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Comparative Evaluation of GCF and Serum Biomarkers in Diabetic and Healthy Adults with Generalized Chronic Periodontitis

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Abstract

Aim of the study: The study was aimed to compare and evaluate GCF and serum biomarkers in Healthy Controls and Non-Insulin Dependent Diabetes Mellitus adults in cases of Generalized Chronic Periodontitis before and after scaling and root planning.

Materials and Method: 40 patients within age group of 30-65 years visiting the OPD at the Department of Periodontology and Oral Implantology, Luxmi Bai Institute of Dental Sciences & Hospital were included in the study Subjects were divided into two groups, group 1 (20 patients)- Healthy non diabetic controls with Generalized Chronic Periodontitis and Group 2 (20 patients)- NIDDM with Generalized Chronic Periodontitis. All the patients were evaluated on the basis of inflammatory biomarkers (IL-8, MMP-9, Osteocalcin and calprotectin) levels in GCF and serum.

Conclusion: It was concluded that inflammation was increased in Diabetic patients that led to an increase in the markers such as IL-8, MMP-9 and Calprotectin. Bone turnover marker, Osteocalcin is reduced in diabetic patients. Furthermore, periodontal therapy was found to enhance clinical parameters.

Keywords: Diabetes Mellitus, Chronic Periodontitis, Gingival Crevicular Fluid.

Introduction

Periodontitis is defined as an immuno inflammatory response induced by microorganisms in dental plaque that contributes to periodontal destruction and tooth loss[1].

Diabetes Mellitus is a metabolic disease that causes abnormal fat, sugar, protein metabolism and resultant hyperglycemia, which can lead to a number of systemic problems. One of the sixth complications of DM is periodontitis.

Worsening glycemic control is linked to periodontal disease. This includes inflammation, immune cell phenotypes, serum lipid levels, and tissue homeostasis.

Biological samples can be collected from serum, GCF, or saliva[10]. GCF is a complex mixture of substances that is derived from serum, host inflammatory cells, structural cells of the periodontium and oral microflora. GCF collection and analysis is currently thought to be a crucial method for finding molecular biomarkers linked to periodontitis.[4] Serum biomarkers are significantly increased in the inflammatory conditions like DM.

IL-8 is a member of the interleukin-8 supergene family, that have the ability to attract particular kinds of leukocyte populations. In inflammatory areas, polymorphonuclear leukocytes are drawn to and activated by IL-8. In the context of periodontal disease, elevated levels of IL-8 were linked to both types of Diabetes Mellitus, indicating their involvement in the pathophysiology of T1DM, T2DM, and Chronic Periodontitis.

MMPs have a vital role in the degradation of collagen during the deterioration of periodontal tissue. Higher levels of metalloproteinases are secreted by cells exposed to growth factors and cytokines during periodontitis. Inflammatory cells also produce MMP-9.

Calprotectin was previously demonstrated to exist in human dental calculus and to be derived from dental plaque, saliva, and GCF. Research has demonstrated that the GCF of patients with periodontitis has a higher amount of calprotectin than that of healthy individuals.

Osteocalcin (OC) is a bone-specific protein secreted by bone-forming osteoblasts and osteocytes indicate changes in bone turnover, resorption and formation.

Materials and Method

Forty patients within age group of 30-65 years visiting the outpatient area at the Department of Periodontology and Oral Implantology, Luxmi Bai Institute of Dental Sciences & Hospital were included in the study. A brief medical history was obtained and fasting blood sugar (FBS) and HbA1c was conducted beforehand as confirmatory tests for diabetes. Subjects were explained about the study purpose and a written consent was obtained.

Group 1 (20patients)- Healthy non diabetic controls with Generalized Chronic Periodontitis (FBS< 126 mg/dl and HbA1c<6.5%) and Group 2 (20 patients)- NIDDM with Generalized Chronic Periodontitis patients (FBS>126mg/dl and HbA1c>6.5%).

Inclusion Criteria: Patients within age group - 30-65 years, patients with more than 30% of sites involved, patients having ≥ 20 teeth, patients with Stage III Grade B periodontitis and patients with Type 2 DM (NIDDM).

