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Original Research Paper

Periodontology

COMPARATIVE EVALUATION OF GLYCOSYLATED HEMOGLOBIN LEVEL IN PERIODONTITIS CASES AND HEALTHY CONTROLS: A CLINICAL AND HEMATOLOGICAL STUDY

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ABSTRACT **Background:** Diabetes mellitus and chronic periodontitis are the common chronic diseases in adults in the world population. The influence of diabetes on the risk of developing periodontal disease has been the subject of much discussion. Both diabetes mellitus and periodontitis are of chronic nature and result from various etiologic factors, wherein environmental and genetic factors play an important role. It is now recognized that a low-grade inflammatory response precedes the development of diabetes mellitus. This concept strengthens the hypothesis that diabetes and periodontal disease are linked via dysregulated inflammatory and immune responses. **Method:** In this study we aimed at determining whether glycosylated hemoglobin (HbA1c) is elevated in individuals with periodontitis but not diagnosed with Type 2 diabetes, and thereby finding the impact of Periodontitis on blood level of Glycosylated Hemoglobin (HbA1c). **Result:** HbA1c values when compared between healthy individuals and individuals with only periodontitis and no diabetes, were statistically significant (p value <0.05). This gives us a strong indication that HbA1c is elevated in cases with established periodontitis, but with no diabetes, as compared to systemically and periodontally healthy controls. **Conclusion:** It can be thus speculated that HbA1c is positively correlated to periodontal destruction, and the chronicity of the periodontal disease can lead to poorer glycemic control (progressive increase in HbA1c values).

KEYWORDS : Glycosylated Hemoglobin (HbA1c), Advanced Glycation End Products (AGEs), Fasting Blood Sugar (FBS).

INTRODUCTION

Periodontitis in adults is a bacterial infection caused by gram-negative anaerobes, which populate the sub gingival plaque. Diabetes mellitus encompasses a heterogeneous group of disorders with the common characteristic of altered glucose tolerance or impaired lipid and carbohydrate metabolism. The clinical manifestations in diabetes are related to the complications of diabetic hyperglycaemic state either directly or indirectly. Both diabetes mellitus and periodontitis are linked via dysregulated inflammatory and immune responses.[1] According to many authors, individuals suffering from diabetes mellitus are at increased risk for the development of periodontal disease.[2] Periodontal disease has been called as the sixth complication of diabetes, along with retinopathy, nephropathy, neuropathy, macro vascular disease, and altered wound healing.[3]

Periodontitis also seems to be a risk factor for incident diabetes.[4] Periodontal diseases can have a significant impact on the metabolic state in diabetes. The presence of periodontitis increases the risk of worsening glycaemic control over time.

Majority of studies conducted on improved glycaemic control after decreasing gingival inflammation have shown conflicting data, and are difficult to interpret. The relationship between diabetes and periodontal diseases has been the subject of more than 200 articles published during the past 50 years. Although many studies reported more severe periodontal disease in subjects with diabetes than those without diabetes[5], relatively few examined the association between periodontitis and glycaemia or the level of glycated/glycosylated hemoglobin (HbA1c) in adults without diabetes. The purpose of this study is to examine the association between periodontitis and glycosylated hemoglobin (HbA1c) in adults.

MATERIAL AND METHODS

A case control study was carried out in a tertiary care dental centre for a duration of twelve (12) months, for estimation and comparison of glycosylated hemoglobin (HbA1c) levels in patients with and without periodontitis.

This study was aimed at determining, whether HbA1c is elevated in individuals with periodontitis but not diagnosed with Type 2 diabetes, and thereby finding the impact of Periodontitis on blood level of HbA1).

A sample size of one hundred and twenty (120) individuals, with age group ≥ 40 years was considered after proper selection following pre devised inclusion and exclusion criteria.

Inclusion Criteria

1. Age of the selected individuals to be equal to or above 40 years.
2. All the selected individuals to have equal to or more than ten natural teeth.
3. All the selected individuals should have no history of long term antibiotic use (≥ 14 days) in the past 6 months.
4. Cases with periodontitis to have at least five teeth with probing depth (PD) ≥ 5mm, bleeding on probing (BOP) ≥ 15 percent, and loss clinical attachment > 1 mm.[6]
5. Healthy controls not to have probing depth (PD) > 4 mm, bleeding on probing (BOP) at ≤ 15 percent, and no periodontal treatment (scaling and root planing or surgery) within the previous 6 months.[6]
6. Diabetic cases to have fasting blood sugar level ≥ 126

mg/dl. Diabetic status to be subsequently confirmed by Oral Glucose Tolerance Test (OGTT) and established by an Endocrinologist/ Medical Specialist. [7]

Exclusion Criteria

1. Non-compliant individuals.
2. Individuals with conditions that shorten erythrocyte survival (e.g., haemolytic anaemia, recent blood loss).
3. Individuals who are positive for rheumatoid factors.
4. Individuals who have received periodontal treatment (scaling and root planing) within the previous 6 months.
5. Pregnant and lactating mothers.
6. Patients presenting with any systemic disease.

