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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF GINGIVAL CREVICULAR FLUID AND SALIVARY SCLEROSTIN LEVELS IN CHRONIC PERIODONTITIS PATIENTS WITH OR WITHOUT TYPE 2 DIABETES MELLITUS: A CROSS-SECTIONAL STUDY

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Chronic Periodontitis, Diabetes Mellitus, Gingival Crevicular Fluid

Abstract

Introduction: Chronic Periodontitis is an infectious disease resulting in inflammation within the supporting tissues of the teeth leading to progressive attachment and bone loss. Biochemical mediators in oral fluids like saliva and gingival crevicular fluid (GCF) are highly beneficial in the determination of current periodontal status.

Aim & objective: A comparative evaluation of GCF and salivary sclerostin levels in chronic periodontitis patients with or without type 2 diabetes mellitus

Methodology: A total of 36 subjects in the age group of 30-60 years were categorised into three groups. Group I (Healthy individuals) Group II (chronic periodontitis patients) and Group III (chronic periodontitis patients with type 2 diabetes mellitus). During the first visit the clinical parameters like Plaque index, Gingival index, Probing pocket depth and Clinical attachment level were recorded. On the subsequent day GCF and saliva samples were collected and subjected for laboratory analysis using ELISA kit. Results were tabulated and subjected to statistical analysis.

Results & Discussion: The differences of Sclerostin levels in Saliva between the groups were not statistically significant (P = 0.089). The differences of Sclerostin levels in GCF between the groups were highly statistically significant (P =0.000).

Conclusion: Sclerostin levels are a promising diagnostic and prognostic biomarker in periodontal diseases.

Introduction:-

Periodontal disease is a chronic multifactorial disease related to the interaction between the microorganisms and the host leading to various immune and inflammatory responses affecting the soft and hard supporting structures of periodontium. Traditional clinical measurements (probing depth, bleeding on probing, clinical attachment loss) used for periodontal diagnosis are often of limited usefulness in that they are indicators of previous periodontal disease rather than present disease activity. Advances in oral and periodontal disease diagnostic research has moved towards methods where by periodontal risk can be identified and quantified by objective measures such as biomarkers.[1]

Biomarker is defined as any substance, structure that can be measured in the body and influence or predict the incidence of disease or outcome.[2]

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Chronic periodontitis is one of most ubiquitous diseases and is characterized by the destruction of connective tissue and bone support following an inflammatory host response secondary to infection by periodontal bacteria.[3]

Diabetes mellitus is a chronic metabolic disease characterized by hyperglycemia due to the inability of insulin dependent cells to absorb glucose effectively. In diabetic patients, advanced glycated end products (AGEs) are said to affect the migration and phagocytic activity of poly morphonuclear neutrophils (PMNs), by binding to the macrophages, resulting in establishment of more pathogenic bacterial bio film, releasing a larger amount of cytokines.[5] Studies have found a high degree of association between diabetes mellitus and periodontal disease which is proposed as the sixth complication of diabetes mellitus. AGEs have detrimental effects on bone metabolism, leading to impaired repair and bone formation and decreased extracellular matrix production. Hence, this relationship is bi-directional with Chronic Periodontitis exerting an effect on diabetes mellitus and vice versa.[6]

Sclerostin is a secreted glycoprotein that binds low density lipoprotein receptor related protein 5 (LRP 5) and blocks the Wnt signalling pathway. [7]Wnt signalling pathway is also impaired in type 2 diabetes mellitus which promotes the deterioration of osteoblastogenesis and increases bone fragility. Thus, Sclerostin levels are increased in patients with type 2 diabetes mellitus.[8] Various studies have shown that Sclerostin also has an affect on other metabolic bone diseases like osteoporosis, arthritis, parathyroidisim disorders, renal osteodystrophy and other bone disorders.[9]

Various body fluids are present to measure biomarkers such as gingival crevicular fluid (GCF), saliva, blood, serum etc. The composition of GCF and saliva somewhat mirrors the blood in various biological aspects and it could be used as a less invasive medium for the estimation of biomarkers. To the best of our knowledge studies pertaining to the salivary Sclerostin levels and comparison of salivary and GCF estimation in Chronic Periodontitis patients with and without type 2 diabetes mellitus is not found in the literature. Hence, the study aims to evaluate and estimate GCF and salivary Sclerostin levels in Chronic Periodontitis patients with and without type 2 diabetes mellitus.