Exclusion Criteria: Patients suffering from systemic conditions such as cancer, renal disease, thyroid disease, Smokers, pregnant/ Lactating mother, patients who used antibiotics or underwent periodontal treatment in the past six months and patients undergoing hormonal replacement or steroid therapy.

Armamentarium

The following clinical parameters were taken at baseline (Day 0) before oral prophylaxis and after 6 weeks- Bleeding on Probing by Sulcus Bleeding Index (Muhlemann H.R and Son. S in 1971) , Pocket Probing Depth using Williams Probe, Plaque Index (Turesky-Gilmore-Glickman modification of the Quigley Hein plaque index in 1970).

Biochemical investigations were done at baseline (Day 0) and after 6 weeks of SRP therapy. GCF and serum samples of both the groups were collected and levels of IL-8, MMP-9, osteocalcin and calprotectin were evaluated using ELISA kits at two different time intervals (Baseline day 0) and at 6 weeks. The GCF samples were collected using Perio absorbant paper using extra crevicular method. The samples were collected and sent to the laboratory for further investigations.

The data was gathered and subjected to statistical analysis.

Method of Collection of GCF and blood sample

For GCF collection, the deepest pocket depths from the teeth were chosen. Each site was carefully examined, and an absorbent paper strip was inserted until little resistance was felt. It swas left for 30 seconds. Following GCF collection, each strip was placed into its corresponding labeled microfuge tube and was immediately placed onto dry ice for transport to laboratory and storage in a -80°C freezer until analysis. For blood sample, 5 ml blood was collected from the anticubital fossa by venipuncture using a 20- gauze needle and immediately transferred to the laboratory. The serum was prepared from the blood sample after 1hr when the blood was allowed to clot by centrifuging at 3000 rpm for 5 min. The serum was immediately transferred to a plastic vial and stored at 4 degree Celcius until transferred to storing facility where it was stored in -80 degree Celcius until the time of assay.

Results

Intragroup comparison of GCF mean IL-8, MMP, Osteocalcin, Calprotectin levels in Group 1(Healthy controls with Generalied Chronic Periodontitis) at baseline and after 6 weeks (post SRP) demonstrated a statistical significant difference p= 0.035, 0.001, 0.019, 0.001 respectively [Table 1] Intragroup comparison of serum mean IL-8, MMP, Osteocalcin, Calprotectin levels in group 1 at baseline and after 6 weeks (post SRP) demonstrated statistical significant difference p= 0.001, 0.001, 0.001, 0.002 respectively [Table 2].

Intragroup comparison of GCF mean IL-8, MMP-9, Osteocalcin, Calprotectin levels in Group 2 (Non-Insulin dependent Diabetes Mellitus) at baseline and after 6 weeks (post SRP) demonstrated a statistical significant difference (p= 0.001, 0.001, 0.001, 0.001) respectively [Table 3]. Intragroup comparison of serum means IL-8, MMP-9, Osteocalcin, Calprotectin levels in group 2 at baseline and after 6 weeks (post SRP) demonstrated a statistical significant difference (p= 0.001, 0.031, 0.001, 0.001) respectively [Table 4].

Intergroup comparison of GCF mean IL-8 levels in group 1 and group 2 at baseline demonstrated a statistical non-significant difference p= 0.132 [Table 5]. Intergroup comparison of GCF mean MMP-9, Osteocalcin, Calprotectin levels in group 1 and group 2 at baseline demonstrated a statistical significant difference (p= 0.001, 0.001, 0.001) respectively [Table 5]. Intergroup comparison of serum mean IL-8, MMP-9, Osteocalcin, Calprotectin levels in group 1 and group 2 demonstrated a statistical significant difference (p= 0.001, 0.001, 0.001, 0.001) respectively [Table 6].

Intergroup comparison of GCF mean IL-8, MMP-9, Osteocalcin, Calprotectin levels in Group 1 and Group 2 after 6 weeks (post SRP) demonstrated a statistical significant difference (p= 0.001, 0.001, 0.001, 0.001) respectively [Table 7]. Intergroup comparison of serum means IL-8, MMP-9, Osteocalcin, Calprotectin levels in group 1 and group 2 after 6 weeks (post SRP) demonstrated a statistical significant difference (p= 0.001, 0.001, 0.001, 0.001) respectively [Table 8].