The study was approved by the ethical committee of the institute. Before carrying out the study, the subjects were explained about the same and an Informed Consent was signed by them.

Demographic parameters namely gender, age, socio-economic status, and educational status of all the subjects were recorded. Baseline clinical examination included assessment of PD, REC, CAL, BOP and Oral Hygiene Index-Simplified (OHI-S) for all the subjects. Chairside fasting blood sugar (FBS) estimation was carried out for each of the subjects just before grouping. Diabetic status of the subjects was confirmed by the Oral Glucose Tolerance Test (OGTT), and analysis by an Endocrinologist/ Medical Specialist, prior to the grouping. These samples after the baseline examination were further divided into four study groups as under:

Group A- 30 subjects without Periodontitis and without Diabetes.
Group B- 30 subjects with Periodontitis and without Diabetes.
Group C- 30 subjects without Periodontitis and with Diabetes.
Group D- 30 subjects with Periodontitis and with Diabetes.

The Glycosylated Hemoglobin (HbA1c) levels of the subjects was estimated in the laboratory by the method of "High Performance Liquid Chromatography" (HPLC). Chair side fasting blood samples of was collected by finger prick method using a lancet, from all the subjects and was subjected to fasting blood glucose estimation by using a handheld portable glucometer. To furthermore confirm the diabetic status of the all the subjects, blood sugar estimation by the Oral Glucose Tolerance Test (OGTT) was carried out at the service laboratory. 2-hour post load glucose ≥200 mg/dl confirmed the diabetic status of the subjects who had already scored a FBS of ≥ 126 mg/dl and were grouped as diabetics. All the reports were established by the Endocrinologist/ Medical specialist. This was in accordance to the report of the expert committee on the diagnosis and classification of diabetes mellitus.[7]

A periodontal probe with Williams marking was used for assessing the Probing Depth (PD), recession (REC), and Clinical Attachment Level (CAL). Bleeding on probing was assessed with the aid of Gingival Bleeding Index (GBI). Baseline oral hygiene of all the subjects were assessed with the help of Oral Hygiene Index- Simplified (OHI-S).

The statistical analysis was done through SPSS for Windows, Version 16.0 Evaluation version (SPSS Inc., New York). 'P' value was set at 0.05 for the statistical significance. The qualitative demographic parameters were subjected to Crosstab tests followed by Pearson Chi-Square tests. The quantitative demographic parameter was subjected to Crosstab tests followed by Oneway ANOVA test. The clinical and biochemical parameters were first evaluated with ANOVA test followed by Post Hoc tests.

RESULT

The demographic parameters of gender, age, socio economic status and education, were all statistically not significant amongst the 04 groups on comparison.

The clinical parameters of probing Depth (PD), recession (REC), clinical attachment level (CAL), bleeding on probing assessed with the aid of Gingival Bleeding Index (GBI) and baseline oral hygiene of all the subjects assessed with the help of Oral Hygiene Index-Simplified (OHI-S), when compared between Group A and C, were statistically non-significant (p value >0.05), but statistically significant results were observed in between Group A and Group B, Group A and Group D, Group B and Group C, Group B and Group D, Group C and Group D.

