Microbiological analysis of oral samples for detection of Mycobacterium tuberculosis by nested polymerase chain reaction in tuberculosis patients with periodontitis

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ABSTRACT

Background: Due to high prevalence of tuberculosis (TB) in India, presence of Mycobacterium tuberculosis in oral samples might influence the periodontal status of patients and also might pose a risk of transmission of TB during dental procedures through aerosols. Hence, this study aims to compare the periodontal status between TB and non-TB patients and to detect the presence of M. tuberculosis in plaque and saliva of TB and non-TB patients.

Materials and Methods: A total of 25 TB and 25 non-TB systemically healthy patients (age 21-49 years) were selected for this Clinico-Microbiological study. The oral hygiene and periodontal status of the patients were measured by using clinical indices and were compared using Mann–Whitney test (P < 0.05 = Significant). Pooled plaque and unstimulated salivary samples were collected and subjected to microbiological evaluation of M. tuberculosis by nested polymerase chain reaction. The detection rates were compared using Chi-square test (P < 0.001 = Highly significant).

Results: No significant difference was observed in periodontal clinical parameters measured between the groups. M. tuberculosis was detected in 92% of saliva and 68% of plaque samples of tuberculosis group, and even in 12% of saliva samples in nontuberculosis group patients.

Conclusion: The TB status of the patient did not influence the periodontal status. However, the presence of M. tuberculosis in plaque and saliva as detected in this study might pose a grave risk of transmission of the disease through aerosols during dental procedures.

Key Words: Dental plaque, nested PCR, periodontitis, saliva, tuberculosis

INTRODUCTION

Tuberculosis (TB) is a chronic granulomatous disease and remains a major cause of morbidity and mortality in developing countries, especially in the Indian subcontinent.¹ World Health Organization reports that India accounts for one-fifth of the global TB incident cases. Each year nearly 2 million people in India develop TB, of which around 0.87 million are infectious cases. It is estimated that annually around 330,000 Indians die due to TB.² Although TB has a definite affinity for the lungs, it can affect any part of the body including the mouth. In order of frequency, the extrapulmonary sites most commonly involved in TB are the lymph nodes, pleura, genito-urinary tract, bones and joints, meninges, peritoneal or gastrointestinal, and pericardium.¹

Oro-facial TB is often difficult to diagnose and it should be an important consideration in the differential diagnosis of lesions that appear in the oral cavity.³ Due to the high prevalence of pulmonary TB in India, and that in active pulmonary TB, the TB bacilli may get deposited passively in the oral cavity contaminating...
the lips, gingiva, teeth, and oral mucous membrane, as well as the saliva during expectoration. Second, oral cavity is one of the sites for extra pulmonary TB.\[^{4-7}\] The periodontal pocket may serve as a good nidus for colonization of TB bacilli, as they are known to survive in low oxygen tension.

The influence of systemic conditions on the periodontium has long been recognized and supported by scientific evidence. Studies on gingival involvement during systemic TB infection are ample,\[^{4,8,9}\] but those on relationship between systemic TB infection and periodontal status are rare. In addition, the presence of TB bacilli in oral samples of both TB and non-TB patients is less documented.

Nested polymerase chain reaction (Nested PCR) is highly sensitive in detecting Mycobacterium tuberculosis and has the advantages of detecting even negligible amount of pathogen deoxyribonucleic acid (DNA) and prevents nonspecific background amplification. Based on the above reasons, there is a need for identifying the presence of TB bacilli in saliva and plaque samples of both TB and non-TB patients using Nested PCR, and to correlate their presence clinically with the periodontal status and also to prevent an occupational hazard of aerosol infection, which is common in Dental clinics.

**MATERIALS AND METHODS**

A total of 50 periodontitis patients\[^{10}\] belonging to both the sexes (24 males and 26 females) were selected for the study and were grouped into two groups. Tuberculosis group (Group I) consisted of 25 patients with pulmonary TB (diagnosed clinically using Revised National Tuberculosis Control Program criteria,\[^{11}\] smear test [Acid fast bacilli (AFB) microscopy] and chest radiographs). Nontuberculosis group (Group II) consisted of 25 patients without active TB (determined clinically and by chest radiographs). The patients were selected from the District Tuberculosis center and from Outpatient Department of Periodontics, CODS, Davangere, India. All the 50 patients selected belonged to rural population and were of lower socio-economic status. The age group of the patients selected ranged from 21 to 49 years.