Table 1: Showing comparison of GCF Biomarkers of Group 1 (HC with GCP)

Non-Diabetic Patients GCF	Conditions	Mean	Std. Deviation	Std. Error Mean	Mean Diff	%age Change	Paired t-test	p value
IL-8	At baseline (Day 0)	14.930	1.583	0.354	0.875	5.861	2.239	0.035 (S)
	After 6 weeks (Post OP)	14.055	1.151	0.257				
MMP-9	At baseline (Day 0)	21.635	2.848	0.637	3.300	15.253	5.357	0.001 (HS)
	After 6 weeks (Post OP)	18.335	1.764	0.394				
Osteocalcin	At baseline (Day 0)	26.510	1.750	0.391	- 1.085	-4.093	2.571	0.019 (S)
	After 6 weeks (Post OP)	27.595	1.942	0.434				
Calprotectin	At baseline (Day 0)	32.930	2.997	0.670	2.205	6.696	5.161	0.001 (HS)
	After 6 weeks (Post OP)	30.725	2.105	0.471				

Table 2: Showing comparison of Serum Biomarkers of Group 1 (HC with Generalized CP) at two different time intervals.

Non-Diabetic Patients Serum	Mean	Std. Deviation	Std. Error Mean	Mean Diff	%age Change	Paired t-test	p value
IL-8	3.435	1.403	0.314	1.150	33.479	4.056	0.001 (HS)
	2.285	0.353	0.079				
MMP-9	269.270	6.552	1.465	7.045	2.616	13.555	0.001 (HS)
	262.225	6.619	1.480				
Osteocalcin	22.495	6.522	1.458	- 2.390	-10.625	8.133	0.001 (HS)
	24.885	6.940	1.552				
Calprotectin	35.630	6.241	1.395	5.000	14.033	3.684	0.002 (S)
	30.630	4.952	1.107				
HBA1C	5.450	0.662	0.148	--	--	--	--
	5.450	0.662	0.148				

Table 3: Showing comparison of GCF Biomarkers of Group 2 (NIDDM with Generalized CP)

Diabetic Patients GCF	Conditions	Mean	Std. Deviation	Std. Error Mean	Mean Diff	%age Change	Paired t-test	p value
IL-8	At baseline (Day 0)	15.900	2.330	0.521	2.930	18.428	4.797	0.001 (HS)
	After 6 weeks (Post OP)	12.970	1.160	0.259				
MMP-9	At baseline (Day 0)	36.380	2.460	0.550	2.250	6.185	5.329	0.001 (HS)
	After 6 weeks (Post OP)	34.130	1.355	0.303				
Osteocalcin	At baseline (Day 0)	13.120	1.973	0.441	- 1.495	-10.709	9.207	0.001 (HS)
	After 6 weeks (Post OP)	14.525	1.764	0.394				
Calprotectin	At baseline (Day 0)	56.250	9.200	2.057	2.900	5.156	4.004	0.001 (HS)
	After 6 weeks (Post OP)	53.350	7.242	1.619				

Table 4: Showing comparison of Serum Biomarkers of Group 2 (NIDDM with Generalized CP)

Diabetic Patients Serum	Mean	Std. Deviation	Std. Error Mean	Mean Diff	%age Change	Paired t-test	p value
IL-8	6.040	1.785	0.399	1.850	30.629	5.286	0.001 (HS)
	4.190	1.052	0.235				
MMP-9	475.580	86.003	19.231	12.550	2.639	2.327	0.031 (S)
	463.030	79.339	17.741				
Osteocalcin	10.255	4.762	1.065	-1.660	-16.187	9.983	0.001 (HS)
	11.915	4.925	1.101				
Calprotectin	43.120	5.865	1.311	6.250	14.494	5.784	0.001 (HS)
	36.870	2.421	0.541				
HBA1C	8.670	1.999	0.447	--	--	--	--

Table 5: Showing comparison of GCF Biomarkers of Group 1 and Group 2 at Baseline.

