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# A Comparative Study of Serum Glycosylated Hemoglobin Levels andPeriodontal Therapy in Smokers and Non-Smokers with Chronic Periodontitis Patient

Running Title: Effect of Non-Surgical Periodontal Therapy in Non-Diabetic Patients.

***Sakshi Gaind[1], Tushar Pruthi[2], Akash Raj Sharma[3], M. Shalini[4], Bikram Singh Brahma[5], Shivani[6]**

1. Assistant Professor, Department of Periodontology, Institute of Dental Studies & Technologies, Modinagar, India. (Corresponding Author) (email id- sakshigaind@gmail.com)
2. Associate Professor, Department of Pediatric and Preventive Dentistry, Subharti Dental College and Hospital, SVSU, Meerut, India.
3. Assistant Professor, Department of Prosthodontics & Crown and Bridge, Subharti Dental College and Hospital, SVSU, Meerut, India.
4. Dental Unit, Department of Prosthodontics, AIIMS Bathinda, Punjab, India.
5. Consultant Periodontist and Implantologist, Guwahati, Assam, India.
6. Assistant Professor, Department of Prosthodontics & Crown and Bridge, Kalka Dental College and Hospital, Meerut, India.

**Abstract.**

*Background and aim:* Smoking is the major risk factor for periodontitis, and affects the extent and severity of disease. Glucose can bind irreversibly to hemoglobin, through a non-enzymatic reaction to form glycosylated hemoglobin. Because it is based on the average life span of an erythrocyte, serum glycosylated hemoglobin levels reflect glycemic control over the previous one to three months. The aim of this study assess the serum glycosylated hemoglobin levels and clinical outcome in smokers and non–smokers with chronic periodontitis following periodontal therapy.

*Methods:* A total of 40 subjects (Group I- 20 Non-Smokers, Group II- 20 Smokers) in the age group of 20-40 years participated in this study. Both groups received initial periodontal therapy. Clinical parameters such as plaque index, gingival index, extent and severity index were evaluated at baseline and 3 months after initial periodontal therapy. Serum glycosylated hemoglobin was investigated at baseline and 3 months in both groups. The statistical tests used were paired t-test.

*Results:* After 3 months initial periodontal therapy both the groups exhibited improvement in clinical periodontal parameters. However, statistically significant results were observed in all the clinical periodontal parameters. When the post treatment clinical periodontal parameters and serum glycosylated hemoglobin levels were evaluated, statistically non-significant differences were obtained in both the groups.

*Conclusions*: Initial periodontal therapy is effective tool in periodontal diseases. This study demonstrated that Group-I and Group II showed significant improvement in all clinical periodontal parameters and serum glycosylated hemoglobin levels after 12 weeks.(www.actabiomedica.it)

**Key words:** Diabetes Mellitus, Glycosylated Hemoglobin, Periodontal Therapy, Smoking

**Introduction** – According to the World Health Organization (WHO), non-communicable diseases (NCDs) accounted for 74% of deaths globally in 2019, of which, diabetes resulted in 1.6 million deaths, thus becoming the ninth leading cause of death globally. The major factors for the global increase in the diabetes epidemic is due to socioeconomic changes, with other associated risk factors