The exclusion criteria consisted of patients with immuno-compromised diseases like human immunodeficiency virus (HIV) and other known systemic diseases; who had undergone periodontal therapy in past 1 year; on antibiotic therapy within last 6 months, smokers, pregnant, and lactating women.

Records for this study were obtained following informed consent from patients. Approval for the study was granted by Institutional Review Board at Rajiv Gandhi University of Health Sciences, Karnataka, India based upon the principles of World Medical Association declaration of Helsinki, 1975.

The oral hygiene status and periodontal status of the patients of both the groups were recorded using Oral Hygiene Index-Simplified (OHI-S) composed of Debris index-simplified (DI-S) and Calculus index-simplified (CI-S)\[^{12}\] and Community Periodontal Index (CPI).\[^{13}\] Clinical parameters like age, sex, rural/urban status, chest radiograph findings, sputum examination results, erythrocyte sedimentation rate, leukocyte count, Enzyme Linked Immunosorbent Assay (ELISA) and Bacille Calmette-Guerin (BCG) vaccination history were also compared between the patients of both the groups.

Unstimulated saliva samples were collected by asking the patient to spit into the sterile vial. Pooled plaque samples were collected from each of the selected patient by using sterile curettes and was immediately transferred into an eppendorf vial containing 0.5 ml of TE buffer (Tris–EDTA buffer) and immediately sent to the microbiological laboratory for PCR analysis.

As the presence of *M. tuberculosis* in oral samples is not well documented, Nested PCR technique was selected for the study. Nested PCR increases the specificity of DNA amplification, by reducing background due to nonspecific amplification of DNA. A total of 10 µl of obtained sample was added to a PCR reaction mixture containing PCR buffer containing 25 mM Mg++, 200 mM dNTP (deoxynucleotide triphosphate), 1 mM specific primers [Figure 1], and 0.025 U/ml of Taq Polymerase. PCR reactions were carried out using Thermocycler PCR System (Corbett research, USA)\[^{TM}\] [Figure 2].

The amplification of a 123 base pair (bp) DNA fragment located within insertion element IS6110, a very specific target sequence in the diagnosis of TB complex by DNA amplification was used in this study. Four sets of primers (Sigma-Aldrich,\[^{8}\] India) found to be highly specific for *M. tuberculosis*\[^{14}\] were utilized. Primary amplification was done using primers TB 1 and TB 2. The amplified product was subjected for secondary amplification using primers TB 28 and TB 29C. The oligonucleotide primers used are:

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1. Palakuru, et al.: Detection of *Mycobacterium tuberculosis* in plaque and saliva of periodontitis patients. Dental Research Journal / November 2012 / Vol 9 / Issue 6
The amplified product was subjected for Agarose gel Electrophoresis and resultant bands were visualized under Transilluminator [Figure 3]. The clinical examination and oral sample collection was done by a single examiner (PSK) while PCR analysis was done by KB. Mann–Whitney test was used for comparison of clinical parameters for periodontal status between the groups. Both the inter group and intra group comparison of the detection of *M. tuberculosis* in plaque and saliva samples by PCR, was done by Chi-square test.

**RESULTS**

Mann–Whitney test was used to compare clinical parameters ($P < 0.05 = \text{significant}$) for periodontal status between the groups [Table 1]. The mean difference in DI-S scores was 0.16 ($P = 0.51$), CI-S scores was 0.08 ($P = 0.72$), OHI-S scores was 0.01 ($P = 0.98$), and CPI scores was 0.36 ($P = 0.19$) and these were statistically not significant. The mean difference in attachment loss scores as measured by CPI was 0.48 ($P = 0.09$) and was statistically not significant.

Comparison of various clinical parameters between the groups showed that all the patients belonging to tuberculosis group had positive findings for TB in chest radiographs were sputum positive and presented with raised erythrocyte sedimentation rate (ESR) and leukocytosis whereas nontuberculosis group patients were all negative for the above clinical parameters. The HIV screening of the selected patients done using ELISA test, was found negative. Only 19 patients in tuberculosis group and 24 patients in nontuberculosis group had a history of BCG vaccination.