GCF	Groups	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		F value	p value
						Lower Bound	Upper Bound		
IL-8	Diabetic Patients	20	15.900	2.330	0.521	14.809	16.991	2.371	0.132 (NS)
	Non- Diabetic Patients	20	14.930	1.583	0.354	14.189	15.671		
	Total	40	15.415	2.027	0.320	14.767	16.063		
MMP-9	Diabetic Patients	20	36.380	2.460	0.550	35.229	37.531	307.063	0.001 (HS)
	Non- Diabetic Patients	20	21.635	2.848	0.637	20.302	22.968		
	Total	40	29.008	7.915	1.251	26.476	31.539		
Osteocalcin	Diabetic Patients	20	13.120	1.973	0.441	12.197	14.043	515.714	0.001 (HS)
	Non- Diabetic Patients	20	26.510	1.750	0.391	25.691	27.329		
	Total	40	19.815	7.026	1.111	17.568	22.062		
Calprotectin	Diabetic Patients	20	56.250	9.200	2.057	51.944	60.556	116.177	0.001

	Non- Diabetic Patients	20	32.930	2.997	0.670	31.527	34.333		(HS)
	Total	40	44.590	13.603	2.151	40.239	48.941		

Table 6: Showing comparison of Serum Biomarkers of Group 1 and Group 2 at Baseline

Serum	Groups	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		F value	p value
						Lower Bound	Upper Bound		
IL-8	Diabetic Patients	20	6.040	1.785	0.399	5.205	6.875	26.331	0.001 (HS)
	Non- Diabetic Patients	20	3.435	1.403	0.314	2.778	4.092		
	Total	40	4.738	2.062	0.326	4.078	5.397		
MMP-9	Diabetic Patients	20	475.580	86.003	19.231	435.329	515.831	114.426	0.001 (HS)
	Non- Diabetic Patients	20	269.270	6.552	1.465	266.203	272.337		
	Total	40	372.425	120.574	19.064	333.863	410.987		
Osteocalcin	Diabetic Patients	20	10.255	4.762	1.065	8.026	12.484	45.950	0.001 (HS)
	Non- Diabetic Patients	20	22.495	6.522	1.458	19.443	25.547		
	Total	40	16.375	8.378	1.325	13.696	19.054		
Calprotectin	Diabetic Patients	20	43.120	5.865	1.311	40.375	45.865	15.298	0.001 (HS)
	Non- Diabetic Patients	20	35.630	6.241	1.395	32.709	38.551		
	Total	40	39.375	7.079	1.119	37.111	41.639		
HBA1C	Diabetic Patients	20	8.670	1.999	0.447	7.734	9.606	46.764	0.001 (HS)
	Non- Diabetic Patients	20	5.450	0.662	0.148	5.140	5.760		
	Total	40	7.060	2.195	0.347	6.358	7.762		

Table 7: Showing comparison of GCF Biomarkers of Group 1 and Group 2 after 6 weeks (post SRP)

GCF	Groups	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	F value	p value

						Lower Bound	Upper Bound		
IL-8	Diabetic Patients	20	12.970	1.160	0.259	12.427	13.513	13.137	0.001 (HS)
	Non- Diabetic Patients	20	14.305	1.169	0.261	13.758	14.852		
	Total	40	13.638	1.334	0.211	13.211	14.064		
MMP-9	Diabetic Patients	20	34.130	1.355	0.303	33.496	34.764	1008.249	0.001 (HS)
	Non- Diabetic Patients	20	18.335	1.764	0.394	17.509	19.161		
	Total	40	26.233	8.147	1.288	23.627	28.838		
Osteocalcin	Diabetic Patients	20	14.525	1.764	0.394	13.699	15.351	496.481	0.001 (HS)
	Non- Diabetic Patients	20	27.595	1.942	0.434	26.686	28.504		
	Total	40	21.060	6.867	1.086	18.864	23.256		
Calprotectin	Diabetic Patients	20	53.350	7.242	1.619	49.961	56.739	179.992	0.001 (HS)
	Non- Diabetic Patients	20	30.725	2.105	0.471	29.740	31.710		
	Total	40	42.038	12.608	1.994	38.005	46.070		

Table 8: Showing comparison of Serum Biomarkers of Group 1 and Group 2 after 6 weeks (post SRP).