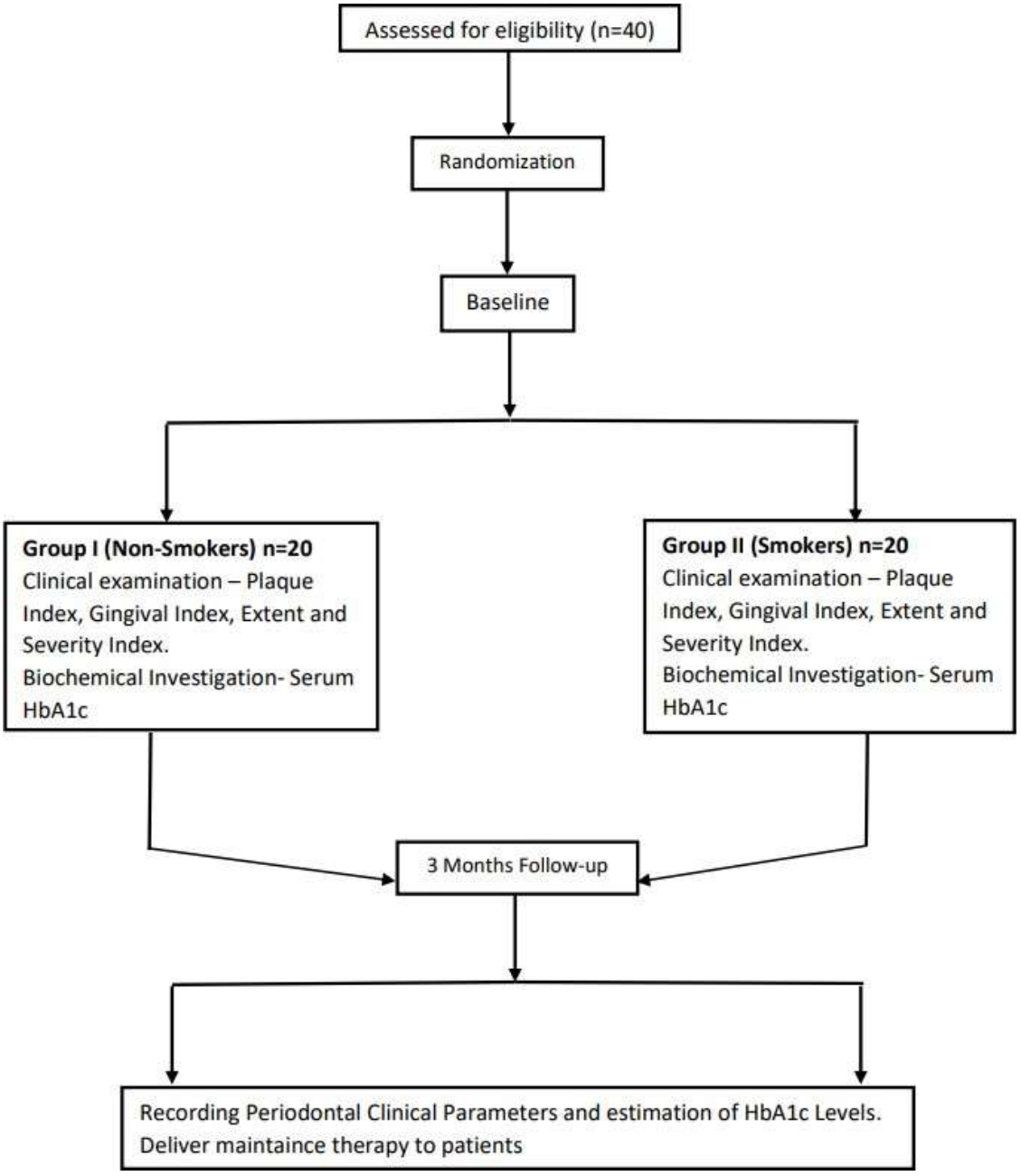
such as population growth, unhealthy eating habits, and a sedentary lifestyle modification.(1) In India, 62.4 million people are suffering from Type 2 Diabetes Mellitus and 77 million are suffering from prediabetes.(2) More than 50% of Indian population are suffering from periodontal diseases.(3)
Periodontal disease is an entity of localized infections that involves tooth supporting tissues, the structure that make up the periodontium. The designation periodontal disease includes both reversible (gingivitis) and irreversible (periodontitis) process.(4) The pathological nature of periodontal disease is certainly based on inflammatory responses to pathogens and destructive materials. In 1993, Loe et al. stated that periodontal disease as the sixth complication of diabetes, after neuropathy, diabetic nephropathy, retinopathy, vascular diseases and delayed healing.(5)
Smoking causes periodontal diseases. Periodontal disease is an entity of localized infections that involves tooth supporting tissues, the structure that make up the periodontium.(6,7) Many authors stated that nicotine which is present in cigarette, has been demonstrated to increase the blood glucose levels.(8) It is the most active substance in tobacco which is absorbed through lung alveoli.(9) Nicotine not only has a direct toxic effect on pancreatic beta cells but also is associated with increased insulin resistance leading to impaired glucose tolerance. Furthermore, the antiestrogenic effect of nicotine could contribute to an increase in visceral adipose tissue accumulation and via this mechanism, insulin resistance. Finally nicotine increases cortisol level and inflammation and has influence on adiponectin a peptide that regulates food intake and body weight, all of which could contribute to higher HbA1c.(5) Nicotine is highly addictive. It may cause rise in blood pressure, increased heart, respiratory rates, and peripheral vasoconstriction which leads to contraction of oral capillaries affecting periodontal tissue and gingival blood flow.(7)
Glycated hemoglobin (HbA1c) is a marker of long-term glucose homeostasis reflecting average blood glucose concentration in the past two to three months.(5) The process of non-enzymatic addition of carbohydrate to polypeptides and proteins is called glycosylation/ glycation and the products that are formed are called as advanced glycated end products (AGE's).(10)
Thus, the aim of this study was to compare the efficacy of serum glycosylated hemoglobin levels and periodontal therapy in smokers and non-smokers with chronic periodontitis patients without Type 2 Diabetes Mellitus.