Aim & Objective:-

A comparative evaluation of GCF and salivary sclerostin levels in chronic periodontitis patients with or without type 2 diabetes mellitus

Material & Methods:-

This is a cross sectional study conducted on outpatients who reported to Department of Periodontics, Dayananda Sagar College of Dental Sciences; Bangalore.

Inclusion Criteria:

Group 1 (Control group): Probing depth ≤ 3mm, Gingival index score <1 and no attachment loss, Mean age 30-60 years.

Group 2 (Chronic periodontitis): Patient diagnosed with chronic periodontitis, as per AAP 1999 criteria, Mean age 30-60 years.

Group 3 (Chronic periodontitis with type 2 diabetes mellitus): Patient diagnosed with chronic periodontitis patients with type 2 diabetes mellitus. Mean age 30-60 years.

Exclusion criteria:

1. Patients with systemic diseases other than type 2 diabetes mellitus. 2. Patients who have undergone periodontal treatment in the last six months. 3. Patients with Use of anti-inflammatory drugs and antibiotics in the last six months. 4. Pregnant and lactating mothers. 5. Radiation or immunosuppressive therapy.6.Smokers, alcoholics and drug abusers.

Data Collection:

A total of 72 samples out of 36 subjects were enrolled in the study. These subjects were divided into 3 groups. Verbal and written informed consent were obtained from all the subjects. 12 GCF and 12 Saliva samples were collected from all the subjects in each group (Figure 1).

Group 1 – 24 samples from 12 periodontally and systemically healthy individuals.

Group 2 – 24 samples from 12 chronic periodontitis patients.
Group 3 – 24 samples from 12 chronic periodontitis patients with type 2 diabetes mellitus.
A proforma was designed to facilitate a systemic and methodological recording of all the observations and information, which included a brief case history and periodontal examination.

Clinical examination included clinical measurement of:
• Plaque index (PI) (Silness P. and Loe H., 1964). [10]
• Gingival index (GI) (Loe H. and Silness P., 1963).[11]

Probing pocket depth (PPD) (measured with a University of north carolina (UNC-15) probe, Hu-Friedy) and CALwas recorded on all the teeth

All the clinical measurements were carried out by the same examiner. Periodontal examination was carried one day before the sample collection. On the subsequent day, patients were asked not to eat, drink or brush teeth for at least 30 minutes prior to the saliva collection. Approximately 4ml of unstimulated saliva was collected using sterile container by spit method. For the GCF collection, the sites from where the sample was taken were well-isolated and collected by placing the microcapillary pipette (Sigma Aldrich, St.Louis) at the entrance of the gingival sulcus (extrasulcular method). A standardized volume of GCF (3µL) was collected. Samples contaminated with saliva and blood was discarded. Samples were transferred to air-tight plastic vials containing Phosphate buffered saline and 0.5% BSA as transport medium & stored at -80°C till they are subjected for laboratory analysis by using ELISA.

The mean optical density of each standard duplicate was calculated (Table 1). A standard curve was drawn on a semi-log paper with the mean optical densities on the Y-axis and the standards concentrations on the X-axis (Figure 2).

Based on the standard curve and the equation **y=mx+c**, the values of the unknown clinical samples were tabulated. Data were examined for normality by Kolmogorov Smirnov test and Shapiro Wilks test and further data that did not achieve normality were analysed using non-parametric tests. The possible correlation between the levels of Sclerostin levels in GCF and Saliva were tested by using Pearson Correlation test. The data was analysed using the Statistical Package for Social Science (SPSS version 10.5) software.

