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Intelectin-1 And Malondialdehyde Levels In Type 2 Diabetic Mellitus Patients With Different Stages Of Periodontitis

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Abstract
Background: periodontitis and diabetes mellitus are two of the most common chronic non communicable diseases, with a bidirectional relationship that exacerbates both conditions. intelectin-1 is an anti-inflammatory adipocytokine produced primarily by visceral adipose tissue, Malondialdehyde (MDA) is a byproduct of lipid peroxidation and serves as a marker for oxidative stress in the body.

Aim of the present study: *To assess the salivary levels of omentin-1 and malondialdehyde in diabetic patients with varying stages of periodontitis compared to a controls group.*

Materials and methods: *One hundred sixty men participated in this study; 32 of them were healthy control groups, while the other 128 men were categorized into four equal groups according to stages of periodontitis (stage-I, stage-II, stage-III, and stage-IV). The clinical periodontal parameters (PPD, CAL, and BOP) were Measured after collecting unstimulated salivary samples from all participants. omentin-1 and malondialdehyde levels in saliva samples were analysis using the enzyme-linked immune sorbent assay (ELISA) method.*

Results: *malondialdehyde (MDA) was maximal in diabetic with periodontitis groups than healthy controls. The level of MDA is increased when periodontitis progresses to higher stages. stage I (323.89 ± 99.15), stage II group (356.74 ± 111.58), stage III group (396.21 ± 105.71), stage IV group (402.44 ± 84.52), control group (157.22 ±116.09) in which their salivary levels were the minimal with significant differences among the groups (p=0.000).*

intelectin-1 level was higher in healthy controls than diabetic patients with all stages of periodontitis. The level of omentin-1 is decreased when periodontitis progresses to higher stages. stage I (216.48±121.46), stage II group (172.90±86.02), stage III group (146.24±78.48), stage IV group (158.05±91.59), control group (263.17±134.34) in which their levels were the highest with significant differences between the groups (p=0.000).

Conclusion*: The oxidative stress marker MDA is increased in diabetic mellitus patients than controls, and when the stage of periodontitis gets worse, while omentin-1 marker is decreased in diabetic mellitus patients than controls when the stage of periodontitis deteriorate.*

Introduction

Periodontitis is characterized by destruction of the teeth-supporting apparatus, including the periodontal ligament and alveolar bone. It is a chronic infection of the oral cavity caused by pathogenic biofilms that cause a persistent inflammatory response in gingival tissue [1,2].

A deficiency in action of insulin, secretion, or both of the two can cause diabetes mellitus (DM), a common metabolic disorder [3]. Regardless of related risk factors, there is a reciprocal association between periodontitis and diabetes, and the two conditions also have an impact on one another [4].

Apart from its ability to store energy, adipose tissue secretes other biological components and mediators of inflammation. Different depots of adipose tissue with regional variability release different chemicals, including "tumour necrosis factor-α (TNFα), interleukin-6 (IL-6), leptin, retinol-binding protein, resistin, adiponectin, omentin, apelin, visfatin, and others" [5]. These secretions affect how fats and carbohydrates are metabolised. They also have a major effect on a number of pathological processes, such as inflammation, atherosclerosis, insulin resistance, T2DM, and vascular endothelium dysfunction [5].

The glycoprotein intelectin-1, commonly referred to as omentin-1, has become an important participant in the intricate interactions that occur between adipose tissue and other physiological processes. The visceral adipose tissue's stromal vascular cells are the main producers of omentin-1[6].

Malondialdehyde (MDA) is a substance that results from polyunsaturated fatty acids peroxiding. It has been applied as a biomarker to assess oxidative stress in a variety of biological samples from patients with a broad spectrum of illnesses.[7]

This study aim to assess the relation of intelectin-1(omentin-1) and oxidative stress marker (MDA) with severity of periodontitis in diabetic patient.

