Compare and Correlate the Presence of Bacterial Pathogen Concentrations and Glycaemic Status in Type 2 Diabetic and Non-Diabetic Patients with Chronic Periodontitis Following Phase 1 Therapy

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ABSTRACT

BACKGROUND
At present, a bidirectional pathway has been suggested between DM and periodontitis, since patients with DM are more predisposed to periodontal diseases, the established periodontitis may simultaneously impair adequate glycaemic control. We wanted to study the change in bacterial pathogen concentrations in plaque as confirmed polymerize chain reaction (PCR) in patients with and without type-2 diabetes before and after phase-1 therapy.

METHODS
Subgingival plaque samples were collected from the deepest part of the pocket by using a sterile curette in 2 different Eppendorf tubes containing tris-EDTA buffer. The plaque samples were then subjected to PCR for detecting bacteria (P. gingivalis, T. forsythia, P. intermedia).

RESULTS
In-vitro microbiological assessment (PCR analysis) showed significant reduction of bacteria within both diabetic and non-diabetic groups. In contrast, the intergroup comparison revealed no significant differences in the microbial counts in both the diabetic and non-diabetic groups thus suggesting that phase-1 therapy may not alter the pathogenic microflora in chronic periodontitis patients with or without diabetes.

CONCLUSIONS
In-vitro microbiological assessment (PCR analysis) showed significant reduction of bacteria within both diabetic and non-diabetic groups. In contrast, the intergroup comparison revealed no significant differences in the microbial counts in both the diabetic and non-diabetic groups thus suggesting that phase-1 therapy may not alter the pathogenic microflora in chronic periodontitis patients with or without diabetes.

KEY WORDS
Chronic Periodontitis, PCR, Oral Pathogens, Diabetes
BACKGROUND

Periodontal disease is a microbial infection involving a variety of microbes that trigger inflammation, loss of connective tissue attachment and alveolar bone around the teeth. The primary etiologic factor of periodontitis is bacterial plaque. The bacteria involved are mainly of gram-negative species, express pathogenic factors that elicit host defense responses resulting in inflammation and tissue destruction. In fact, the propensity of periodontitis to proceed with periods of exacerbation and remission could suggest that the presence of other organisms contributes in the disease progression. The development of periodontitis may depend upon cooperative interactions among specific pathogenic bacteria and tissue destructive inflammatory mediators. Various evidences has shown relationship between type 2 diabetes and periodontal disease. However, the effect of periodontal intervention on these elements is yet to be ascertained.

Diabetes is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycaemia.

At present, a bidirectional pathway has been suggested between DM and periodontitis, since patients with DM are more predisposed to periodontal diseases, the established periodontitis may simultaneously impair adequate glycaemic control. Moreover, poorly controlled diabetic subjects, presenting elevated levels of glycosylated haemoglobin (HbA1c), show higher attachment and alveolar bone loss and local inflammatory cytokines than well controlled patients. Exacerbated local inflammation in diabetic subjects with inadequate glycaemic control could modify the subgingival environment, and consequently, the subgingival microbial profile. Evidence has shown variations in the micro flora in non-diabetic and diabetic individuals; and also, within the diabetic individuals as well, based on their glycaemic control.

We wanted to assess, compare and correlate the change in bacterial pathogen concentrations in plaque as confirmed polymerize chain reaction (PCR) in patients with and without type-2 diabetes before and after phase-1 therapy in patients with chronic periodontitis.

METHODS

This study was conducted among 120 patients of chronic periodontitis visiting the Department of Periodontics, Maharishi Markandeshwar College of Dental Sciences and Research, Mullana, Ambala and Department of Medicine, Maharishi Markandeshwar Medical College, Mullana Ambala. Ethical approval was obtained.

Method of Collection of Data

In this study, which was of 4 months duration, following initial screening, 120 patients with chronic periodontitis (Mild to moderate) were divided into 2 main groups based on their glycaemic status. Glycated haemoglobin levels were assessed using standardized assay- High Pressure Liquid Chromatography (HPLC).

Group-1-Control (n=30)

30 non-diabetic patients with chronic periodontitis.

