indicates, firstly, that the oral cavity might be a source of tuberculosis and, secondly, it is possible for the bacteria to be transmitted to other individuals. However, further studies in this area are necessary.

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