**Materials And Methods** - This study was carried out in Department of Periodontology, Seema Dental College & Hospital, Rishikesh, Uttarakhand, India with the approval of institutional ethical committee. The reference number of the ethical clearance was SDC/2018/A-115. The study was conducted over a duration of 3 months starting from March 2018 and extending to June 2018 at Dehradun, Uttarakhand, India.
Sample Size: A sample size of 40 were required to achieve 80% power and 5% significance. During this study, 40 patients reporting to the outpatient department were selected who were found to have who were smokers/ non-smokers with chronic periodontitis. The study subjects were categorized into two groups consisting of twenty subjects each.

**Selection Criteria:**

A total of 40 subjects (Group I- 20 Non-Smokers, Group II- 20 Smokers) in the age group of 20-50 years participated in this study. Clinical parameters such as Plaque Index, Gingival Index, Extent and Severity Index were evaluated at baseline and 3 months after initial periodontal therapy. Serum glycosylated hemoglobin was investigated at baseline and 3 months in both groups. Each patient was given detailed verbal and written description of the treatment.
*Inclusion Criteria*: Patients who were smokers and non-smokers with chronic periodontitis. Patients who were systemically healthy with no history of periodontal therapy in last six months.

*Exclusion Criteria*: Medically compromised patients with underlying systemic disease and those patients who were not willing to report for follow-up were excluded from this study.
The selected patients were randomly allocated to two groups (Flowchart 1)
Group I (20 patients): Patients who were non-smoker with chronic periodontitis
Group II (20 patients): Patients who were smoker with chronic periodontitis
The patients were scheduled for laboratory and periodontal examinations before treatment (baseline), and after nonsurgical treatment (3-month recall visits).




Flowchart 1: Schematic diagram of the study design

**Clinical Procedure**

In Group I (non-smoker) patients who received a full-mouth scaling and root planing at baseline by performing the supra and subgingival scaling using an ultrasonic scaler and root planning using Gracey curettes. Oral hygiene instructions were given to all patients. Patients were instructed to brush