Inter group comparison of the detection of *M. tuberculosis* in saliva samples by PCR [Table 2] revealed that saliva from 23 patients in the tuberculosis group and 3 patients belonging to nontuberculosis group, showed the presence of *M. tuberculosis* in saliva. Plaque from 17 of the patients in the tuberculosis group showed the presence of *M. tuberculosis*, whereas none of the patients belonging to nontuberculosis group showed the presence of *M. tuberculosis* in plaque [Table 3]. The Chi-square test performed to compare the intra group detection rates in saliva and plaque samples [Table 4] showed a statistically highly significant result ($P < 0.001$) in tuberculosis group, whereas the non-tuberculosis group showed nonsignificant results ($P = 0.23$).
DISCUSSION

*M. tuberculosis* is one of the pathogens causing the greatest amount of chronic disease and death throughout the world[15] and its occurrence in oral cavity is well documented in the literature.[4,6] As much as 20% of the world’s population is infected by this organism.

This study aimed to compare and evaluate the periodontal status of periodontitis patients with and without TB and to identify the presence of *M. tuberculosis* in plaque and saliva of periodontitis patients with and without TB. All the selected patients (both tuberculosis and non-tuberculosis groups) belonged to rural population and were of low socio-economic status.[16] This study being the initial effort to evaluate the presence of *M. tuberculosis* in oral secretions of non-TB patients along with TB patients; the rural population was selected by choice as TB is highly prevalent in nonurbanized Indian population with lower socio-economic status.[17] All the selected patients were HIV negative as screened by ELISA test. Six patients in tuberculosis group and one patient in nontuberculosis group were not BCG vaccinated. However, BCG vaccination was not found to be protective in south Indian trials, which might be due to prior exposure to some nontubercular *Mycobacterium* or exposure to other species of *Mycobacteria*.[17]

The oral hygiene status and periodontal status of both the groups were recorded and compared using Mann–Whitney test. The mean difference in DI-S, CI-S and OHI-S, CPI, and attachment loss scores among the two groups were not statistically significant. At present, the results of the present study is suggestive that TB as a disease entity has not influenced the periodontal status. Scanty literature exists to compare these results.

There are several methods for identifying *M. tuberculosis*.[18,19] However, the traditional culture method is still considered a gold standard[18] with disadvantages like delay in results for up to 21 days or failure of organisms to grow. Even modern culture tests like BACTEC 460 TB culture system need 10-14 days for detection.[19] It has been shown that nucleic acid amplification methods such as PCR provide an alternative approach in the detection of microorganisms and thus offer new possibilities for a more rapid and accurate diagnosis of TB.[18] The

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### Table 1: Comparison of clinical parameters for periodontal status between the groups

| Measurement        | Group I (tuberculosis group) | Group II (nontuberculosis group) | Group I vs. Group II |
|--------------------|-----------------------------|---------------------------------|---------------------|
|                    | Mean±SD | Median | Mean±SD | Median | Mean difference | Z* | P      |
| DI-S               | 1.67±0.68 | 1.80    | 1.51±0.67 | 1.66    | 0.16          | 0.65 | 0.51 NS |
| CI-S               | 1.49±0.64 | 1.50    | 1.57±0.60 | 1.60    | 0.08          | 0.35 | 0.72 NS |
| OHI-S              | 3.15±1.10 | 3.50    | 3.16±1.12 | 3.5     | 0.01          | 0.02 | 0.98 NS |
| CPI score          | 3.24±0.93 | 4.0     | 3.60±0.65 | 4.0     | 0.36          | 1.31 | 0.19 NS |
| Attachment loss    | 1.64±0.99 | 2.0     | 2.12±0.83 | 2.0     | 0.48          | 1.71 | 0.09 NS |

*Mann-Whitney Test. P>0.05 is not significant (NS). OHI-S: Oral hygiene index-simplified, DI-S: Debris index-simplified, CI-S: Calculus index-simplified, CPI: Community periodontal index.