Group-2-Test: (n=90)

These 90 diabetic patients were further subdivided into 3 subgroups-

- 2 (A) 30 diabetic patients with chronic periodontitis. (Good control (HbA1c<5%))
- 2 (B) 30 diabetic patients with chronic periodontitis. (Moderate control (HbA1c<7%))
- 2 (C) 30 diabetic patients with chronic periodontitis. (Poor control (HbA1c>8%))

Inclusion Criteria

- Patients with chronic periodontitis (mild to moderate with up to 4 mm (CAL) clinical attachment loss and with the presence of true periodontal pocket (up to 6 mm) in ≥30% of sites in the mouth.
- Systemically healthy patients (for Group 1).
- Diabetic patients having chronic periodontitis with varied levels of glycaemic control classified as good, moderate and poor (Group 2).

Exclusion Criteria

- Patients with any systemic diseases (Group 1)
- Presence of any systemic or debilitating diseases apart from diabetes in group.

The following parameters were assessed in all the patients at baseline (0), 1 months and 4 months

1) Clinical Parameters
   1. Gingival index (Loe and Silness),(1963)(GI)
   2. Plaque index (Silness and Loe),(1967)(PI)
   3. Probing Pocket Depth (PPD) and relative Attachment Level (RAL)

2) Microbiological Parameters
   1. Subgingival plaque samples were collected from the deepest part of the pocket by using a sterile curette in 2 different Eppendorf tubes containing tris-EDTA buffer.
   2. The plaque samples were then sent for detecting bacteria (P. gingivalis, T. forsythia, P. intermedia, )
TREATMENT PROCEDURE

Clinical Aspect- All the 120 patients were subjected to phase 1 therapy. After assessing the clinical parameters ultrasonic scaling was done followed by thorough root planing at baseline. Patients were recalled after 1 month and 4 months during which time, plaque samples were collected, and clinical parameters were recorded.

Microbiological Aspect- The plaque samples were subjected to PCR. The main components of the PCR Master Mix were HemoklenTaq buffer 5x, dNTP (2.5 mM), HaemoklenTaq enzyme and PCR water. Additives like DMSO, glycerol and formamide were also added for getting better results.

PCR Protocol
For PCR, bacterial DNA from the plaque samples was amplified in 50 µl of a reaction mixture containing 3 µl of the DNA sample, 1X PCR buffer (50 mM KCl, 1.5 mM MgCl2, 10 mM Tris-HCl, pH 9.0), 2.5 mM deoxyribonucleotide mixture 1 µl of each primer, and 0.5-1 µl of haemoklenTaq DNA polymerase (NAB) in a thermal cycler for 30 cycles. After amplification, 20 µl of the PCR product was analyzed by agarose gel electrophoresis by incorporating propidium iodide on 1% gel. The newly synthesized DNA fragments were then visualized under ultraviolet light. The size of the PCR products was estimated from the electrophoretic migration of products relative to a 1 kb ladder marker. (Thermoscientific).

Statistical Analysis
All the clinical, microbiological and biochemical parameters recorded were statistically analysed using SPSS software version 13, and Paired t test.

RESULTS

P. gingivalis (PG) - Intra Group (Table IA)
Group-1 (Control): The mean difference in P. gingivalis counts between baseline and 1 month was 4926000000.0+5081385637.78 and baseline and 4 months was 6731000000.0+58166329.08 at all-time intervals i.e. from baseline to 1 month, baseline to 4 months & from 1 month to 4 months.

Group-2A (Test): The mean difference in P. gingivalis counts between baseline and 1 month was 4286666666.67+4733278140.36 and baseline and 4 months was 6443333333.33+473278140.37 and baseline and 4 months was 6731000000.0+58166329.08. This difference was found to be statistically significant (p <0.05) from baseline to 1 month and baseline to 4 months but not from 1 month to 4 months, (p>0.05).

P. gingivalis (PG) - Inter Group (Table IB)
Intergroup comparisons of Pg counts at the end of 1 month showed no significant differences between group 1 and 2A, 2B and 2C. On comparing group 2A and 2B & 2C and group 2B, 2A and 2C & 2B and 2C (p >0.05). In contrast, at the end of 4 months, significant reduction was observed in group 1 when compared with group 2C. Group 2A in comparison with group 2C & group 2B when compared with group 2C (p <0.05). However, no significant differences in Pg counts were observed between group 1 and 2A, group 1 and 2B and also between group 2A and 2B (p >0.05) at the end of 4 months.
T. forsythia (TF)- Intra Group (Table 2A)
Group-1 (Control): The mean difference in T. forsythia scores between baseline and 1 month was 70000000.00± 255653321.30, at baseline and 4 months was 76333333.33±252675112.83 and 1 month and 4 months was 63333333.33±24280449.54. This difference was not found to be statistically significant (p>0.05) at all time intervals i.e. from baseline to 1 month, baseline to 4 months and 1 month and 4 months.

