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Research Article

Influence of Glycemic Status on Periodontal Condition and Specific Periodontal Pathogens in Controlled and Uncontrolled Diabetics Before and After SRP

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Abstract

Introduction: Diabetes and periodontitis are inflammatory diseases. Their chronic nature increases the systemic inflammation and they share a two way relationship. Treating one of them improves the condition of the other.

Aims:

1. To assess the influence of glycemic status on the prevalence of *P. gingivalis* and *P. intermedia* in controlled and uncontrolled diabetics before and after SRP.
2. To assess the relationship between glycemic status and periodontal condition.
3. To assess the short-term effect of SRP on glycemic status.

Settings and Design: 40 patients were grouped as controlled (Group A) and uncontrolled diabetes (Group B), based on their HbA_{1c} levels. PI, GI, PPD and CAL were assessed. HbA_{1c} and subgingival plaque samples were analysed for *P. gingivalis* and *P. intermedia* at baseline and 6 weeks following SRP.

Methods and Material: Microbiological analysis was done using PCR.

Statistical analysis: Statistical analysis was done using SPSS for windows and included Descriptive analysis, Contingency Table analysis, Independent samples '*t*' test and Paired samples '*t*' test.

Results: No differences in periodontal parameters were observed between the groups. *P. gingivalis* and *P. intermedia* were more frequently detected in in Group B (9 and 9 subjects respectively) than in Group A (6 and 4 subjects respectively), but was statistically non-significant. A decrease in detection was seen in both groups at recall. Change in HbA_{1c} in both groups at recall was statistically non-significant.

Conclusions: Poorer glycemic control increased the prevalence of *P. gingivalis* and *P. intermedia*, with non-significant reduction after SRP. No significant short-term influence of SRP on glycemic status was observed in 6 weeks.

Keywords: Diabetes; Periodontitis; Glycemic Status; Periodontal Pathogens; Two-Way Relationship; SRP

Key Messages

The prevalence of *P. gingivalis* and *P. intermedia* did not significantly differ among controlled and uncontrolled diabetic periodontitis individuals. The host response or other destructive pathogens could be implicated in diabetic individuals for periodontal destruction. No statistically significant changes were observed following SRP in diabetic individuals in 6 weeks.

Introduction

Periodontal infections cause chronic inflammation of the periodontium, which includes the gingiva, cementum, periodontal ligament (PDL), and the alveolar bone. This causes progressive alveolar bone loss and PDL destruction [1] and triggers an immune-inflammatory response [2].

The classic model put forward by Page and Kornman in 1997 draws a clear perspective on the initiation and progression of periodontal pathology, throwing significant light on the influence of systemic diseases and conditions on the risk for periodontal disease [3-5]. Conversely, periodontitis can regulate/modify the pathogenesis some systemic diseases [6]. Biologically, diabetes and periodontitis share various similarities in pathobiology, with inflammation being a crucial factor [7].

The elevated gingival crevicular fluid (GCF) glucose concentration in diabetic patients act as a source of nutrition altering the environment to favour the more pathogenic species [19]. Diabetic individuals also have an impaired immune response to periodontal pathogens [8].

Diabetic patients and patients with chronic periodontitis have similar subgingival microorganisms [9-11] irrespective of the duration, type or metabolic control [12]. Most investigations used culture and immunoassays.

Though culture is the 'gold standard' in microbiology, its limitations include the inability to detect non-viable bacteria, the difficulty to culture certain species, and insufficient sensitivity that requires a larger number of target organisms to give a positive reaction [13]. Polymerase Chain Reaction (PCR), is simple, highly sensitive and specific. Multiple and a varying spectrum of putative periodontal pathogens can successfully be detected [14].

Diabetics with periodontitis have a high degree of systemic inflammation with higher serum IL-6, TNF-α, and CRP levels. Elevated pro-inflammatory cytokines cause an increase in insulin resistance and aggravate glycemic control. Periodontitis evidently results in poor glycemic control in diabetics [15]. Conversely, better glycemic control has followed periodontal therapy in diabetic individuals [16,17].

Methodology

This is a prospective study where 20 patients with controlled diabetes and 20 patients with uncontrolled diabetes were recruited. Ethical clearance was obtained and the study was completed in a year.

Selection of subjects was based on:

Inclusion criteria

- Routine patients presenting to clinic with history of diabetes.
- Age > 18 years.
- Patients having at least 20 natural teeth.
- No other systemic/medical complications.