### Table 2: Inter group comparison of the detection of *M. tuberculosis* in saliva samples by PCR

| Saliva       | Group I (tuberculosis group) | Group II (nontuberculosis group) |
|--------------|------------------------------|---------------------------------|
| Positive (%) | 23 (92)                      | 3 (12)                          |
| Negative (%) | 2 (8)                        | 22 (88)                         |
| Total        | 25                           | 25                              |

χ²=32.1, P<0.001 HS: Highly significant, sensitivity=92%, specificity=88%

### Table 3: Inter group comparison of the detection of *M. tuberculosis* in plaque samples by PCR

| Plaque       | Group I (tuberculosis group) | Group II (nontuberculosis group) |
|--------------|------------------------------|---------------------------------|
| Positive (%) | 17 (68)                      | 0 (0)                           |
| Negative (%) | 8 (34)                       | 25 (100)                        |
| Total        | 25                           | 25                              |

χ²=28.8, P<0.001 HS: Highly significant sensitivity=68%, specificity=100%

### Table 4: Intra group comparison of the detection of *M. tuberculosis* between saliva and plaque samples

| Sample     | Detection of *M. tuberculosis* | Group I (tuberculosis group) | Group II (nontuberculosis group) |
|------------|-------------------------------|------------------------------|---------------------------------|
| Saliva     | Positive                      | 23                           | 3                               |
|            | Negative                      | 2                            | 22                              |
| Plaque     | Positive                      | 17                           | 0                               |
|            | Negative                      | 8                            | 25                              |

χ²=28.9, P<0.001 HS

χ²=28.9, P<0.23 NS

HS: Highly significant, NS: Not significant
nested PCR uses two sets of primers targeting specific DNA sequence. The first pair of PCR primers amplify a fragment similar to a standard PCR. However, a second pair of primers called nested primers (as they lie/are nested within the first fragment) bind inside the first PCR product fragment to allow amplification of a second PCR product which is shorter than the first one. Using more detailed knowledge of DNA sequence increases the specificity of the detection rate. Hence nested PCR analysis being more specific and sensitive when compared with conventional PCR was selected.

In the present study, the nested PCR analysis revealed the presence of *M. tuberculosis* in saliva of 23 (92%) TB patients, whereas 3 (12%) nontuberculosis group patients showed the presence of *M. tuberculosis* in saliva. The presence of TB bacilli in oral samples of TB patients could be due to swallowing of sputum with direct seeding and hematogenous spread.[1,20,21] The presence of *M. tuberculosis* in salivary samples of 3 (12%) patients in the clinically negative nontuberculosis group could be due to the Latent Infection. Patients with latent TB are noninfective until they develop active TB. However, in the study by Eguchi et al.,[14] the detection rates were 92% (34 out of 37 samples) for plaque in TB patients and 0% in non-TB healthy patients, which may be due to the difference in study population selected. However, the PCR technique does not reveal the viability of the organism, which can be a disadvantage of this technique.

*M. tuberculosis* has been found in aerosol particles generated by high-speed hand piece used during simulated dental procedures on patients with active TB.[22] The presence of *M. tuberculosis* in saliva and plaque potentiates the risk of transmission of TB in the indoor environment with prolonged exposure as in Dental clinic. Although occupational infection is uncommon, an outbreak of oral TB following dental treatment at two clinics has been reported.[4,23] The findings of this study warrant for an effective antimicrobial rinse to reduce the aerosol contamination. Povidone iodine at a final concentration of 0.2% killed all of the strains within 120 seconds, and at 0.1% killed 99.9% or more bacilli within 60 seconds.[24] Povidone iodine can be an effective antiseptic to be used as preprocedural rinse especially in areas with high prevalence of TB.

**CONCLUSION**

In conclusion, this study highlights and establishes the presence of *M. tuberculosis* in saliva and plaque of TB patients, which are sources of airborne microbial load through aerosols. Although PCR technique is not cost effective, it may be necessitated in suspicious patients in areas with high prevalence of TB. The need for proper medical screening with preestablished guidelines, strict aseptic conditions and measures to control aerosol generation and transmission during various dental procedures are highly recommended. Considering the limitation of the present study like smaller sample size, further studies with larger sample size are required to reinforce the results.

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