Results:-

The mean Plaque index (PI) score for Group 1, Group 2 and 3 were 0.428, 1.35 and 1.79 respectively. The difference in PI scores between the groups was highly statistically significant (P = 0.000)(Table 4). On pair wise comparison between all the three groups, the difference in PI score was highly statistically significant (P = 0.000)(Table 5).

The mean Gingival index (GI) score for Group 1, Group 2 and Group 3 were 0.24, 1.48 and 2.15 respectively. The difference in GI scores between the groups was highly statistically significant (P =0.000)(Table 4). On pair wise comparison between all the three groups, the difference in GI score was highly statistically significant (P = 0.000)(Table 5).

The mean Probing pocket depth (PPD) for Group 1, Group 2 and Group 3 were 1.69, 3.21 and 5.40 respectively. The differences in PPD between all the groups were highly statistically significant (P = 0.000)(Table 4). On pair wise comparison between all the three groups, the differences in PPD was highly statistically significant (P = 0.000)(Table 5).

The mean clinical attachment level (CAL) for Group 1, Group 2 and Group 3 were 1.51, 3.17 and 4.66 respectively. The differences in CAL between all the groups were highly statistically significant (P= 0.000)(Table 4). On pair wise comparison between all the three groups, the differences in CAL was highly statistically significant (P =0.000)(Table 5).

The mean Sclerostin levels in Saliva for Group 1, Group 2 and Group 3 were 8.17, 11.81 and 10.51 respectively(Table 2). The differences of Sclerostin levels in Saliva between the groups were not statistically significant (P = 0.089)(Table 4). On pair wise comparison between all the three groups, the differences in Sclerostin levels were also not statistically significant (Table 5).

The mean Sclerostin levels in GCF for Group 1, Group 2 and Group 3 were 25.28, 28.32 and 40.17 respectively(Table 3). The differences of Sclerostin levels in GCF between the groups were highly statistically significant (P =0.000)(Table 4). On pair wise comparison, the differences in Sclerostin levels between Group 1 and Group 3, also Group 2 and Group 3 were highly statistically significant (P = 0.000) while Group 1 and Group 2 were not statistically significant (Table 5).

A negligible correlation was found in Group 1, strong negative correlation was found in Group 2 and moderate positive correlation was found in Group 3 between Sclerostin and PPD values in GCF. A strong negative correlation was found in Group 1, strong negative correlation was found in Group 2 and weak negative correlation was found in Group 3 between Sclerostin and PPD values in Saliva. The correlation was statistically significant in Group 1 between Sclerostin and PPD values in Saliva (P = 0.023).

A moderate positive correlation was found in Group 1, negligible correlation was found in Group 2 and weak negative correlation was found in Group 3 between Sclerostin and CAL values in GCF. A moderate negative correlation was found in Group 1, moderate positive correlation was found in Group 2 and very strong negative correlation was found in Group 3 between Sclerostin and CAL values in Saliva (P >0.05).

A negligible correlation was found in Group 1, weak positive correlation was found in Group 2 and weak negative correlation was found in Group 3 between Sclerostin and PI values in GCF. A strong positive correlation was found in Group 1, negligible correlation was found in Group 2 and strong negative correlation was found in Group 3 between Sclerostin and PI values in Saliva (P >0.05).

A negligible correlation was found in Group 1, negligible correlation was found in Group 2 and negligible correlation was found in Group 3 between Sclerostin and GI values in GCF. A negligible correlation was found in Group 1, negligible correlation was found in Group 2 and strong negative correlation was found in Group 3 between Sclerostin and GI values in Saliva. The correlation was found to be statistically significant in Group 3 between Sclerostin and GI values in Saliva (P = 0.05)(Table 6).