Exclusion criteria

- Received antibiotics in the past 3 months.
- Patients who were subject to periodontal therapy over the past 6 months.
- Use of steroids or other medications that may alter immune response.
- Tobacco/pan chewers and smokers.
- Pregnant and lactating women.

40 diabetic patients were divided into 2 groups after their glycated hemoglobin levels (HbA_{1c}) have been established. The first group (Group A) comprised of 20 individuals who had controlled diabetes (HbA_{1c} <8.0%) [12]. The second group (Group B) comprised of 20 individuals who had uncontrolled diabetes (HbA_{1c} ≥8.0%). All recruited patients were briefed about the study and a signed consent was obtained.

Assessment of clinical parameters:

1. Plaque Index (PI) - Silness and Loe (1964) using an explorer.
2. Gingival index (GI) - Loe and Silness (1963) with a Williams periodontal probe.
3. Periodontal status using a Florida Probe®
    a. Depth of the pocket (PD)
    b. Clinical Attachment Level (CAL)

Plaque sample collection

Diabetic patients having gingivitis and chronic periodontitis were eligible for the study. Supragingival deposits were removed before plaque sample collection. The area was isolated using cotton rolls. A sterile curette was inserted into the periodontal pocket as less traumatically as possible and plaque sample from the root surface of 2 teeth per quadrant was collected employing a gentle pull-stroke. Thorough scaling and root planing (SRP) was performed and oral hygiene instructions (OHI) were explained. The collected specimens were transferred to marked Ependorff tubes (Tarsons Pvt, Ltd, New Delhi, India) containing transport media which was 200 μl of 10X TE Buffer (Tris-HCL EDTA Buffer - Applied Biosystems, USA) and then sent to the laboratory immediately and stored at -70^{0}C in anultra-low temperature freezer until processing.

On day 20, OHI were reinforced via personal phone calls to the patient. The patients were recalled after 6 weeks and HbA_{1c} evaluation was done, following which, subgingival plaque was collected in a similar manner as described above and sent to the laboratory.

DNA extraction

DNA extraction from the plaque samples was done using highly purified Invitrogen DNA isolation kit (Purelink™ DNA extraction kit). The standard "proteinase K" method was followed for DNA isolation. The water bath was set at 55°C. 20 µl of proteinase K was added to a sterile micro-centrifuge tube.

200 µl of sample was transferred to the tube containing proteinase K and incubated at 55°C for 30 minutes. 20 µl of RNAse-A was added to the lysate and mixed well by briefly vortexing and was incubated at room temperature for 2 minutes.

200 µl of Purelink™ Genomic Lysis/Binding buffer was added and vortexed to obtain a homogenous solution. 200 µl of 96-100% ethanol was then added and vortexed for 5 seconds to obtain homogeneity and was subjected to purification protocol immediately.

Purification method

The purification of genomic DNA was done using Purelink™ kit. It was a spin-column based centrifugation procedure of 10-15 minutes.

1. Spin-column was removed from the package and placed in a clean collection tube.
2. The entire prepared lysate was added to the column.
3. It was centrifuged at 10000 rpm for a minute.
4. The column was then placed into a clean collection tube.
5. 500 µl of prepared wash-buffer was added.
6. Centrifugation for 3 minutes was done at maximum speed.
7. The column was placed in a sterile 1.5 ml micro-centrifuge tube.
8. 25-200 µl of genomic elution buffer was added and incubated at room temperature for 1 minute.
9. The column was centrifuged at maximum speed for 1 minute at room temperature thus obtaining purified genomic DNA in the tube.
10. Purified DNA was stored at -20°C until further processing.

PCR Protocol

Custom SYBR® Green assay reagents for *Porphyromonas gingivalis* and *Prevotella intermedia* (Life Technologies, Applied Biosystems, India) were used in this study.

The specific primer sequence were as follows:

- *P. gingivalis* forward- 3'-TGCAACTTGCCTTACAGAGGG -5'
- *P. gingivalis* reverse- 5'-ACTCGTATCGCCCGTTATTC- 3'
- *P. intermedia* forward- 3'-CCACATATGGCATCTGACGTG-5'
- *P. intermedia* reverse- 5'-TCAATCTGCACGCTATGGG-3'

In brief, a solution containing master mix (10 µl) and primers (1 µl each) for *P. gingivalis* or *P. intermedia*, extracted DNA of unknown sample (5 µl) and nucleus free water were mixed. A complete reaction 20 µl by volume was made.