Materials and method:

Study design

A complete of one hundred sixty (160) male participant , Among the ages range from 40-60 years old were included in this case-control study, One hundred twenty-eight patients with Type-2 diabetes mullets and periodontitis(were collected from Diabetes and Endocrinology Center at Marjan Hospital – Babylon, Thirty two Controls people were collected from people who visit dental school for regular dental check-ups who do not have any disease .

The Mustansiriyah university-college of dentistry-institutional Ethics Committee Board granted ethical approval.(approval number and date" REC145"on 01/December/2023). participants signed a consent form indicating their agreement in the study.

Exclusion criteria

Patients undergone periodontal management within the previous six months, those who had taken an antibiotic within that same time frame, those with systemic diseases other than diabetes mellitus, female patients, those who smoked or had smoked cigarettes within the previous three years, patients with edentulous teeth, and patients with fewer than twenty teeth were not included in this study.

Saliva sample collection

Un-stimulated saliva was collected in the morning [8]. saliva was collected for 10 minutes from each patient, into sterile tubes according to Navazesh method[9]. The samples that were collected underwent centrifugation at 5000 rpm for 10 minutes.[10] The obtained supernatant was then transferred into Eppendorf tubes using a micropipette and kept in the laboratory freezer at -20°C until the day of analysis

After saliva was collected, a comprehensive periodontal examination was carried out, which included measuring "bleeding on probing (BOP) [11], probing pocket depth (PPD), and clinical attachment loss (CAL)". All clinical periodontal parameters were measured with a "periodontal probe (the University of Michigan O probe with Williams labelling)". Every tooth, with the exception of the wisdom teeth, had six surfaces inspected. Every subject underwent the identical clinical evaluation conducted by the same examiner.

Salivary omentin-1 and malondialdehyde analysis

Salivary concentrations of omentin-1 and malondialdehyde in the saliva of the diabetic with periodontitis group and the control group were determined using an ELISA kit from Elk Biotechnology company.

Statistical analysis

To characterize, analyses, and present the data, Statistical Package for Social Science (SPSS) version 24 (USA, Illinois) was utilized. The two types of statistical analyses are inferential, which includes the one -way ANOVA test and Tukey's test, and descriptive, which includes the mean and standard deviation (SD).

Result:

Table 1: Mean values and standard deviation of clinical periodontal parameters for controls group and stages of diabetic with periodontitis groups .

groups	Periodontal parameters	mean	Standard Deviation
controls	PPD	0.61	0.13
	CAL	0.42	0.25
	BOP	0.80	0.14
STAGEI	PPD	1.61	0.18
	CAL	1.77	0.44
	BOP	1.15	0.19
STAGE II	PPD	1.98	0.31
	CAL	2.08	0.41
	BOP	1.22	0.22
STAGE III	PPD	2.15	0.46
	CAL	2.61	0.70
	BOP	1.33	0.20
STAGE IV	PPD	2.43	0.55
	CAL	2.89	0.84
	BOP	1.41	0.20

Table 1shows mean values of PPD, CAL, and BOP were increased from controls group till diabetic with periodontitis groups , the mean value of PPD for control was (0.61±0.13), for stage I (1.61±0.18), for stage II (1.98±0.31), for stage III (2.15±0.46), for stage IV (2.43±0.55) .the mean value of CAL for control was (0.42±0.25), for stage I (1.77±0.44), for stage II (2.08±0.41), for stage III (2.61±0.70), for stage IV (2.89±0.84). the mean value of BOP for control was (0.80±0.14), for stage I (1.15±0.19), for stage II (1.22±0.22), for stage III (1.33±0.20), for stage IV (1.41±0.20) .