Group-2A (Test): The mean difference in T. forsythia scores between baseline and 1 month was 199533333.33± 40429606715.80, at baseline and 4 months was 1696551724.13±3768050154.98 and 1 month and 4 months was -23103448.28±170589368.89. This difference was found to be statistically significant (p<0.05) from baseline to 1 month and baseline to 4 months but not from 1 month to 4 months, (p>0.05).

Group-2B (Test): The mean difference in T. forsythia scores between baseline and 1 month was 162600000.00±3617409627.60, at baseline and 4 months was 1663333333.33±3699998446.72 and 1 month and 4 months was 373333333.33±182736541.71. This difference was found to be statistically significant (p<0.05) from baseline to 1 month and baseline to 4 months but not from 1 month to 4 months, (p>0.05).

Group-2C (Test): The mean difference in T. forsythia scores between baseline and 1 month was 1099633333.33±3020280170.64, at baseline and 4 months was 1044000000.00±3052032222.90 and 1 month and 4 months was -556333333.33±243353648.70. This difference was found to be statistically significant (p<0.05) at baseline to 1 month but not from baseline to 4 months & from 1 month to 4 months, (p>0.05).

T. forsythia (TF)- Inter Group (Table 2B)
Inter group comparisons of TF counts at the end of 1 month showed no significant differences between group 1 and 2A, 2B and 2C respectively and also between group 2A and 2B and 2A and 2C and also between groups 2B and 2C. (p>0.05).
Similarly, no significant differences were found between group 1 and 2A and 2B respectively, and group 2A and 2B and 2A and 2C and also group 2B and 2C at the end of 4 months, (p>0.05). In contrast, significant difference was found in the TF counts at the end of 4 months between group 1 and group 2C (p<0.05)

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<th>Dependent Variable</th>
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<th>Std. Error</th>
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<td>NS[2A v/s 2B]</td>
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</tbody>
</table>

P. intermedia (PI)- Intra Group (Table 3A, Graph 3A)
Group-1 (control): The mean difference in P. intermedia counts between baseline and 1 month was 175533333.33± 371564092.27, at baseline and 4 months was 1757500000.00± 3750355155.60 and 1 month and 4 months was 19666666.67± 37644779.82. This difference was found to be statistically significant (p<0.05) at all time intervals i.e. from baseline to 1 month, baseline to 4 months & from 1 month to 4 months.

Group-2A (Test): The mean difference in P. intermedia counts between baseline and 1 month was 1821333333.33± 3716936756.33, at baseline and 4 months was 179766666.67±365283634.53 and 1 month and 4 months was -21666666.67± 310428883.90. This difference was found to be statistically significant (p<0.05) at baseline to 1 month and baseline to 4 months but not from 1 month to 4 months, (p>0.05).

Group-2B (Test): The mean difference in P. intermedia counts between baseline and 1 month was 21466666.67±3927763014.60, at baseline and 4 months was 2169000000.00±39883031297.71 and 1 month and 4 months was 443333333.33±184216576.69. This difference was found to be statistically significant (p<0.05) at baseline to 1 month, baseline to 4 months but not from 1 month to 4 months, (p>0.05).

Group-2C (Test): The mean difference in P. intermedia counts between baseline and 1 month was 2872666670.30±421938784.74, at baseline and 4 months was 292633336.97±501342091.10 and 1 month and 4 months was 53666666.67±370736404.49. This difference was found to be statistically significant (p<0.05) at baseline
Periodontitis usually results from the complex interactions of the microbial communities with the host elements in the gingival sulcus that leads to inflammation, loss of connective tissue attachment and alveolar bone around the teeth. \(^6\)

Diabetes mellitus (DM) is well recognized as a risk factor for periodontal disease, while periodontitis is thought to influence the systemic inflammatory condition, lipid and glucose metabolism and insulin resistance. \(^7\) Diabetes mellitus, especially when uncontrolled, appears to be an important risk factor for periodontal destruction, since an alteration in host-response and microbiological aspects occurs in diabetic subjects. \(^8\) Periodontal disease leads to an increase in insulin resistance, which could impair glycaemic control. Thus, the control of periodontal disease is necessary for better systemic health in these individuals. Periodontal treatment relies on biofilm disruption and plaque control to prevent recolonization and recurrence of the disease. \(^8\)

