SALIVARY ENDOTHELIN-1 AS A SURROGATE BIOMARKER FOR PERIODONTITIS AND TYPE II DIABETES MELLITUS A CLINICO-BIOCHEMICAL STUDY

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Abstract

An important etiological factor responsible for diabetic vascular complications is endothelial dysfunction, which is characterized by an imbalance between endothelial-derived vasoconstrictor factor, Endothelin-1 (ET-1). The levels of Endothelin-1 are characteristically increased in diseased periodontal tissues. Considering these factors , the current study evaluated and compared salivary Endothelin-1 levels among healthy, periodontitis, Type II diabetic, and Type-II diabetes with periodontitis subjects.100 individuals fulfilling the inclusion and exclusion criteria were divided into 4 groups. Group 1 (Systemically healthy subjects without generalized periodontitis), Group 2 (Subjects with generalized periodontitis), Group 3 (Periodontally healthy subjects with type-2 Diabetes), and Group 4 (Subjects with Type-II diabetes with generalized periodontitis). Saliva samples were collected from all four groups. Salivary Endothelin-1 levels were determined through ELISA analysis. The data obtained was then subjected to statistical analysis. Results indicated significantly elevated Salivary Endothelin-1 levels in group 4 (diabetes along with periodontitis) when compared to the healthy group. Overall comparative analysis revealed raised salivary Endothelin -1 levels in group 2, group 3, and group 4 when compared to the healthy group ($p< 0.0015$**). The results of this study suggest that patients suffering from periodontitis and diabetes have higher salivary levels of Endothelin-1 than subjects with periodontitis and healthy controls indicating its fundamental role in the bidirectional relationship between diabetes and periodontitis. Therefore, Endothelin-1 can be considered a surrogate Salivary biomarker for Type-II Diabetes Mellitus and periodontitis.

Key words: Endothelin-1, ET-1, Generalized Stage II Grade B periodontitis, Type-2 Diabetes Mellitus

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Introduction

Periodontitis is a common chronic inflammatory disease characterized by the destruction of the supporting structures of the teeth. Epidemiological data confirm that diabetes is a major risk factor for periodontitis and there is a bidirectional relationship between the degree of hyperglycemia and the severity of periodontitis [1,2].

Diabetes mellitus (DM) is a chronic condition characterized by chronic hyperglycemia that results from unregulated endocrine and metabolic pathways in glucose utilization [3]. The altered glucose metabolism associated with type II Diabetes mellitus (Type II DM) leads to microvascular, neurologic, and immunological changes. In clinical studies, increased levels of several pro-inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin (IL)-1, IL-6, and IL-18 were associated with various diabetic complications such as diabetes-associated coronary artery disease, peripheral arterial disease, nephropathy, neuropathy, retinopathy, and periodontitis [4].

The primary etiology responsible for diabetic vascular complications is endothelial dysfunction, which is characterized by an imbalance between endothelial-derived vaso-dilator and -constrictor substances, such as the vasoconstrictor factor, Endothelin-1 (ET-1) [5].

Endothelin-1 is one of the most frequent findings of ET in the human body expressed within tissues during the progression of inflammation. ET-1 is secreted by endothelial cells after exposure to pathogenic bacteria and it represents a potent mediator of vascular inflammation and a vasoconstrictor [6,7]. The levels of Endothelin-1 are characteristically increased in diseased periodontal tissues and are thought to be a critical determinant of periodontitis outcome [8]. Recent studies have assessed plasma and serum endothelin-1 levels in Type II Diabetes Mellitus subjects [9,10]. To date, salivary ET-1 level assessment and its association with DM have not been investigated to the best of our knowledge.

Therefore, this study evaluated and compared salivary Endothelin -1 levels in periodontitis subjects with and without Type II Diabetes mellitus.

Materials and Methods

Study Design and patient selection:

This cross-sectional study was conducted in the Department of Periodontology, School of Dental Sciences, KIMSDU, Karad.

The sample size was obtained by the following formula:

$$n = \frac{Z_{1-\alpha/2}^2 \, SD^2}{(M \times \varepsilon)^2} \quad (1)$$

Based on the above calculation a minimum sample of 22 was required in each group. However, considering the possibility of dropouts 25 samples were considered in each group. A purposive sampling technique was used to allocate a total of 100 patients equally to four groups. Group 1 consisted of 25 Systemically and periodontally healthy subjects, Group 2 consisted of 25 subjects with Generalized Stage II grade B periodontitis, Group3 consisted of 25 subjects with Type II Diabetes mellitus without periodontitis and Group 4 consisted of 25 subjects with Type II Diabetes mellitus with Generalized Stage II grade B periodontitis.