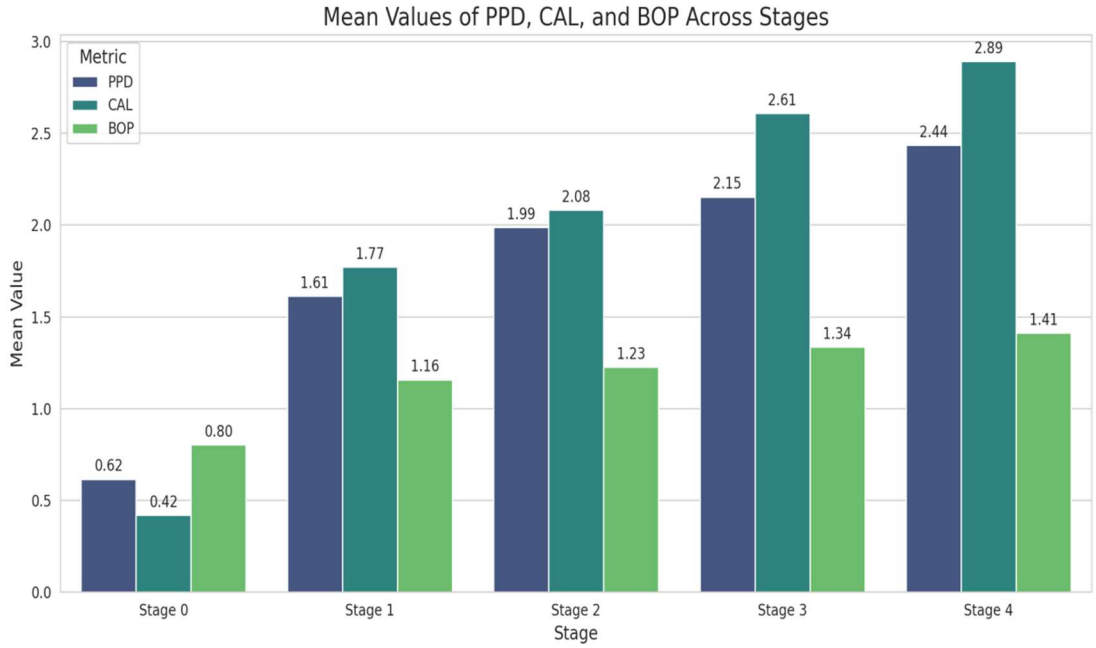

Figure 1: bar chart visualizes the mean values of PPD (Probing Pocket Depth), CAL (Clinical Attachment Level), and BOP (Bleeding on Probing) for controls group and stages of diabetic with periodontitis groups

Table 2: A: Mean ,standard deviation and anova test of salivary intelectin-1 for study groups and B: among groups.

Table 2A: Mean ,standard deviation and anova test of salivary intelectin-1 for study groups

	groups	**Mean ± SD**	**F**	**P value**
intelectin-1 (pg/ml)	**I**	216.48±121.46	6.78	0.000
	II	172.90±86.02		
	III	146.24±78.48		
	IV	158.05±91.59		
	controls	263.17±134.34		

SD: standard deviation ; **F**: ANOVA

in table 2A the mean value of intelectin-1 was decreased from controls till stage IV with a significant difference (0.00) (ANOVA test was used), the mean value of intelectin-1 for controls was (263.17±134.34), for stage I mean value was(216.48±121.46),for Stage II mean value was(172.90±86.02),for Stage III mean value was(146.24±78.48),for Stage IV mean value was (158.05±91.59).

Following multiple pairwise comparisons (using Tukey's Honest Significant Difference (HSD) test) among groups as seen in **table 2B** the results of controls group and group II, control group and group III , control group and group IV were significant difference ($p<0.05$) , And the rest of the results were non-significant ($p>0.05$)

Table 2B: Tukey's Honest Significant Difference (HSD) test of salivary intelectin-1 among study groups.