their teeth twice daily and rinse with 0.2% chlorhexdine gluconate solution twice daily. Oral hygiene control and reinstruction were reviewed each recall visit. Venous blood samples were taken for each patient to measure HbA1c (glycosylated hemoglobin) value at baseline, 3 months, after treatment.
In Group II (smoker) patients who received a full-mouth scaling and root planing at baseline by performing the supra and subgingival scaling using an ultrasonic scaler and root planning using Gracey curettes. Oral hygiene instructions were given to all patients. Patients were given instructions to maintain oral hygiene and motivated to stop smoking. Patients were instructed to brush their teeth twice daily and rinse with 0.2% chlorhexdine gluconate solution twice daily. Oral hygiene control and reinstruction were reviewed each recall visit. Venous blood samples were taken for each patient to measure HbA1c (glycosylated hemoglobin) value at baseline, 3 months, after treatment.
**Parameters Assessed**- The following clinical parameters were assessed at baseline before the periodontal therapy and at 3 months. Clinical periodontal parameters such as Plaque Index, Gingival Index, Extent and Severity Index were taken in the both groups. Serum Glycosylated Hemoglobin Levels were taken in both groups. Clinical parameters were carried out by a precalibrated examiner with the appropriate armamentarium.

**Results** – Demographic details are given in Table 1. There was no statistically significant difference between the two groups in terms of age, gender ($p$ >0.05). The statistical tests used were paired t-test and chi square test.

Table 1: Age, Gender distribution of the Group I and Group II.

| **Variable** | **Group-I (Non-smokers) N=20** | **Group-II (Smokers) N=20** | **df** | ***P* value** |
|---|---|---|---|---|
| Age (Mean ± SD) | 38.05 ± 8.00 | 39.95 ± 8.86 | 39.01 ± 8.86 | >0.05* |
| Gender (Male:female) | 16:14 | 16:14 | 32:28 | >0.05* |

SD-Standard deviation
df- difference
**P* value obtained is non significant

**Change of Clinical Parameters after Nonsurgical Periodontal Treatment** (Table 2)

At baseline, there was statistically significant difference in clinical periodontal parameters between the two groups (p >0.05). After nonsurgical treatment, the periodontal clinical parameters of Group I and Group II showed statistically significant changes. At third month follow-up, the results showed that there was a reduction in clinical periodontal parameters. (Table 2)

Table 2: Statistical comparison of mean values of Clinical Periodontal parameters at different intervals for Group-I (Non-Smokers) and Group-II (Smokers).

| | Group-I (Non-smokers) | | | | | Group-II (Smokers) | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Parameter** | **Different Interval** | **Mean ± SD** | **Mean Difference** | **t** | ***P* value** | **Mean ±SD** | **Mean Difference** | **t** | ***P* value** |
| Plaque index | Baseline | 1.87 ±0.49 | 0.33 ±0.30 | 4.939 | **<0.001*** | 2.25±0.363 | 0.52 ± 0.32 | 7.12 | **<0.001*** |
| | 3 months | 1.54 ±0.31 | | | | 1.72 ±0.28 | | | |
| Gingival index | Baseline | 2.23 ±0.29 | 0.34 ± 0.19 | 7.85 | **<0.001*** | 1.94 ±0.407 | 0.36 ± 0.303 | 5.327 | **<0.001*** |
| | 12 weeks | 1.88 ±0.36 | | | | 1.58 ±0.27 | | | |
| Extent | Baseline | 84.20±11.62 | 5.33 ± 1.59 | 14.9 | **<0.001*** | 90.64 ±6.61 | 5.52 ± 2.43 | 10.16 | **<0.001*** |
| | 12 weeks | 78.87±11.69 | | | | 85.12 ±7.22 | | | |
| Severity | Baseline | 3.52 ± 0.53 | 0.18 ± 0.17 | 4.949 | **<0.001*** | 4.01 ±0.61 | 0.31 ± 0.25 | 5.53 | **<0.001*** |
| | 12 weeks | 3.33 ± 0.54 | | | | 3.70 ±0.57 | | | |

SD - Standard Deviation

*$p$ value obtained is significant

**Change of Hba1c Level after Nonsurgical Periodontal Treatment (**Table 3)

At baseline, there was statistically significant difference in glycosylated hemoglobin at baseline and 3 months of both groups.