Subjects with Generalized Stage II Grade B Periodontitis, Type II Diabetes Mellitus and between 35-65 years of age were included in the study. Subjects with periodontitis were selected as per criteria by the 2017 AAP World Workshop on Classification of Periodontal and Peri-implant Diseases and Conditions. Subjects with Diabetes Mellitus were selected after following the criteria of diagnosis by the American Diabetes Association (2022). Pregnant and lactating females, subjects with a history of periodontal therapy within six months, patients under medications known to influence periodontal tissues, and tobacco chewers/ smokers were excluded from the study.

A flowchart representing recruitment and allocation of the study population is given as shown in **Figure 1.**

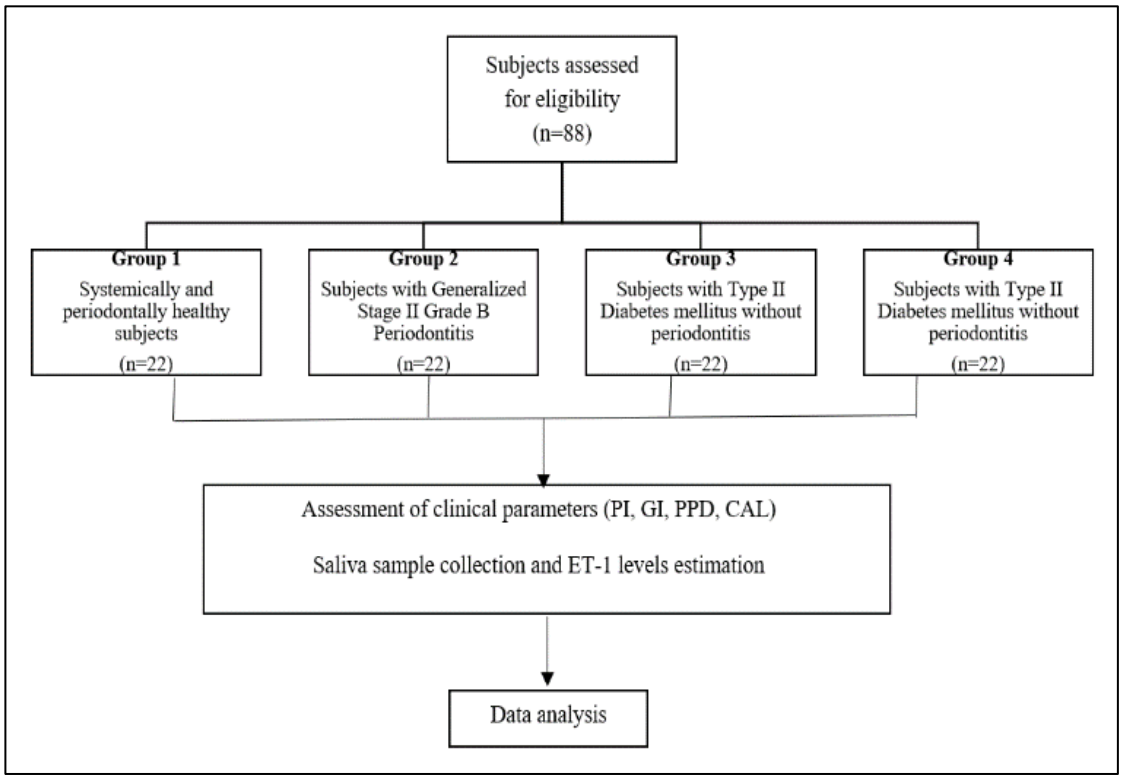

Figure 1: Flowchart representing recruitment and allocation of the study population

***Collection of Saliva samples*:** To standardize the collection, two ml of unstimulated whole saliva sample was collected under aseptic conditions using the spitting method two hours after the last meal from 10 am to 12 pm. Two ml of the saliva sample was pipetted out in an Eppendorf tube. To remove the cell debris samples were centrifuged at 1000 rpm at 2-8^{o}C for 20 minutes. For further analysis, half ml of the supernatant was stored in a one-and-a-half ml plastic Eppendorf tube with a tracking number at -80^{o} C at the Department of Genetics, KIMSDU, Karad.

Assessment of clinical parameters: Baseline measurements of the Gingival index (GI) (Loe H and Silness 1963), Plaque index (PI) (Silness J. and Loe H 1964), Probing pocket depth (PPD) using the Williams periodontal probe and Clinical attachment loss (CAL) were recorded.

