Detection of acid fast bacilli in the saliva of patients having pulmonary tuberculosis

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ABSTRACT

Background: Tuberculosis is a serious disease caused by bacteria called Mycobacterium tuberculosis. The disease is readily detected by demonstration of the bacteria in a clinical specimen. The purpose of this study was to determine the density of acid fast bacilli in the mixed and parotid saliva samples and to compare them with the sputum, in addition, to find out the efficacy of the saliva samples in the diagnosis of pulmonary tuberculosis.

Subject and Methods: A sample of 25 patients of both sexes, Age ranged from 17-70 participated in this study. Unstimulated mixed saliva and the parotid saliva was collected for direct smear of acid fast bacilli by Ziehl-Nelson acid fast stain. Five samples were inoculated on Lowenstein Jensen media and stoenbrink media to determine the presence of the bacilli in the samples.

Results: Concerning the mycobacterium tuberculosis, about 60% of unstimulated mixed saliva revealed positive acid fast bacilli, while all samples of parotid saliva showed negative acid fast bacilli. There was no significant relationship between the duration of signs and symptoms of disease and the detection of mycobacterium tuberculosis in the collected specimens. The density of mycobacterium tuberculosis in the mixed saliva mainly was scanty which mean it was not more than 2-9 bacilli in at least 100 fields. This confirms the fact that the body fluids commonly contain only small number of mycobacterium tuberculosis. The five samples of saliva which were inoculated on Lowenstein Jensen media and stoenbrink media showed positive cultures.

Conclusion: Mixed saliva was less efficient than sputum by direct smear of sputum.

Keywords: Mycobacterium tuberculosis, saliva. (J Bagh Coll Dentistry 2012;24(3):59-62).

INTRODUCTION

Tuberculosis is a serious disease caused by bacteria called Mycobacterium tuberculosis. The disease usually affects the lungs but other organs may also be affected (1). The variable nature of its manifestation, as well as its ability to involve almost every organ system, either singly or multiply, makes it essential that the possibility of extra pulmonary tuberculosis be included in the differential diagnosis of any infectious process in the body (2,3). The disease is considered a worldwide problem. Almost one-third of the world’s population is infected with TB, although a healthy immune system can prevent active disease. (4) The disease is readily detected by skin test, chest x-ray, or by demonstration of M. tuberculosis bacteria in a clinical specimen. There are two distinct stages of TB:

1. TB infected individuals are those who are tuberculin test positive, but do not have the bacteria in their saliva and are without clinical symptoms.
2. TB diseased persons have M. tuberculosis bacteria in their saliva and are symptomatic for the disease. (5)

Since the disease is infectious in nature, the clinical signs and symptoms of tuberculosis are common to many other diseases which are:

- Loss of weight.
- Loss of energy.
- Poor appetite.
- Fever and wet cough.

TB is transmitted through the air from exposure to germs in the saliva of infected person from their lungs. (1,6) There are two kinds of active TB:

Primary TB: Occurs soon after a person is first exposed to TB. Reactivation TB: occurs in people who were previously exposed to TB if their immune system is weakened, TB can breakout of the tubercles and cause active disease. Most of the cases of TB in people with HIV disease are due to reactivation of a previous TB infection. (7,8)

TB may be of concern to the dentist from at least three standpoints:

First: It is an infectious disease and as such is communicable in its active state. The dentist is in a high-risk population and may contract the disease from a patient or patients may contract the disease from the dentist who might have an active case.

Second: On rare occasions tuberculosis lesion may be found in the oral cavity; thus the dentist must be alert to include tuberculosis in the differential diagnosis of oral lesions.

Third: The dentist may be the first person to discover that a patient has Tuberculosis. (1)
The treatment usually consists of combination of drugs. Generally, TB drugs are taken daily for 5 to 12 months. It is important that the exact medication plan should be decided by qualified health care providers. If left untreated, an individual with TB disease can become severely ill and also transmit the disease to others. Untreated, TB disease can be fatal. (9)

The purpose of this study was finding out the density of acid fast bacilli in the mixed and parotid saliva samples and to compare them with the sputum, in addition, to determine the efficacy of the saliva samples in the diagnosis of pulmonary tuberculosis.