Study groups		**Mean difference**	**P value**
controls group	group I	46.6845	0.3863
controls group	group II	90.2661	0.000
controls group	group III	116.9258	0.000
controls group	group IV	105.1178	0.000
group I	group II	43.5816	0.4581
group I	group III	70.2413	0.0608
group I	group IV	58.4333	0.1729
group II	group III	26.6596	0.8463
group II	group IV	14.8517	0.9795
group III	group IV	-11.808	0.9914

Table 3 :A: Mean , standard deviation and anova test of salivary Malondialdehyde for study groups and B:among study group.

Table 3 :A: Mean , standard deviation and anova test of salivary Malondialdehyde for study groups.

	groups	**Mean ± SD**	**F**	**P value**
MDA (pg/ml)	**group I**	323.89 ± 99.15	29.74	0.000
	group II	356.74 ± 111.58		
	group III	396.21 ± 105.71		
	group IV	402.44 ± 84.52		
	controls	157.22 ±116.09		

MDA: Malondialdehyde**; SD**: standard deviation ; **F**: ANOVA

in table 3A the mean of Malondialdehyde was increased from controls till stage IV with a significant difference (0.00) (ANOVA test was used), mean value of Malondialdehyde for controls was (157.22 ±116.09), for stage I mean value was(323.89 ± 99.15),for Stage II mean value was(356.74 ± 111.58),for Stage III mean value was(396.21 ± 105.71),for Stage IV mean value was (402.44 ± 84.52).

Following multiple pairwise comparisons (using Tukey's Honest Significant Difference (HSD) test) among

groups as seen in **table 3B** All the results were significant difference ($p<0.05$) ,except group I with group II and group II with group III , group II with group IV, group III with group IV were no significant difference ($p>0.05$)

Table 3B: Tukey's Honest Significant Difference (HSD) test of salivary Malondialdehyde among study groups.

Study groups		**Mean difference**	**P value**
Controls group	group I	-166.6824	0.000
controls group	group II	-199.5322	0.000
controls group	group III	-238.9924	0.000
controls group	group IV	-245.2189	0.000
group I	group II	-32.8498	0.7139
group I	group III	-72.31	0.000
group I	group IV	-78.5365	0.000
group II	group III	-39.4602	0.5526
group II	group IV	-45.6867	0.4024
group III	group IV	-6.2264	0.9993

Discussion

ajjIn current study, the relationship between the severity of periodontitis and some salivary biomarkers in diabetic patients was studied, as diabetes and periodontitis have a bidirectional relationship[4], and researchers are still searching for this relationship.

In current study , the results show mean value of BOP ,PPD,CAL were increased from the control group to diabetic with periodontitis groups . These findings was in consisitent with (Sreenivasulu et al.,2022) who found that the diabetic group with periodontitis had considerably more bleeding on probing than the control group, suggesting a link between periodontal health and diabetes in the study.[12] Those results also were in consistent with Ali and Ahmed,2018 [13]who demonstrated that both probing pocket depth and clinical attachment loss mean values were increased in periodontitis with increased severity, and also Those findings were in agreement with Nazdar et al ,.2020 [14] who find Elevated probing pocket depth and clinical attachment loss values were observed in diabetic patients with increased periodontal disease severity. These outcomes were clarified by the control group's people 'that have healthy periodontium use of toothbrushes and other interdental cleaning devices for good oral hygiene and plaque control Zahraa and Ayser ,2023 and also good glycemic control[15].

Diabetes is a significant risk factor for periodontitis, with diabetics nearly three times more susceptible to the condition. Diabetes is linked to higher levels of systemic inflammatory markers [16]. It has been established that

hyperglycemia may be implicated in the activation of pathways that enhance inflammation, oxidative stress, and apoptosis [17]. This elevation in diabetic inflammation contributes to both micro and macro vascular problems. Diabetes, in turn, raises the inflammation of periodontal tissue. if periodontitis-related tissue damage is not identified and treated effectively, it can result in the breakdown of both hard and soft tissues and eventually tooth loss[18].

concerning the salivary level of intelectin-1, the results of this study showed that the level of intelectin-1 was significantly maximal in controls than all diabetic with periodontitis groups . There are few studies about salivary omentin-1in diabetic patients with periodontitis where (Patil et al ,2022)[19] in their research concluded: salivary intelectin-1 levels are significantly lower in patients with chronic periodontitis compared to healthy individuals. this suggests that lower levels of intelectin-1 are associated with increased periodontal disease severity. These results agree with the results of our study.