Discussion:-

The findings of our study demonstrated a statistically significant difference in PI, GI, PPD and CAL between periodontally healthy, chronic periodontitis with and without type 2 diabetes mellitus patients. On comparing periodontally healthy group with chronic periodontitis with and without type 2 diabetes mellitus group, there wasa significant difference between all the clinical parameters. Similar findings were seen in the study done by Balli et al in 2015 [7] where found significant difference was found between the clinical parameters i.e., PPD, CAL, PI and GI. Also, similar findings were seen in a study done by Tiantian et al in 2018. [12]

In our study, on comparing Sclerostin levels in saliva between the periodontally healthy and chronic periodontitis with and without type 2 diabetes mellitus group, no significant difference were observed. These findings were in contrast with a study done by Tiantian et al in 2018 [12] wherein the salivary Sclerostin levels were significantly higher in chronic periodontitis group when compared to the non-periodontitis group.

In our study, there is a significant difference found between Sclerostin levels in GCF between periodontally healthy group and chronic periodontitis with and without type 2 diabetes mellitus group. Similar findings were seen in a study done by Balli et al in 2015 [7] and Tiantian et al in 2018. [12]

Studies done by Balli et al[7] and Tiantian et al [12] have supported the applicability of Sclerostin as a biomarker in Saliva and GCF respectively, but no longitudinal data is available to prove Sclerostin as a predictor of bone loss. In our study, a statistically significant difference was observed in GCF Sclerostin levels but not in Salivary Sclerostin levels between the chronic periodontitis with and without type 2 diabetes mellitus group. The explanation for this response may be due to the assessment of pathological status of the periodontium in a site specific manner.[1]

Also, in accordance with the salivary Sclerostin levels, the difference between the present and previously published studies may be related to the complex structure and collection method of the saliva. In the present study, we used unstimulated technique in order to avoid any disruption in the Sclerostin levels in saliva.

In study by Martin et al [8] and Gennari et al in 2012,[13] they found that circulating Sclerostin levels were increased in type 2 diabetes mellitus. However after extensive literature search therewas no published data till date available related to the evaluation of Sclerostin levels in Saliva and GCF in chronic periodontitis with type 2 diabetes mellitus group. Hence, this is the first kind of a study where we observed that the Sclerostin levels in GCF were significantly higher in chronic periodontitis with type 2 diabetes mellitus group when compared to the chronic periodontitis group alone.

List of Figures and Tables

Figure 1:- The study design is represented in the flow chart.