PCR was programmed as follows:

- **Holding stage:** 95°C, 10 seconds
- **Shuttle heating:** 40 cycles, 95°C, duration of 15 seconds followed by 40 cycles, 60°C, duration of 1 minute.
- **Melt curve stage:** 95°C for 15 seconds, 60°C for 1 minute and 95°C for 15 seconds.

16S RNA was used as an endogenous control. (SYBR® Green assay reagents, Applied Biosystems, India).

- 16S RNA forward- 3'-TCCTACGGGAGGCAGCAGT-5'
- 16S RNA reverse- 5'- GGACTACCAGGGTATCTAATCCTG-TT-3'

Detection of the microorganisms was based on the number of PCR cycles necessary to obtain the threshold signal of fluorescence.

All the calculations were done using Applied Biosystems Software.

Statistical analysis

SPSS for windows (v 16.0) was used for all analysis and included descriptive, crosstab, independent samples '*t*' test and paired samples '*t*' test analysis.

Results

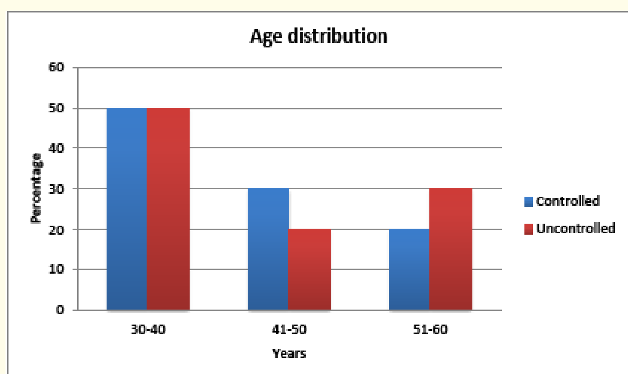
All participants in the study were known diabetics having type 2 diabetes, were on medications for the same, and had no other systemic conditions or complications. No alterations in medications or doses were reported during the entire course of the study.

Age distribution

The age of patients who were recruited in the study ranged between 30-58 years [(mean: 42±8.15 years in Group A, 44±8.8 years in Group B, respectively (Table 1 and Graph 1)].

Glycemic Status	Age (years)			Significance (%)
	30-40	41-50	51-60	
Controlled (Group A)	10	6	4	0.670
Uncontrolled (Group B)	10	4	6	

Table 1: Age distribution between the groups.



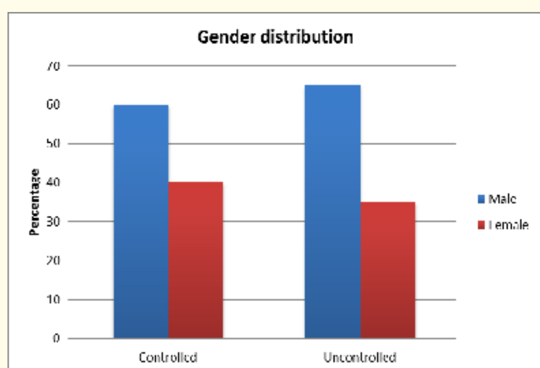
Graph 1: Age distribution between the groups.

Gender distribution

Out of 40 patients in all the groups 25 were males and 15 were females (62.5% and 37.5%, respectively). Group A consisted of 12 males (60%) and 8 females (40%) and Group B consisted of 13 males (35%) and 7 females (35%). There was no statistically significant intergroup gender distribution (Table 2 and Graph 2).

Glycemic Status	Gender		Significance
	Male	Female	(%)
Controlled (Group A)	12	8	0.483
Uncontrolled (Group B)	13	7	

Table 2: Gender distribution between the groups.



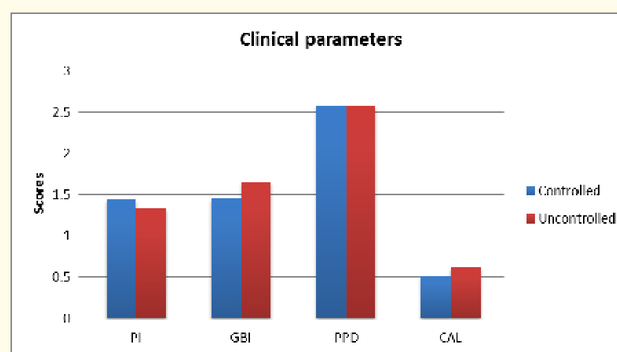
Graph 2: Gender distribution between the groups.