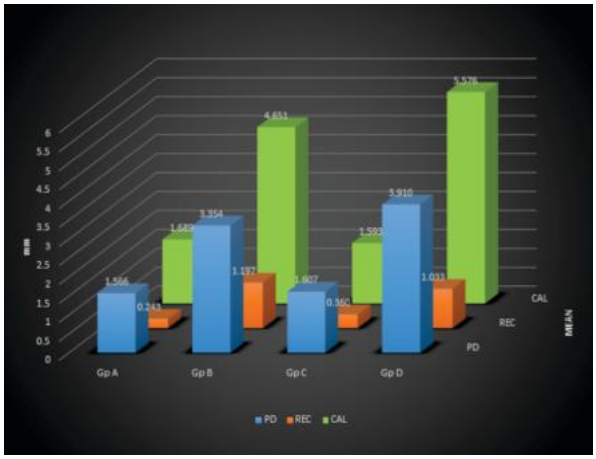

Chart: Clinical Variables: Intragroup/ Intergroup Charting: PD/ REC/ CAL

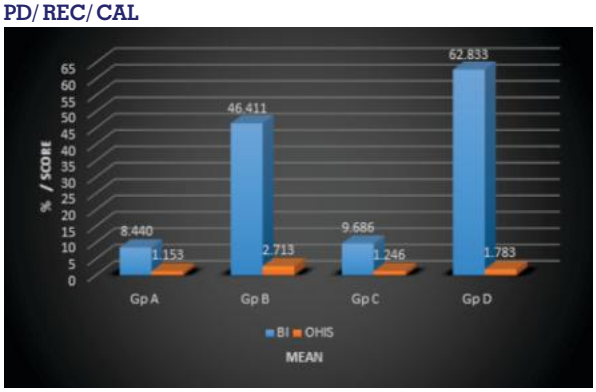

Chart: Clinical Variables: Intragroup/ Intergroup Charting: BI/ OHIS

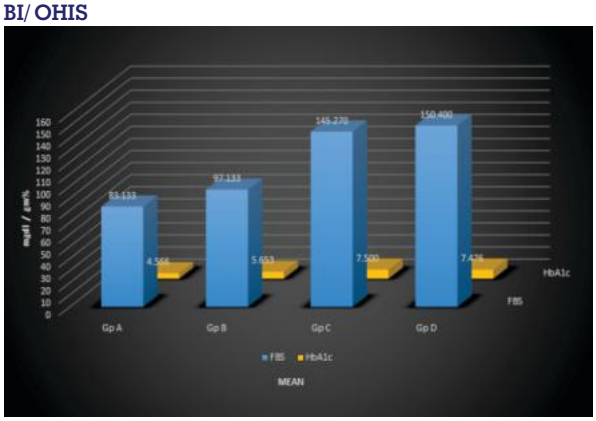

Chart: Biochemical Variables: Intragroup/ Intergroup Charting: FBS/ HbA1C

The biochemical parameters of fasting blood sugar (FBS) and Glycosylated Hemoglobin (HbA1c) levels of the subjects were statistically evaluated, and the results were:

FBS

Intragroup- The mean FBS of Group A, B, C and D were 83.133+/- 7.824 mg/dl, 97.133+/- 9.354 mg/dl, 145.270+/- 16.492mg/dl and 150.400+/- 18.475 mg/dl respectively (Table 17). The least value of FBS range was found in Group A

patients (min 72.00 mg/dl & max 105.00 mg/dl), with a mean (83.133+/- 7.824 mg/dl) which was lower than that of Group B (97.133+/- 9.354 mg/dl). The highest value of FBS range was found in Group D patients (min 128.00 mg/dl & max 187.00 mg/dl), with a mean (150.400+/- 18.475 mg/dl) which was higher than that of Group C (145.270+/- 16.492mg/dl). Group D had maximum average FBS of150.400+/- 18.475 mg/dl, and Group A had minimum average FBS of83.133+/- 7.824 mg/dl. The least recorded FBS value was 72.00 mg/dl in Group A, and a maximum value was 187.00 mg/dl in Group D.

Intergroup- FBS when compared between Group A and B (p value 0.001), were statistically significant (p value <0.05). Similar results were observed with the FBS values in between Group A and Group C (p value 0.000), Group A and Group D (p value 0.000), Group B and Group C (p value 0.000), Group B and Group D (p value 0.000). Statistically insignificant results were observed with the FBS values in between Group C and Group D (p value 0.477).

HBA1c

Intra Group- The mean HbA1c values of Group A, B, C and D were 4.566+/- 0.389 %, 5.653+/- 0.312 %, 7.500+/- 0.854 %, and 7.476+/- 0.789 % respectively. The least value of HbA1c range was found in Group A patients (min 3.80 % & max 5.50 %), with a mean (4.566+/- 0.389 %) which was lower than that of Group B (5.653+/- 0.312 %), with a range of min 5.00 % & max of 6.30 %. The highest value of HbA1c range was found in Group C and Group D patients (min 6.20 % & max 8.90 % in both). Group C had maximum average HbA1c of 7.500+/- 0.854 %, and Group A had minimum average HbA1c of 4.566 +/- 0.389 %.

Inter Group- HbA1c values when compared between Group A and B (p value 0.000), were statistically significant (p value <0.05). Similar results were observed with the HbA1c values in between Group A and Group C (p value 0.000), Group A and Group D (p value 0.000), Group B and Group C (p value 0.000), Group B and Group D (p value 0.000). Statistically insignificant results were observed with the HbA1c values in between Group C and Group D (p value 0.999).