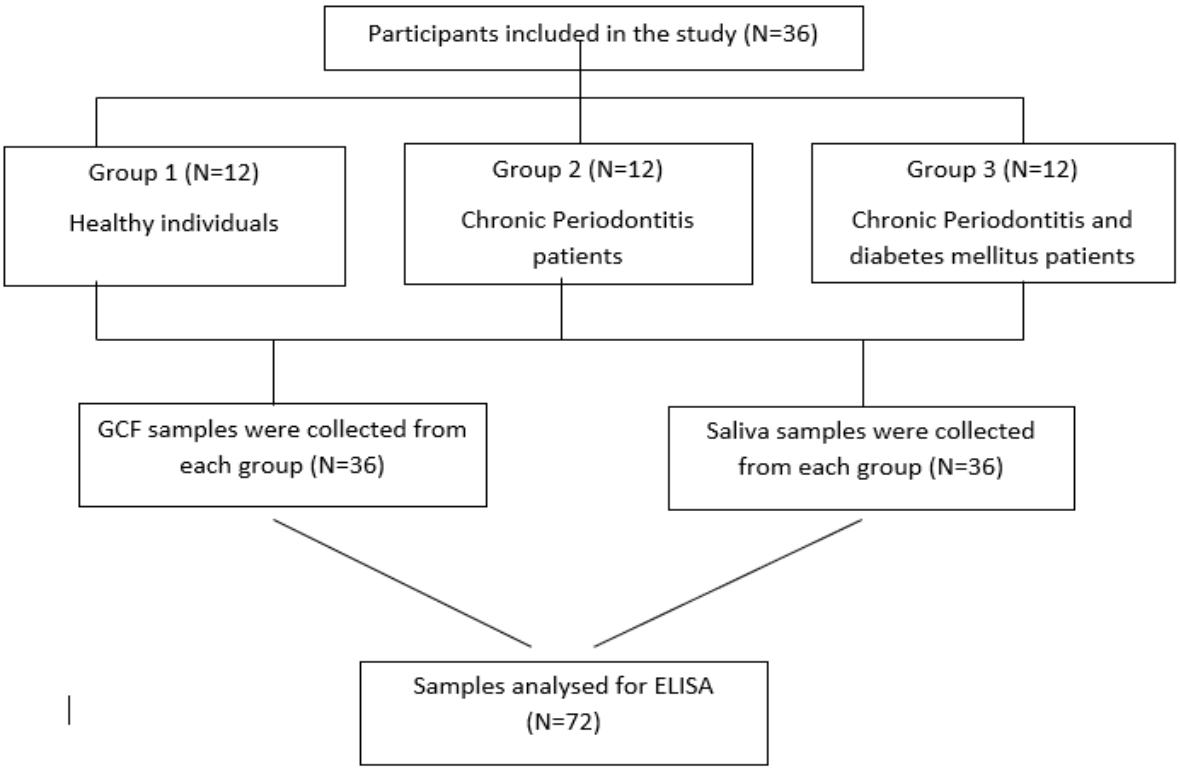
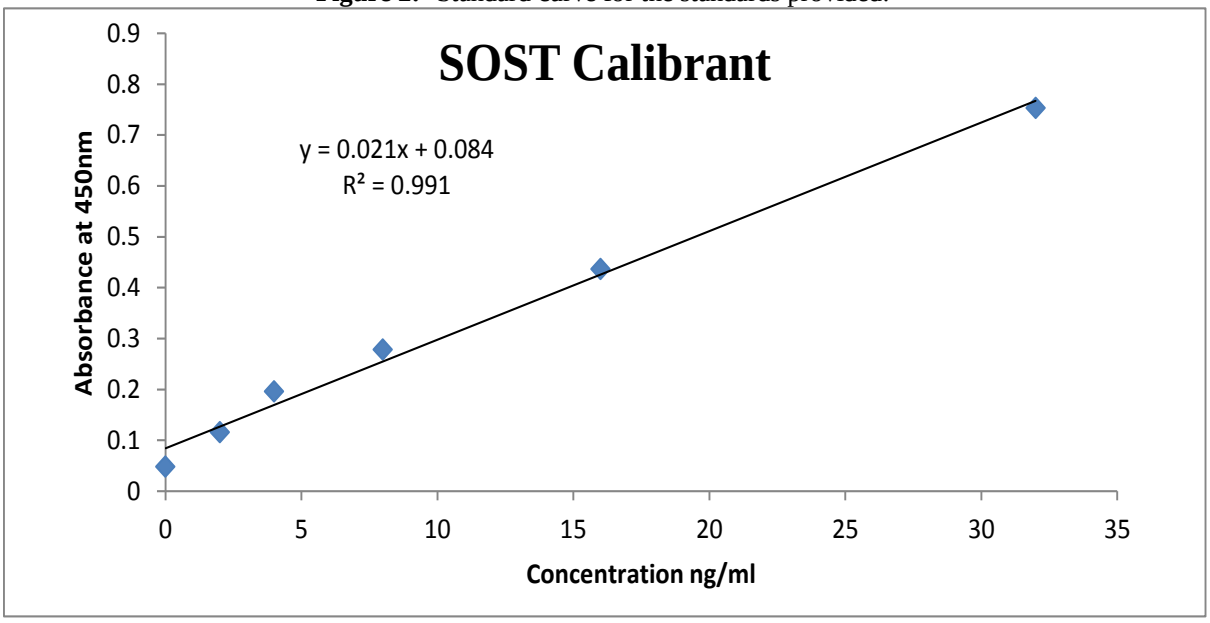


Figure 2:- Standard curve for the standards provided.