Comparison of PI, GI, PD and CAL in controlled and uncontrolled diabetic patients

The mean PI, GI, PPD and CAL in Group A were 1.43±0.53, 1.45 ±0.49, 2.58±0.95 and 0.51±0.80, respectively. In Group B, the mean PI, GI, PPD and CAL were 1.33 ± 0.50, 1.65 ± 0.53, 2.58 ± 0.67 and 0.61 ± 1.00, respectively. The differences, however, in the mean PI, GI, PPD and CAL between Group A and Group B were not statistically significant (*p*>0.05) (Table 3 and Graph 3).

Glycemic Status	Clinical Parameters			
	PI	GI	PPD	CAL
Controlled (Group A)	1.43±0.53	1.45±0.49	2.58±0.95	0.51±0.80
Uncontrolled (Group B)	1.33±0.50	1.65±0.53	2.58±0.67	0.61±1.00
Significance (*p*)	0.535	0.213	0.994	0.741

Table 3: Comparison of clinical parameters between the groups.



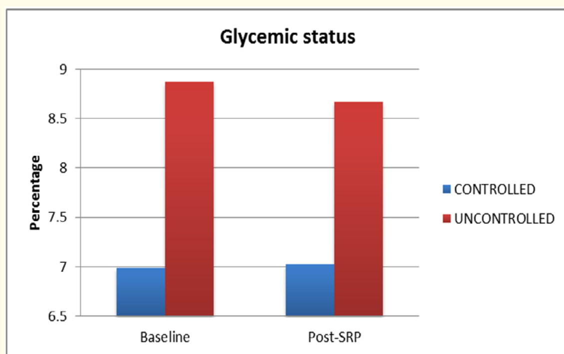
Graph 3: Comparison of clinical parameters between the groups.

Comparison of HbA_{1c} levels in controlled diabetic patients before and after SRP

The HbA_{1c} values at baseline in Group A ranged from 6.3% to 7.9%, with highest value recorded being 7.9%. The HbA_{1c} values at recall ranged from 6.1 % to 8.1 %, with highest value recorded being 8.1%. The changes in the mean HbA_{1c} values before and after SRP in Group A were not statistically significant (*p*>0.05). The baseline HbA_{1c} values in Group B ranged from 8.1% to 11%, with 11% being the highest recorded value. The HbA1c values at recall ranged from 7.7% to 10.7%, with lowest value being 7.7%. No statistical significance was seen in the changes in the mean HbA_{1c} values between both the groups before and after SRP (*p*>0.05) (Table 4 and Graph 4).

Glycemic Status	HbA_{1C} (%)		Significance (p)
	Baseline	Post-SRP	
Controlled (Group A)	6.99±0.57	7.03±0.66	0.839
Uncontrolled (Group B)	8.87±0.75	8.67±0.68	0.387
Inter-group significance (p)	0.839	0.387	

Table 4: Comparison of glycemic status between the groups.



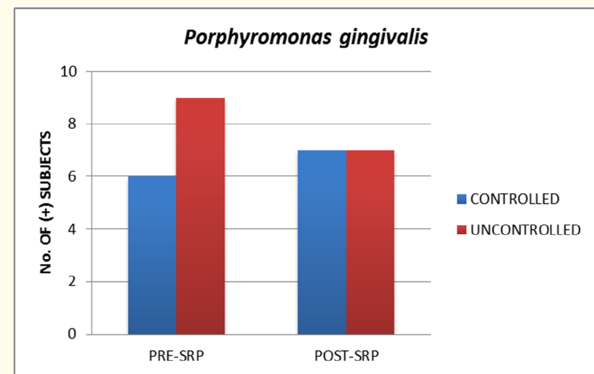
Graph 4: Comparison of glycemic status between the groups.

Comparison of the frequency of detection of P. gingivalis among controlled and uncontrolled diabetic patients before and after SRP

P. gingivalis was detected in a higher number of subjects in Group B (9 subjects) than in Group A (6 subjects) at baseline. There was a decrease in the number of subjects in whom *P. gingivalis* was detected after 6 weeks following SRP in both groups (1 and 2, respectively. However, intra-and inter-group baseline and follow-up changes and intergroup changes all findings were not statistically significant before or after (p>0.05) (Table 5 and Graph 5).

Glycemic Status	*Porphyromonas gingivalis* (number of subjects detected positive)		Significance (p)
	Baseline	Post-SRP	
Controlled (Group A)	6	7	1.000
Uncontrolled (Group B)	9	7	0.162
Inter-group significance (p)	0.277	1.000	

Table 5: Comparison of *P. gingivalis* levels between the groups.