Also Suchetha and Aghanashini, 2023[20] in their research on "Estimation and Correlation of Salivary and Serum intelectin-1 in Chronic Periodontitis: An Interventional Study", concluded: Patients with chronic periodontitis had much lower baseline saliva and serum levels of intelectin-1.Three months following scaling and root planning (SRP), there was a significant rise in intelectin-1 levels in saliva and serum, suggesting a successful outcome of the intervention. also These results agree with the results of our study.

As well Avexilla et al.,2022[21] in their research on" Analysing adipokine Omentin-1 in periodontal disease and type-2 diabetes mellitus: An interventional comparative study" , concluded: A study found that salivary intelectin-1 levels were significantly lower in both chronic periodontitis patients and those with T2DM before treatment. Post-scaling and root planning (SRP), levels increased, indicating a potential role as an inflammatory marker for T2DM and periodontal disease. Elevated salivary omentin-1 post-treatment may reflect improved glycemic control and periodontal health, emphasizing its potential as a therapeutic target[21].

Salivary intelectin-1 anti-inflammatory adipokine, inhibits inflammatory process via cellular signaling pathways and molecular mechanisms. This adipokine inhibits tumor necrosis factor alpha (TNF-α)-induced cyclooxygenase-2 (COX-2) [22] and the expression of adhesions molecules in endothelial cells, blocking the extracellular regulated protein kinase (ERK)/nuclear factor kappa B (NF-κB) pathway.[23] Moreover, intelectin-1 plays an anti-inflammatory role in endothelial cells by promoting the AMPK/AKT pathway directly via suppressing the expression of proinflammatory mediators, including TNF-α, IL-6 and monocyte chemotactic protein-1 (MCP-1) in macrophages.[24] In addition, intelectin-1 promotes the PI3K/AKT signaling pathway that induces the proliferation of human osteoblasts .[25] Therefore, salivary intelectin-1 appears to be a significant regulator of bone remodeling.

Also Regarding the salivary level of malondialdehyde, the results of this study showed that the level of MDA was significantly increased in all diabetic with periodontitis groups than in controls. These results were consistent with result of (Tanja et al.,2022)[26] in their search on Lipid Peroxidation Levels in Saliva and Plasma of Patients Suffering from Periodontitis found that Patients with periodontitis had significantly higher levels of salivary and plasma malondialdehyde (MDA), indicating increased lipid peroxidation

In addition Deepthi et al.,2019 [27] in their search on Malondialdehyde as a marker of oxidative stress in periodontitis patients found that Higher salivary MDA levels were detected in patients with periodontitis compared to healthy controls, indicating increased oxygen radical activity during periodontal inflammation ,furthermore Jelena Mirnic et al.,2022[28] in their search on Evaluation of Lipid Peroxidation in the Saliva of Diabetes Mellitus Type 2 Patients with Periodontal Disease found that The presence of diabetes exacerbates oxidative stress, leading to even higher MDA levels. A study focusing on diabetic patients with chronic periodontitis found that these individuals had elevated levels of MDA, indicating that the combination of diabetes and periodontal disease significantly increases oxidative stress markers[28].aslo Abbas & El-Samarrai, 2016 found that TBARS level was significantly higher in T2DM patients than in controls[29].

The interaction between diabetes and periodontitis implies that diabetic patients may experience enhanced oxidative stress, potentially leading to even greater MDA levels. Specific research on diabetic patients were not addressed.

Conclusion

The findings of this study showed that intelectin-1 was decreased and MDA was increased when stages of periodontitis progressed.

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