Serum	Groups	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		F value	p value
						Lower Bound	Upper Bound		
IL-8	Diabetic Patients	20	4.190	1.052	0.235	3.698	4.682	58.924	0.001 (HS)
	Non-Diabetic Patients	20	2.285	0.353	0.079	2.120	2.450		
	Total	40	3.238	1.237	0.196	2.842	3.633		
MMP-9	Diabetic Patients	20	463.030	79.339	17.741	425.898	500.162	127.230	0.001 (HS)
	Non-Diabetic Patients	20	262.225	6.619	1.480	259.127	265.323		
	Total	40	362.628	115.876	18.322	325.569	399.686		

Osteocalcin	Diabetic Patients	20	11.915	4.925	1.101	9.610	14.220	46.455	0.001 (HS)
	Non-Diabetic Patients	20	24.885	6.940	1.552	21.637	28.133		
	Total	40	18.400	8.855	1.400	15.568	21.232		
Calprotectin	Diabetic Patients	20	36.870	2.421	0.541	35.737	38.003	25.630	0.001 (HS)
	Non-Diabetic Patients	20	30.630	4.952	1.107	28.312	32.948		
	Total	40	33.750	4.979	0.787	32.158	35.342		
HBA1C	Diabetic Patients	20	8.670	1.999	0.447	7.734	9.606	46.764	0.001 (HS)
	Non-Diabetic Patients	20	5.450	0.662	0.148	5.140	5.760		
	Total	40	7.060	2.195	0.347	6.358	7.762		

Discussion

The present study evaluated the GCF and serum biomarkers of diabetic and healthy adults with generalied chronic periodontitis. The total duration of study was 6 weeks, with visits to the department at baseline (Day 0) and at 6 weeks. Subjects were explained about the study purpose and a written consent was obtained from each one of them. These patients were subjected to oral examination and GCF and serum samples were collected at baseline (day0) and then scaling and root planing (SRP) was performed. These samples were then sent to the laboratory for the ELISA testing of IL-8, MMP-9, Osteocalcin and Calprotectin biomarkers. The patients were recalled at 6 weeks and after assessing their clinical parameters GCF and serum samples were collected again and sent to the lab for ELISA testing and evaluation.

The comparison of scores of IL-8 in GCF before SRP and after 6 weeks (post SRP) of group 1 and group 2 was significant. These results were in accordance with the study by Goutoudi P, Diza E and Arvanitidou M (2004). IL-8 levels were markedly decreased by both the treatment modalities such as scaling and root surface debridement and surgical therapy. The comparison of scores of IL-8 in GCF of Group 1 and Group 2 patients at 6 weeks (post SRP) was highly significant. However, the comparison of scores of IL-8 in serum before SRP and after 6 weeks (post SRP) of Group 1 and group 2 was highly significant. These results were in accordance with the study by Borilova Linhartova P et al (2018). This study evaluated that low-grade inflammation is a symptom of both DM and chronic periodontitis patients, suggesting a connection between the two conditions. IL-8 is one of the most significant chemoattractants and activators of neutrophils, a chemokine that plays a role in the development and intensification of acute inflammatory reaction.

The comparison of scores of MMP-9 in GCF before SRP and after 6 weeks (post SRP) of group 1 and group 2 was highly significant. These results were in accordance with the study by Gonçalves PF et al (2013). The comparison of scores of MMP-9 in GCF of Group 1 and Group 2 at baseline was highly significant. These results were in

accordance with the study by Peniche-Palma DC (2019), Nędzi-Góra M, Kostrzewa-Janicka J and Górska R (2014). The comparison of scores of MMP-9 in GCF of Group 1 and Group 2 patients after 6 (post SRP) weeks was highly significant. These are in accordance with Ingman et al. Seguier and colleagues. Also, the comparison of scores of MMP-9 in serum of Group 1 and Group 2 patients at baseline and after 6 weeks (post SRP) was highly significant. These results were in accordance with the study by Derosa G et al (2007). So, this study concluded that MMPs are known to activate osteoclasts, initiate cytokine activity, and break down the collagen in the extracellular matrix. The identification of different MMP types in GCF, as well as their inhibitors, in saliva or blood, may serve as markers of chronic periodontitis.