List of Tables

Table 1:-

Concentration (ng/ml)	Optical density at 450nm (OD)
0	0.0481
2	0.1161
4	0.196
8	0.2782
16	0.4366
32	0.7532

Optical Density values for the standards provided

Table 2:-

Sclerostin levels in Saliva	Minimum	Maximum	Mean	Std. Deviation
Group 1	0.00	12.67	8.17	3.63
Group 2	9.84	14.77	11.81	1.43
Group 3	1.24	19.85	10.51	5.62

Mean Distribution Of The Groups Based On Sclerostin Levels In Saliva

Table 3:-

Sclerostin levels in GCF	Minimum	Maximum	Mean	Std. Deviation
Group 1	6.68	34.11	25.28	6.99
Group 2	23.32	37.61	28.32	4.16
Group 3	28.26	65.36	40.17	9.92

Mean Distribution Of The Groups Based On Sclerostin Levels In GCF

Table 4:-

	F value	p value
PI	35.326	0.000*
GI	66.830	0.000*
Probing depth	222.019	0.000*
CAL	74.912	0.000*
Saliva	2.607	0.089
GCF	13.518	0.000*

***denotes statistically significant**

Comparision Of The Clinical Parameters And Sclerostin Levels In Saliva And GCF Using Anova

Table 5:-

Dependent Variable	Group	Groups	Mean Difference	p value
Probing depth	Group 1	Group 2	-1.52	0.000*
		Group 3	-3.71	0.000*
	Group 2	Group 3	-2.19	0.000*
CAL	Group 1	Group 2	-1.66	0.000*
		Group 3	-3.14	0.000*
	Group 2	Group 3	-1.48	0.000*
PI	Group 1	Group 2	-0.92	0.000*
		Group 3	-1.36	0.000*
	Group 2	Group 3	-0.43	0.038*

GI	Group 1	Group 2	-1.24	0.000*
		Group 3	-1.90	0.000*
	Group 2	Group 3	-0.66	0.001*
GCF	Group 1	Group 2	-3.03	0.970
		Group 3	-14.89	0.000*
	Group 2	Group 3	-11.85	0.001*
Saliva	Group 1	Group 2	-3.63	0.093
		Group 3	-2.33	0.473
	Group 2	Group 3	1.30	1.000

***denotes statistically significant**

Pair Wise Comparison Of Clincial Parameters And Sclerostin Levels In Saliva And Gcf Using Post-Hoc Bonferroni Test

Table 6:-

Group		GCF		Saliva	
	Clinical parameters	r value	p value	r value	p value
Group 1	Probing depth	0.19	0.554	-0.647	0.023*
	CAL	0.357	0.255	-0.32	0.31
	PI	-0.007	0.982	0.062	0.848
	GI	-0.021	0.948	-0.067	0.837
Group 2	Probing depth	-0.43	0.163	-0.541	0.069
	CAL	-0.008	0.98	0.391	0.209
	PI	0.292	0.356	0.003	0.992
	GI	-0.184	0.567	-0.157	0.626
Group 3	Probing depth	0.329	0.296	-0.299	0.345
	CAL	-0.248	0.438	-0.078	0.809
	PI	-0.248	0.438	-0.492	0.104
	GI	0.022	0.945	-0.568	0.05*

***denotes statistically significant**

Correlation Of Sclerostin Levels In Gcf And Saliva With Clinical Parameters

Conclusion:-

Sclerostin levels are a promising diagnostic and prognostic biomarker in periodontal diseases.

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