Table 3: Statistical comparison of mean values of glycosylated hemoglobin at different intervals for Group-I (non-smokers) and Group-II (smokers).

| | Group-I (Non-smokers) | | | | | Group-II (Smokers) | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Parameter** | **Different Interval** | **Mean ± SD** | **Mean Difference** | **t** | ***P* value** | **Mean ± SD** | **Mean Difference** | **t** | ***P* value** |
| Glycosylated Hemoglobin | Baseline | 5.43 ±0.39 | 0.43 ± 0.27 | 6.93 | **<0.001*** | 5.95 ± 0.604 | 0.41 ± 0.23 | 8.068 | **<0.001*** |
| | 12 weeks | 5.005 ±0.27 | | | | 5.53 ± 0.457 | | | |

SD- Standard Deviation,

*$P$ value obtained is significant

**Comparison of mean difference for clinical periodontal parameters and HbA1c at different time intervals for non-smokers and smokers group.**

The intragroup comparison of the clinical periodontal parameters (Plaque index, gingival index, extent and severity index) of group I (non-smokers) and group II (smokers) the mean values were not statistically significant (Table 4). Regarding glycemic control, In intergroup comparison Group I (non-smokers) and group II (smokers) glycosylated hemoglobin at baseline and 3 months of both groups the mean values were not statistically significant showed an insignificantly difference (Table 4).

Table 4: Statistical Comparison of mean difference for plaque index, gingival index, extent, severity and HbA1c at different time intervals for non-smokers and smokers group.

| | **Group-I (Non-smokers)** | **Group-II (Smokers)** | | |
|---|---|---|---|---|
| **Parameter** | **Mean ± SD** | **Mean ± SD** | **t** | **P value** |
| Difference in plaque index | 0.332 ± 0.3006 | 0.52 ±0.32 | -1.919 | 0.063* |
| Difference in gingival index | 0.348 ± 0.198 | 0.36 ± 0.303 | -0.167 | 0.869* |
| Difference in extent | 5.33 ± 1.59 | 5.527 ± 2.43 | -0.303 | 0.764* |
| Difference in severity | 0.188 ± 0.170 | 0.313 ± 0.253 | -1.831 | 0.075* |
| Difference in HbA1c | 0.43 ± 0.277 | 0.415 ± 0.230 | 0.186 | 0.853* |

SD- Standard Deviation,

**P* value obtained is non significant

**Discussion –** Glycohemoglobin is formed by a non-enzymatic interaction between glucose and the amino groups of the valine and lysine residues in hemoglobin. Formation of glycohemoglobin is irreversible and the level in the red blood cell depends on the blood glucose concentration.(11) In this study glycemic status was evaluated in smokers and non-smokers at baseline and 3 months with higher mean values in smokers. In this study total of 40 patients (Group I- 20 Non-Smokers, Group II- 20 Smokers) in the age group of 20-50 years were distributed to two groups, each patient received nonsurgical periodontal treatment. Clinical parameters related to chronic periodontitis include plaque index, gingival index, extent and severity index were evaluated at baseline and 3 months after initial periodontal therapy. By recording these parameters, previous periodontal destruction, ongoing disease and prediction of disease progression may be monitored and are essential for treatment planning and to evaluate treatment outcomes. Serum glycosylated hemoglobin levels was investigated at baseline and 3 months after initial periodontal therapy in both groups. Smoking affects active periodontal therapy and responds less favorably to treatment outcomes. Smokers have less gingival scores as nicotine causes vasoconstriction in peripheral blood vessels and reduces the clinical signs of gingival inflammation. In the current study, it was observed that there was significant reduction in plaque and gingival index at 3 months after non-surgical periodontal therapy in Group I (non-smokers) which is in accordance with the study of Indurkar (12) who reported the clinical effects of mechanical non-surgical periodontal therapy in subjects with chronic periodontitis over a period of six weeks and concluded that decrease in scores of plaque and gingival index. On comparison of mean difference between the groups, there was no statistically significant difference observed in PI and GI with reduction in scores of non-smokers. This observation indicates that non-smokers maintained optimum level of hygiene throughout the course of study which was in accordance with the study of Aziz AS et al.(13) who assessed the short-term effectiveness of scaling and root planing on clinical parameters, systemic inflammatory and oxidative stress markers between smokers and non-smokers and concluded that smokers exhibited more periodontal damage and higher systemic inflammatory and oxidative stress burden than non-smokers. In the current study Extent and Severity Index was evaluated to assess the periodontal destruction in smokers and non-smokers. Since there is lack of literature regarding extent and severity index the results have been compared with other similar studies Thomson KFR et al,(14) Carlos JP et al.(15) Thomson KFR et al.(14) assessed clinical attachment loss to determine prevalence, extent and severity of severe periodontitis and concluded that current smokers had significantly higher extent of periodontal destruction. Another study by Carlos JP et al(15) evaluated extent and severity index in 369 aged 17-32yrs old. 22% of sites