Intergroup Analysis: Biochemical Variables

Table: Intergroup Analysis FBS Multiple Comparisons: ANOVA (Post Hoc Tests: Tukey HSD)

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Low Bound	High Bound
FBS (mg/dl)	A	B	-14.00000*	3.56387	0.001	-23.2898	-4.7102
		C	-62.13333*	3.56387	0.000	-71.4231	-52.8435
		D	-67.26667*	3.56387	0.000	-76.5565	-57.9769
	B	A	14.00000*	3.56387	0.001	4.7102	23.2898
		C	-48.13333*	3.56387	0.000	-57.4231	-38.8435
		D	-53.26667*	3.56387	0.000	-62.5565	-43.9769
	C	A	62.13333*	3.56387	0.000	52.8435	71.4231
		B	48.13333*	3.56387	0.000	38.8435	57.4231
		D	-5.13333	3.56387	0.477	-14.4231	4.1565
	D	A	67.26667*	3.56387	0.000	57.9769	76.5565
		B	53.26667*	3.56387	0.000	43.9769	62.5565
		C	5.13333	3.56387	0.477	-4.1565	14.4231

*. The mean difference is significant at the 0.05 level.

Table: Intergroup Analysis HbA1c Multiple Comparisons: ANOVA (Post Hoc Tests: Tukey HSD)

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Low Bound	High Bound
HbA1C (gm %)	A	B	-1.08667*	0.16347	0.000	-1.5128	-0.6606
		C	-2.93333*	0.16347	0.000	-3.3594	-2.5072
		D	-2.91000*	0.16347	0.000	-3.3361	-2.4839
	B	A	1.08667*	0.16347	0.000	0.6606	1.5128
		C	-1.84667*	0.16347	0.000	-2.2728	-1.4206
		D	-1.82333*	0.16347	0.000	-2.2494	-1.3972
	C	A	2.93333*	0.16347	0.000	2.5072	3.3594
		B	1.84667*	0.16347	0.000	1.4206	2.2728
		D	0.02333	0.16347	0.999	-0.4028	0.4494
	D	A	2.91000*	0.16347	0.000	2.4839	3.3361
		B	1.82333*	0.16347	0.000	1.3972	2.2494
		C	-0.02333	0.16347	0.999	-0.4494	0.4028

*. The mean difference is significant at the 0.05 level.

DISCUSSION

Our study pattern was an effort towards evaluating that whether the blood level of glycosylated hemoglobin (HbA1c) is elevated in systemically healthy individuals with periodontitis as compared to healthy controls. The results were encouraging, where we found statistically significant elevated HbA1c values in Group B i.e. individuals with periodontitis but without diabetes, when compared to Group A i.e. individuals without periodontitis and without diabetes.

Various demographic, clinical, and biochemical parameters were obtained from each subject. The demographic parameters comprised of gender, age, socioeconomic status and the educational qualification. The clinical parameters comprised of probing depth (PD), gingival recession (REC), clinical attachment level (CAL), bleeding on probing (BOP) which was measured with the help of Gingival Bleeding Index (GBI), and oral hygiene status measured with OHI (S). The biochemical parameters recorded consisted of fasting blood sugar (FBS), and glycosylated hemoglobin (HbA1c) level. The groupings of the subjects were justified by their recorded parameters.

Age was an insignificant variable amongst the 4 groups in our study (p value of 0.308). Gender was an insignificant variable (p value 0.533), amongst the 4 groups in our study model, referring to almost equal distribution of sexes in the groups. The statistical analysis revealed the socio-economic status and education insignificant amongst the groups of our study, which were important to rule out any intergroup bias when comparing the periodontal status and the systemic condition of the subjects.

Amongst the clinical variables in our study probing depth (PD) compared in between Group B (subjects with periodontitis and without diabetes) and Group D (subjects with periodontitis and with diabetes) [p value 0.001], with mean PD of 3.354+/- 0.652 mm and 3.910+/- 0.834 mm respectively. This gives us a clear indication that increased PD is associated with diabetes mellitus, when compared between groups, both having established periodontitis.

The recession (REC) when compared between Group A and C (p value 0.895) was statistically non-significant (p value >0.05), probably as the diabetic status in Group C was leading to increase in PD but was not advanced enough to cause soft tissue recession, nor periodontitis. Again, statistically significant results were obtained comparing the recession values between Group B and Group C (p value 0.000), Group C and Group D (p value 0.001), as the subjects of both the Group B and Group D had periodontitis without and with diabetes respectively, while those of Group C did not have periodontitis.

In the present study the clinical attachment level (CAL) when compared between Group B (subjects with periodontitis and without diabetes) and Group D (subjects with periodontitis and with diabetes), was statistically significant [p value 0.000], suggesting greater attachment loss in already established diabetic population.