Estimation of salivary Endothelin*-1 level:* Levels of salivary Endothelin-1 were determined using Fine Test® Human Endothelin-1 (ET-1) enzyme-linked immunosorbent assay (ELISA) kit. The assay was carried out using an ELISA reader (LisaQuant, Department of Microbiology, KIMSDU, Karad, India) according to the instructions and directions of the manufacturer.

Data analysis: Data obtained was entered into an Excel sheet and subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 20.0. The difference in the salivary Endothelin-1 level was determined using the Quadratic Regression Equation. Mean and standard deviation was calculated. A one-way ANOVA test was used for overall comparative analysis and a Tukey-Kramer Multiple Comparison test was used to analyze the intergroup variability.

Study Ethics and Safety: This clinical biochemical study was approved by an Ethical Committee and Institutional Review Board of Krishna Institute of Medical Sciences, Karad (Protocol No: KIMSDU/IEC/06/2022). Subjects were explained in detail about the study and included in the study after obtaining their consent.

Results and Discussion

Eighty-Eight subjects in the age range of 35-65 years, participated in this study. Analysis of general demographic data has been expressed in **(Table 1)**.

Table 1: Age and Gender wise Distribution

Study Group	**Gender**	**Age (in years)**		
	Males (%)	Females (%)	Mean	SD
Healthy Group (n=25)	10 (40%)	15 (60%)	42	5.180
Periodontitis Group (n=25)	16 (64%)	9 (36%)	47.76	6.616
Diabetes Group (n=25)	13(52%)	12(48%)	50.92	6.843
Diabetes + Periodontitis (n=25)	10 (40%)	15 (60%)	52.68	4.964

SD: Standard deviation, %= Percentage

Table 2: Descriptive statistics of Salivary Endothelin -1 levels in Group 1, Group 2, Group 3, and Group 4

Salivary Endothelin-1 Levels	**Mean (pg/ml)**	**SD**	**SE**	**Minimum (pg/ml)**	**Maximum (pg/ml)**	**p-value significance,**
Group 1	201.742	35.165	6.216	175.80	297.03	p< 0.0015**
Group 2	230.24	34.93	6.176	133.55	298.16	
Group 3	232.265	42.23	7.465	163.55	312	
Group 4	239.066	46.54	8.228	145.91	320	

p>0.05 – no statistically significant difference *p<0.05 significant **p<0.001 – highly significant

After assessment of periodontal parameters higher mean PI, GI, PPD, and CAL levels were reported in subjects with diabetes along with periodontitis than in subjects with periodontitis alone. The mean Salivary Endothelin-1 level in the healthy group was 201.742 pg/ml while it was 230.24 pg/ml in periodontitis subjects. The mean salivary Endothelin-1 level was found to be 232.265 pg/ml in Diabetes, while 239.066 pg/ml in patients with diabetes and periodontitis. **(Table 2)**

The intergroup comparison revealed that Salivary Endothelin-1 levels raised significantly in diabetes patients and in patients with diabetes along with periodontitis when compared with healthy subjects (p< 0.0015). Further comparative analysis showed that in group 4 salivary ET-1 levels were significantly higher compared to healthy subjects (p<0.01**). **(Table 3)**

Table 3: Overall comparative statistics of Salivary Endothelin-1 levels in Group 1, Group 2, Group 3, and Group 4

Group	**Comparison Group**	**p-value Significance**
Group 1 (Healthy) vs	Group 2 (Periodontitis only)	p<0.05*
	Group 3 (Diabetes)	p<0.03*
	Group 4 (Diabetes + Periodontitis)	p<0.01**
Group 2 (Periodontitis) vs	Group 3 (Diabetes)	p>0.05
	Group 4 (Diabetes + Periodontitis)	p>0.05
Group 3 (Diabetes)	Group 4 (Diabetes + Periodontitis)	p>0.05

p>0.05 – no statistically significant difference *p<0.05 -significant **p<0.001 – highly significant

Both periodontal disease and type II diabetes mellitus are recognized as chronic inflammatory diseases and a two-way relationship between them has been suggested in many reviews and epidemiological studies. Possible mechanistic links between DM and periodontitis have been proposed, including altered polymorphonuclear cell (PMN) function, increased adipokine production, and altered apoptosis, which could result in increased inflammatory cytokine production in both patients with periodontitis and DM. This was proposed to contribute to the exaggerated immune responses and impaired tissue integrity, making diabetic patients more susceptible to periodontitis. Patients with DM have an increased risk of developing periodontitis, and those with untreated periodontitis seem to have poorer glycemic control. Also, periodontitis is

considered the sixth complication of DM [11]. DM influences periodontitis initiation and progression by causing a hyperinflammatory response, impairing bone repair processes, and producing advanced glycation end products [12]. Periodontitis as a local focus of infection can cause IL-6, TNF-a, and CRP levels to increase in systems, resulting in increased systemic inflammation, which contributes to insulin resistance [13]. The current study reported raised GI, PI, PPD, and loss of clinical attachment in diabetes with periodontitis subjects as compared to subjects with periodontitis only. This is in accordance with the study by Mohammad HG et al., however, Dağ A et al. did not find a significant difference in periodontal parameters (PPD, CAL, GI, and PI) between both groups [14,15].