SUBJECTS AND METHODS
A total of 25 subjects who were diagnosed as having pulmonary tuberculosis participated in this study. Their age ranged between 17-65 years. The subjects were not having systemic disease other than tuberculosis. Those patients were collected from Chest and Respiratory Disease Institute, TB Lab Reference. The patients were having duration of the disease between 2-12 months. The samples of the saliva were collected from the patients under standardized condition (between 10-12 a.m., at least 2hrs after eating and oral hygiene procedure). The mixed saliva samples were taken from the floor of the mouth, while parotid saliva was taken after localization of the orifice of the parotid salivary gland duct. The area was dried with a piece of cotton, and then the gland milked gently with the finger. The milked saliva was collected with blunt instrument and distributed on a glass slide for a direct smear. The slides were then processed and stained with Ziehl Nelson acid fast stain and examined under oil immersion (1000x) for the presence of acid fast bacilli. Culture of mycobacterium was done by digestion-decontamination method to confirm the presence of the micro-organism.

Statistical analysis was done with the assessment of the values at the P>0.05 levels.

RESULTS
The results of mixed saliva showed microorganisms in 15 patients. However, 10 patients showed negative results (absence of microorganisms) in the samples collected. The microorganisms could not be isolated from the parotid saliva of the total number (25 patients with pulmonary tuberculosis) so the results considered negative. However the microorganisms were isolated from the sputum of the whole number of the patients, as shown in table 1.

In the specimens examined, the presence of 2-9 bacilli in 100 fields was considered scanty positive) which were observed in saliva samples, while in the sputum, samples the microorganisms were scanty to sever; as shown in figure 1. The duration of signs and symptoms of the total TB patients ranged between 1-12 months. In order to compare the duration of signs and symptoms with the results of direct smear of saliva we divided the duration into four groups. The highest percentage of positive acid fast bacilli found in patients with 1-3 months duration of the disease, while the lowest percentage was found in 7-9 months. There was no significant relationship between the duration of signs and symptoms of the disease and the presence of microorganisms (Table 2).

DISCUSSION
In this part of study about 25 patients having pulmonary tuberculosis were diagnosed by direct smear of sputum for acid fast bacilli. By using mixed saliva from those patients with pulmonary tuberculosis, about 15 (60%) revealed the presence of mycobacterium tuberculosis in the saliva. This confirms the fact of presence of mycobacterium tuberculosis in the saliva of patient having pulmonary tuberculosis. (10)

We did not identify mycobacterium tuberculosis in the parotid saliva because the microorganism which was identified in the mixed saliva was not present in the saliva primarily but it results from contact of the oral tissue with infected sputum. Up to our knowledge we did not find any study performed on the saliva as a sample for diagnosis pulmonary tuberculosis to compare our finding with these studies, so we did our comparison between the sensitivity of saliva and the sensitivity of sputum.

In our study the sensitivity of direct smear of saliva for acid fast bacilli is equal to 60% of the sensitivity of sputum. The sensitivity of direct smear of sputum for the microorganism ranged from 22-80%. (11,12)

The density of mycobacterium tuberculosis in the mixed saliva mainly is scanty which mean it was not more than bacilli in at least 100 fields. (2,9)

This confirms the fact that the body fluid commonly contains only small number of mycobacterium tuberculosis. (10) Therefore, the mixed saliva was less efficient than sputum because by direct smear of sputum the quantity of the bacilli observed on the smear could be
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provided which serve in the demonstration of the severity of disease.

To overcome this shortage, we had to concentrate the sample of saliva by using, cytocentrifugation, or sequential layering of several drops of uncenterfuged fluid, on-slide, or polycarbonate membrane.

**Cultivation**

To explain the ability of using saliva as sample for culture, five samples of mixed saliva selected randomly to be inoculated on the Lowenstein Jensen media and stone brink media. All samples revealed positive culture. This means that the mycobacterium tuberculosis which was recovered well by Lowenstein Jensen media and mycobacterium bovis which was recovered by stone brink media were present in the saliva. So we can conclude that the sample of saliva can be inoculated on different media and we can use it if the sputum is unavailable.

Therefore, we can summarize the difference between sample of saliva and sputum as shown in Table 3.

Table 3: Comparison between the saliva and sputum

<table>
<thead>
<tr>
<th></th>
<th>Always available</th>
<th>Sometimes not available</th>
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<tbody>
<tr>
<td>Less efficient than sputum because is like other body fluids commonly contain only small numbers of mycobacteria</td>
<td>More efficient than the body fluid</td>
<td></td>
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<tr>
<td>Can be concentrated to maximize the yield of mycobacterium before inoculation on media and direct smear</td>
<td>Also can be concentrated to maximize the yield of mycobacterium before inoculation on media and direct smear</td>
<td></td>
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<tr>
<td>Can be inoculated to liquid and solid media</td>
<td>Can be inoculated to liquid and solid media</td>
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<tr>
<td>The sample which revealed positive is always scanty so we cannot measure the severity of disease</td>
<td>The sample which revealed positive may be scanty or moderate or severe so we can measure the severity of disease</td>
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**REFERENCES**