Our study revealed an increased bleeding from gingival tissues seen in diabetic cases with periodontitis (Group D mean BI 62.833+/- 27.791 %) when compared to patients having only periodontitis (Group B mean BI 46.411+/- 31.738 %).

Statistically significant results (p value 0.000) were observed with the OHIS values in between Group B (mean OHIS 2.713+/- 1.114) and Group D (mean OHIS 1.783+/- 0.704). This suggests that amongst both the groups having periodontitis, subjects with diabetes (Group D) were more aware of their periodontal problems (more severe than Group B), thus maintaining oral hygiene better than those with periodontitis but no diabetes.

Fasting blood sugar (FBS) when compared between Group A (mean FBS 83.133 +/- 7.824 mg/dl) and B (mean FBS 97.133+/- 9.354 mg/dl) (p value 0.001), were statistically significant (p value <0.05). This is probably due to impaired glucose metabolism seen in Group B without diabetes but with periodontitis. The impaired glucose metabolism may either be due to decreased insulin production or due to increased insulin resistance, both as a direct result of circulating cytokines originating from the periodontal inflammation.

These results are in accordance with the study by Saito T et al, where they studied whether periodontitis is associated with impaired glucose tolerance (IGT) in Japanese men. The results were suggestive of that the degree of alveolar bone loss is significantly associated with IGT, suggesting that periodontitis is associated with impaired glucose tolerance.[8]

HbA1c values when compared between Group A (mean value 4.566+/- 0.389 %) and B (mean value 5.653+/- 0.312 %) [p value 0.000], were statistically significant (p value <0.05). This gives us a strong indication that HbA1c is elevated in cases with established periodontitis, but with no diabetes, as compared to systemically and periodontally healthy controls. It can be thus speculated that HbA1c is positively correlated to periodontal destruction, and the chronicity of the periodontal disease can lead to poorer glycemic control (progressive increase in HbA1c values). Though a very few studies have been carried out to ascertain the direct effect of periodontitis on HbA1c levels, a study by Wolff RE, Wolff LF, and Michalowicz BS, was undertaken to assess the glycosylated hemoglobin (HbA1c) levels in periodontitis cases and healthy controls. In the study HbA1c was assessed using a chairside test in 59 adults without diabetes but with periodontitis, and 53 healthy controls. After adjustments for age, gender, BMI, and current smoking, mean HbA1c was significantly higher (p value 0.046) in cases (with periodontitis, but without diabetes) than controls (without periodontitis and without diabetes). They concluded that periodontitis was associated with a slight elevation in glycosylated hemoglobin. [6] Similar results were observed, and statistically proven in our study.

Similar results were observed with the HbA1c values in between Group A and Group C (p value 0.000), Group A and Group D (p value 0.000), Group B and Group C (p value 0.000), Group B and Group D (p value 0.000). This was as expected as the subjects in Group C and D were having diabetes, whereas in Group A and B they were without diabetes. Statistically insignificant results were observed with the HbA1c values in between Group C and Group D (p value 0.999), both with established diabetes.

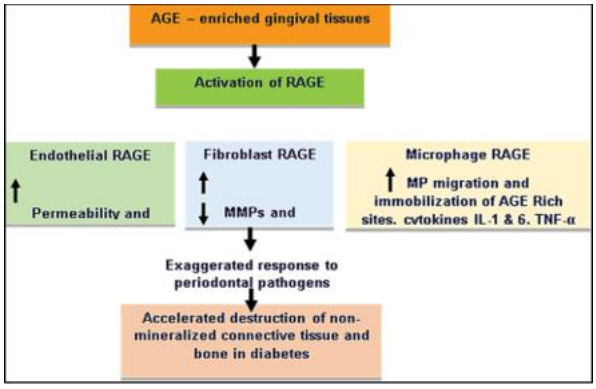

Figure: Relationship Between AGEs and Periodontitis

CONCLUSION

The study suggested that periodontitis creates a state of elevated pro-inflammatory cytokines in the systemic circulation. These cytokines further interact with various tissues and organ systems throughout the body, leading to a functional malady. The cytokines are known to target tissues responsible for carbohydrate metabolism in the pancreas, liver and muscles, leading to deficiency of insulin secretion caused by pancreatic -cell dysfunction and/or insulin resistance in liver and muscle. This predisposes a condition of hyperglycemia and may lead to diabetes later in life.Thus, prima facie evidence in this cross-sectional study has given an indication towards a positive correlation between periodontitis and diabetes through the estimation of glycosylated hemoglobin.

Conflict of Interest

The authors have none to declare.

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