examined showed evidence of disease, with an average severity of 1.48 mm attachment loss per diseased site. When re-examined approximately three years later, both extent and severity had increased significantly during the three-year period. In this study glycemic status was evaluated in smokers and non-smokers at baseline and 3 months with higher mean values in smokers. Ohkuma T et al.(16) reported that glycosylated hemoglobin increased significantly with increase in number of cigarettes per day compared with non-smokers, indicating a dose-response relationship.(11) There was statistically significant reduction in glycated hemoglobin at three months after non-surgical periodontal therapy in group I (non-smokers) and Group II (smokers) which was in accordance with the Study of Vaghani H et al.(17) Muthu J et al.(18) Vaghani H et al.(17) evaluated the glycated hemoglobin levels in healthy patients and patients with periodontitis, before and after non-surgical periodontal therapy and concluded that glycated hemoglobin of patients with periodontitis were significantly reduced after three months of non-surgical periodontal therapy. Muthu J et al.(18) compared HbA1c in subjects with periodontitis and healthy controls and evaluate the effect of non-surgical periodontal therapy on the glycemic control in periodontitis and concluded that HbA1c was higher in periodontitis. 3 months following periodontal therapy there was improvement in periodontal parameters and decreased in HbA1c levels. There was statistically significant reduction in HbA1c at 3 months after non-surgical periodontal therapy in Group II (smokers) which is contraindicatory to study by Verma N(19) who found no significant difference in mean values of HbA1c in cigarette smokers and Bidi smokers and no correlation between smoking index and HbA1c.

Study by Jyothirmayi B et al.(10) evaluated the association between cigarette smoking and glycated hemoglobin levels and found a significant association between smoking and glycated hemoglobin levels. This was similar to Torrungruang K et al.(20) determine the effect of cigarette smoking on the severity of periodontitis in older Thai adults and concluded that former smokers were more likely to have severe periodontitis than non-smokers. Ali OH et al.(21) evaluated the clinical parameters plaque index, gingival index, probing depth, clinical attachment level in smokers and non-smokers and concluded that smokers exhibited more amount of plaque and lesser amount of gingival index. Study by Urberg M et al.(22) is contradictory to present study who reported the effects of smoking on plasma glucose by comparing the glycosylated hemoglobin levels of subjects who smoked one pack per day or more with those of non-smokers and concluded that smokers have average blood glucose that is higher than that of non-smokers. On comparison of mean difference between the groups, there was no statistically significant difference observed in PI and GI with reduction in scores of non-smokers. This observation indicates that non-smokers maintained optimum level of hygiene throughout the course of study. Thus, Bacteria can survive and grow in the complex ecosystem of biofilm as they produce of various virulence factors to evade the host immune defense system and destroy host periodontal tissues. (23) Thus, diagnosis and management of periodontal disease is essential for treatment and maintenance of periodontitis-susceptible patients. (24,25) Further long term study with large sample size and follow up areneeded to confirm the findings of this study.

**Conclusion** - This study demonstrated that both groups showed significant improvement in clinical periodontal parameters and serum glycosylated hemoglobin after non-surgical periodontal therapy. On comparison Group-I (non-smoker) showed better treatment outcome as compared to Group-II (smokers).

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**Conflict of Interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

**Authors Contribution:** all authors contributed to conceptualization, methodology, software, validation, formal analysis, investigation, data curation, as well as writing of original research.

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