To the best of our knowledge, this is the first study to evaluate and compare Salivary Endothelin-1 levels in Diabetic patients and in patients with diabetes along with periodontitis. Endothelin-1 (ET-1) is the main member of the endothelin peptide family. It was discovered by Yanagisawa et al. in 1988 [16]. ET-1 is produced by vascular endothelial and smooth muscle cells, airway epithelial cells, macrophages, fibroblasts, cardiac myocytes, brain neurons, and pancreatic islets [17]. ET-1 is induced in various tissues during inflammatory processes. It is a potent mediator of vascular inflammation and a vasoconstrictor. In this regard, it has been shown that several proinflammatory cytokines, including IL-1, -6, and -8, have been reported to upregulate the secretion of ET-1 [18].

Diabetes mellitus (DM) is a chronic condition characterized by chronic hyperglycemia that results from unregulated endocrine and metabolic pathways in glucose utilization [19]. The major microvascular complications of diabetes are nephropathy, retinopathy, neuropathy, and skin microangiopathy. Evidence based literature suggests that the ET-1 system may contribute to diabetic vascular disease [20]. In addition to its direct vasoconstrictor effects, enhanced levels of ET-1 may contribute to endothelial dysfunction through inhibitory effects on nitric oxide (NO) production.

ET-1 may contribute to the development of endothelial dysfunction and insulin resistance by increasing the production of reactive oxygen species, mainly superoxide anion, in the vasculature. This is mainly dependent upon the activation of NADPH oxidase protein expression and activity. ET-1 levels are found to be elevated in diabetic patients in various studies. Jochen G. Schneider et al. and Claudete M. Zanatta et al. reported that plasma ET-1 concentrations were significantly higher in subjects with type II diabetes compared to healthy controls [22]. A study by Arifur Rahman et al. suggested that serum ET-1 levels in diabetic subjects appeared to be higher [10]. Also, a study by Cervantes MH et al. reported raised urinary ET-1 levels in diabetic patients with microalbuminuria [23]. A similar trend was observed in the current study as this study revealed that Salivary ET-1 levels were raised in diabetic patients when compared to healthy subjects.

Our study also revealed that higher salivary ET-1 levels were associated with periodontitis. This is in line with the study by Waleed Khalid et al. and Sahar S. Kadhim et al. as they reported significantly higher Serum ET-1 levels in chronic periodontitis subjects when compared to healthy subjects [24,25]. Also, A study by Gaetano Isola et al. recently showed that subjects with periodontitis had higher salivary ET-1 levels compared to healthy controls [26].

Similarly, previous studies have reported an upregulation of ET-1 in inflamed gingival tissues and periodontal tissues. Yamamoto E et al., Ansai T et al., Tamil Selvan T et al., and Chen et al. found an increased concentration of ET-1 in gingival tissue samples in the chronic periodontitis group when compared with the periodontally healthy group [27-30]. Similarly, two studies assessed the ET-1 levels in GCF. Fujioka D et al. found a higher concentration of ET-1 in chronic periodontitis than in periodontally healthy subjects. But, the study by Pradeep AR et al., did not detect ET-1 in GCF [31,32].

The current study revealed higher Salivary ET-1 levels in diabetics with periodontitis. This may be explained by the fact that Diabetes mellitus and periodontitis are both chronic inflammatory conditions. Inflammatory vascular changes that occur in DM subjects with periodontitis further aggravate inflammatory response thus increasing salivary ET-1 levels.

Since there are no studies available on the comparison of salivary ET-1 levels in patients with diabetes along with periodontitis, a direct comparison of our results is not possible.

Conclusion

Periodontitis and diabetes patients expressed elevated salivary Endothelin-1 levels. Therefore,

Endothelin-1 can be considered as a surrogate Salivary biomarker for Type II Diabetes Mellitus and periodontitis.

Limitations

Limitations of the present study include a relatively small sample size.

Future Perspectives:

Future studies with a modified study design and larger sample size can be employed to establish the exact role of Endothelin-1 in the pathogenesis of periodontal diseases and diabetes. It will also help to further explain the bidirectional relationship between periodontal diseases and diabetes by utilizing ET-1 as a salivary biomarker.

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Conflict of interest: None

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