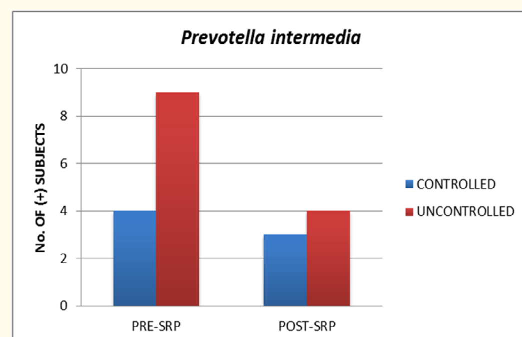
Graph 5: Comparison of *P. gingivalis* levels between the groups.

Comparison of the frequency of detection of *P. intermedia* among controlled and uncontrolled diabetic patients before and after SRP

P. intermedia was detected in a more patients belonging to Group B than in Group A (9 and 4, respectively) at baseline. There was not statistical significance (p>0.05). Fewer subjects showed detection of in both the groups 6 weeks after SRP was performed. Though Group B showed a greater difference than Group A as compared to baseline (5 subjects and 1 subject respectively), the intra- and inter-group variability was not statistically significant (p > 0.05) (Table 6 and Graph 6).

Glycemic Status	*Prevotella intermedia* (number of subjects detected positive)		Significance (p)
	Baseline	Post-SRP	
Controlled (Group A)	4	3	0.330
Uncontrolled (Group B)	9	4	0.210
Inter-group significance (p)	0.073	0.686	

Table 6: Comparison of *P. intermedia* levels between the groups.



Graph 6: Comparison of *P. intermedia* levels between the groups.

Discussion

Diabetes is a major risk factor for periodontal disease [18]. Inter- group age and gender showed no significant variability. Numerous studies have reported the similarities in the composition of the subgingival biofilm of periodontally healthy and diseased sites between diabetic and non-diabetic individuals. In this study, inter-group variability in PI scores was not significant at baseline. There were, however, a higher number of subjects in Group B in whom detection of *P. gingivalis* and *P. intermedia* was positive at baseline, though to a non-significant degree, with no statistically significant reduction in detection frequency at recall. This increase in baseline frequency of detection is consistent with the reports of Tervonen *et al.* [12] who investigated the prevalence of periodontal pathogens using an immunoassay. They observed that most inflammatory sites harboured *P. gingivalis* and *P. intermedia* to a significant degree. Other clinical parameters like GI, PPD and CAL also showed no significance in variability among the groups, nor were there significant differences in correlations of clinical parameters and the glycemic status at baseline in both the groups.

PCR was used in the present study for microbiological analysis and the presence of the organism was expressed based on the number of positive detections in the groups.

Kiran *et al.* [19] reported that diabetic patients showed a statistically significant improvement in the glycemic status after SRP without systemic antibiotic therapy on periodontal health and glycaemic control similar outcomes were observed in other studies [16,20-24]. However, the present study shows no such changes in HbA_{1c} following periodontal treatment at 6 weeks. The absence of immediate effects of SRP on glycemic status indicates that the beneficial effects of periodontal therapy may have only long term benefits on the glycemic status which may hypothetically be attributed to the longer duration of time required for resolution of the systemic inflammatory burden.

Though not statistically significant, marginal changes in glycaemic control were observed in some patients at 6 weeks. This individual variability in the HbA_{1c} values can be attributed to the varying baseline values. Patients having higher baseline values of HbA_{1c} had greater reductions of glycemic values than those with lower baseline values.

Lastly, the glycated hemoglobin test to determine glucose in plasma is independent of factors like diet, physical activity and/or medication. It provides an average estimation of the glucose levels over the preceding 1 to 3 months. It does not account for short-term fluctuations in plasma glucose levels [25], thereby making it a more reliable test for longer durations of assessment.

Conclusion

From the results obtained in this study, a marginal short-term improvement on glycemic status immediately following SRP over a short period as early as 6 weeks could be anticipated. However, it was evident in this study that individuals with a poorer glycemic status could nurture more periodontal pathogens than those with good glycemic control thereby increasing the risk of periodontitis which could be controlled by scaling and root planing. Further interventional and longitudinal studies with adequate sample size are warranted to corroborate substantial changes in the glycemic status and the subgingival microflora in different diabetic states.

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