The relationship between plaque accumulation and HbA1c levels remains controversial. \(^9,11\) Some reports have observed an improved compliance with oral hygiene amongst diabetic subjects with better glycaemic control, while others have not found this association. \(^12-14\) Some studies have shown the impact of type 1 and type 2 DM on tooth loss, \(^15\) but the association between metabolic control and the number of remaining teeth has not been considered. Previous studies have shown that the microbiota associated with diabetes does not appear different from the microbiota of non-diabetic patients. \(^15,16\) Although the microbiology of periodontal disease in DM is fairly well understood. The sample size of 120 patients evaluated in this study was arrived at following consultation with the statistician which was in accordance with the vast majority of clinical periodontal treatment studies in humans. \(^16\) In order to understand this phenomenon with better sensitivity and specificity, it was decided to carry out the study by dividing the 120 patients into 4 groups based on the level of glycaemic control.

The clinical parameters (GI, PI, PPD, RAL) were assessed to evaluate the disease severity at regular intervals and also to understand whether phase-1 therapy has an effect on patients with chronic periodontitis with and without diabetes.

The gold standard for recording changes in periodontal status is the longitudinal measurements with relative attachment level (RAL) from CEJ to the base of the pocket. Due to the relative inconsistencies in determining CEJ accurately at the selected sites, it was decided to use a customized acrylic stent and use the base of the stent as the fixed reference point and evaluate relative attachment level (RAL).

In addition, the microbiological analysis with the help of multiplex PCR technique was done to evaluate the effect of phase-1 therapy on bacterial concentrations in patients with chronic periodontitis with and without diabetes. Single-stage PCR was used to detect P. gingivalis, P. intermedia, and T. forsythia. It has been suggested that pooling of plaque samples increases the probability of detecting existing pathogens. \(^21\)

Inter-group comparison revealed, a significant improvement in glycated Hb levels in the non-diabetic group as compared to the diabetic groups at 1 month following phase-1 therapy. Additionally significant improvement was also observed in the well-controlled and moderately controlled diabetic group when compared with the poorly controlled group. This is in accordance with Taylor et al 2008, \(^23\) Shlissman 1990 \(^23\) who concluded that phase-1 therapy improves the glycaemic status of diabetic patients with chronic periodontitis.

With regard to the microbiologic parameters, intra group comparison revealed a significant improvement in Pg counts, Tf count, Pi count, count with in both the test (Diabetic) and control (non-diabetic) group from baseline to 1 month but not from 1 month to 4 months except in poorly controlled diabetic group at 1 month and 4 months. This is in accordance with the findings of Sandholm et al 1989, \(^24\) Contreras at al 1999, \(^25\) Saygun et al...
2008\textsuperscript{26} who concluded that phase-1 therapy results in reduction of bacterial counts in patients with chronic periodontitis and diabetes and also with Hintao et al 2007\textsuperscript{27}. IbahimuMdal 2013\textsuperscript{28} who concluded that phase-1 therapy results in reduction of bacterial counts in patients with chronic periodontitis

Inter group comparison revealed significant reduction in Pg counts, Tf count, Pi count, in the non-diabetic group compared to the poorly controlled diabetic group and well controlled diabetic group compared to poorly-controlled diabetic group at the end of 4 months. But interestingly no such differences were observed at 1 month. This is accordance with Makiura et al\textsuperscript{2008} who concluded that Pg counts were reduced in the patients with diabetes following phase-1 therapy.

**CONCLUSIONS**

In-vitro microbiological assessment (PCR analysis) showed significant reduction of bacteria within both diabetic and non-diabetic groups. In contrast, the intergroup comparison revealed no significant difference in the microbial counts in both the diabetic and non-diabetic groups thus suggesting that phase-1 therapy may not alter the pathogenic microflora in chronic periodontitis patients with or without diabetes. Correlation assessments of clinical parameters with the microbiological and biochemical parameters showed significant correlation at various time intervals again suggesting that phase 1 therapy results in improved clinical and microbiological status and glycaemic status in chronic periodontitis patients with and without diabetes.

**REFERENCES**