The comparison of scores of osteocalcin in GCF before SRP and after 6 weeks (post SRP) of group 1 and group 2 was significant. These results were in accordance with the study by Bullon P et al (2007). The comparison of scores of osteocalcin in GCF of Group 1 and Group 2 patients after 6 weeks (post SRP) was highly significant. These results were in accordance with the study by Wildan MW, Syaify A, Herawati D (2023). Also the comparison of scores of osteocalcin in serum of Group 1 and Group 2 patients at baseline and after 6 weeks (post SRP) was highly significant. These results were in accordance with the study by Maddaloni E et al (2014). This study concluded that lower concentration of osteocalcin is correlated with high levels of hemoglobin A1c (HbA1c) and insulin resistance as poor metabolic control in diabetes mellitus leads to compromised bone metabolism.

The comparison of scores of calprotectin in GCF before SRP and after 6 weeks (post SRP) of group 1 and group 2 was highly significant. The comparison of scores of calprotectin in GCF of Group 1 and Group 2 patients at baseline and after 6 weeks (post SRP) was highly significant. These results are in accordance with the study by Gao H et al (2020). However, the comparison of scores of calprotectin in serum before SRP and after 6 weeks (post SRP) of group 1 was significant. The comparison of scores of calprotectin in serum before SRP and after 6 weeks (post SRP) of group 2 was highly significant. The comparison of scores of calprotectin in serum of Group 1 and Group 2 patients at baseline and after 6 weeks (post SRP) was highly significant. These results were in accordance with the study by Gao H et al (2020). This study concluded that oxidative stress caused by hyperglycemia raises the levels of inflammatory cytokines in the blood. Calprotectin's plasma concentration is consistently much higher in patients with type 2 diabetes and impaired glucose tolerance than in healthy controls. It also has a strong positive correlation with insulin resistance indicators, glucose and lipid metabolism parameters, and inflammatory biomarkers.

It was concluded that inflammation is aggravated in diabetic patients that leads to the increase in inflammatory markers such as IL-8, MMP-9, Calprotectin. Lower osteocalcin levels are correlared with high levels of HbA1c and insulin resistance as poor metabolic control in diabetes mellitus leads to compromised bone metabolism. It is also concluded that clinical parameters such as pocket probing depth, bleeding on probing and plaque index were improved with periodontal treatment as elimination of irritants and toxins allows the periodontal tissues to heal leading to significant improvements in the clinical parameters. Our results suggest that compared to non-diabetic patients with comparable periodontal conditions, diabetic patients may have noticeably higher levels of systemic and periodontal inflammation.

Within the limitations of this study, longer follow up and bigger sample size is required. Moreover, the procurement and handling of GCF is highly technique sensitive. With the advances in biochemical testing techniques and better collection methods, more accurate results can be achieved.

Conclusion

Hence, it can be concluded that there maybe a positive correlation between chronic periodontitis and diabetes mellitus. The inflammatory biomarkers such as IL8, MMP-9 and calprotectin increase in GCF and serum of type 2 diabetic patients with generalied chronic periodontitis as compared to the non- diabetic patients. It was also observed that there is an inverse correlation between HbA1c and osteocalcin levels suggesting that a poor metabolic control in type 2 diabetes leads to decreased osteocalcin level. Also, the levels of inflammatory markers such as IL-8, MMP-9, calprotectin and clinical parameters such as bleeding on probing, plaque index and pocket probing depth decrease in both the groups after scaling and root planing and the osteocalcin levels increase after SRP.

Thus, our study evaluated the role of biomarkers such as IL-8, MMP-9, osteocalcin and calprotectin as diagnostic markers in the inflammatory conditions such as Diabetes.

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