Received: 16 December 2021 | Revised: 29 January 2022 | Accepted: 12 February 2022
DOI: 10.22034/ecc.2022.325049.1307
Eurasian Chemical Communications SAMI

FULL PAPER

Evaluating Osteocalcin and Osteonectin in serum male patients with type 2 Diabetes mellitus and periodontitis

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Biomarkers play an essential role since they are good predictor factors for several diseases. Accordingly, we attempted to assess the level of Osteocalcin and Osteonectin in patients suffering from periodontitis in type 2 diabetes mellitus compared to healthy control. 120 male persons were included in the current study collected from the Albaladyat specialist center for dentistry in Iraq. Patients have been divided into three groups (30 patients with type2 Diabetic Mellitus, 30 patients with periodontitis, 30 patients with Diabetic Mellitus with periodontitis), and 30 healthy subjects in a control group. Clinical periodontal parameters were determined for all the studied groups. Additionally, serum was collected from each subject to determine Osteocalcin and Osteonectin levels by the ELISA method. The results revealed a significant difference between study groups and controls regarding the levels of Osteonectin and Osteonectin at the P-value (0.003 and 0.0001). Moreover, the current study results showed a direct association between each of Osteonectin, Osteocalcin with clinical periodontitis parameters. Moreover, analysis by the ROC curve showed that both Osteonectin and Osteocalcin might represent the strongest markers for diagnosis of periodontitis in diabetic patients. Levels of the studied biomarkers suggest suitable biomarkers for early detection of the sixth diabetic complication (periodontitis).

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KEYWORDS

Type 2 diabetes mellitus; periodontitis; serum; Osteocalcin; Osteonectin; clinical periodontal parameters.

Introduction

Diabetes mellitus (DM) is considered one of the most important diseases worldwide. Global estimates forecast that the proportion of the adult population with diabetes will rise by 69% by 2030 [1]. It defines as a complex and chronic disease that needs continuous medical attention, with a high disease burden on the patients [2]. Acute hyperglycemia may progress to life-threatening diabetic ketoacidosis while persistent hyperglycemia is correlating with macrovascular complications, raising myocardial infarction, stroke, and microvascular complications risk that contributes to diabetic nephropathy, retinopathy, and neuropathy [3-5], the sixth complication of DM is periodontitis, which means that diabetes considered as an essential risk factor for development and progression of periodontitis [6].

Periodontitis is the most prevalent health disease that impairs esthetic and social life and can result in tooth loss. It is a multifactorial chronic inflammatory disease

marked by increasing attachment loss, bone resorption, pocket formation, and gingival bleeding [7]. Periodontal disease is characterized by a local accumulation of pathogenic bacteria in dental plaque, and their toxic metabolic products, which affect the function of epithelium and drive its growth and synthesis of tissue-destructive proteinases [8]. Periodontitis severity can be assessed by clinical characteristics such as periodontal probing pocket depth, loss of clinical attachment, and amount of bleeding in the mouth [9]. Biomarkers are released into the tissues and blood through the inflammatory and immunological cells during the illness process, many of which go into the gingival fluid, the blood, and the saliva and are thus easy to analyze [10]. Osteocalcin (OCN), a biomarker of bone growth, has developed. A recent survey has shown the bone/energy metabolism relationship, which showed that the osteoblast indicates an insulin receptor. Osteoclastic bone resorption releases osteocalcin under insulin signaling [11]. Osteocalcin is then excluded from circulation. It affects goal tissues like adipose tissue and the beta cells of the pancreas by increasing insulin manufacture and beta-cell proliferation [12]. Added to this, osteonectin (ON) is an osteoblast and dentoblast-binding phosphorylated glycoprotein of 32 kDa that binds at the same time to the type I of collagen and hydroxyapatite [13]. So the research question for the current study is the serum level of Osteocalcin and Osteonectin associated with periodontitis in type 2 diabetes mellitus compared to healthy and whether these biomarkers can be used as a diagnostic biomarker for periodontitis in diabetic patients as compared to healthy.

Materials and methods

An observational case-control study was proposed to answer the research question. All patients and controls were selected from subjects attending the Albaladyat specialist center for dentistry. Before enrolling in this study, each patient was given an informed consent form that included written information detailing the nature of the trial. Additionally, all patients' medical and dental histories were documented using a questionnaire. Furthermore, the current study strictly conforms with ethical principles, including the World Medical Association Declaration of Helsinki. The current study included 120 male subjects (patients and healthy) who were divided into three study groups (30 patients with type 2 diabetes mellitus with clinically healthy periodontium, 30 patients with periodontitis and systemically healthy, and 30 patients with type 2 diabetes mellitus with periodontitis) and 30 systemically healthy subjects as a control group. Periodontitis groups are classified as follows: (Tonetti *et al.,* 2018) [14] Interdental CAL is observable at two non-adjacent teeth, or buccal or oral CAL 3 mm with pocketing more than 3mm is detected at two teeth. While clinically healthy periodontium will be as follows as the clinical criteria for periodontal health: Healthy periodontium has a BOP of 10%, a PPD of 3mm, and is intact (Chapple *et al*., 2018) [15]. Furthermore, the diabetes criteria were type 2 with Hba1c greater than 7 [10].

Eligibility criteria

- **Inclusion criteria** will be systemically healthy patients (excluding the case definition criteria) eligible to be included in the study, have a minimum of 20 teeth.
- **The Exclusion criteria** include individuals with systemic conditions such as liver and/or kidney dysfunction, inflammatory bowel disease, specifically Crohn's disease, a history of organ transplant or cancer therapy, or any cardiovascular disease or disorder. Additional exclusion criteria include any previous extensive periodontal therapy or being currently under active periodontal treatment. Patients were receiving antibiotic treatment

or immunosuppressant medication within the last 3 months.

The periodontal examination was performed in a dental chair, and a calibrated examiner recorded the periodontal variables for all teeth except the third molar tooth. The parameters recorded include: plaque index (PI) [16], gingival index (GI) [17], probing pocket depth (PPD), clinical attachment loss (CAL), and bleeding on probing (BOP) [18]. After periodontal examination, fasted samples were obtained at least 10 h overnight fasting in the morning. Briefly, after a tourniquet was applied to the arm, the skin was rubbed with alcohol, and blood would be collected from one of the superficial veins. Once a suitable vein was identified, the needle was inserted into the vein. 5 mL of venous blood was collected from each subject (patients and controls) in serum separating tubes at room temperature. The serum was obtained by centrifuging blood at 4000 rpm for 10 min. Then serum was removed and transferred into Eppendorf tubes and stored at -20 C° until the assayed time, in which serum blood glucose measured by an enzymatic method. At the same time, osteocalcin and osteonectin were measured by the ELISA technique.

Statistical analysis

Descriptive statistics, including mean ±SD, were used for the continuous data, while frequency and percentage were used for categorical variables. Data distribution was checked by using Shapiro–Wilk test. An ANOVA test followed by post-hoc analysis was used for parametric continuous variables. Pearson's correlation test will perform correlation between clinical and biochemical parameters. The sensitivity and specificity of the biomarkers, single or as combinations, were investigated using the receiver operating characteristic (ROC) curve. All analyses will be performed by using SPSS (version 25) software. The statistical difference will be considered when $p < 0.05$.

Results

The descriptive statistics between two study groups, periodontitis (P) and diabetic with periodontitis (DP) for clinical periodontal parameters (PLI, GI) Mean ± SD in the study group; was described in (table 1) while (BOP, PPD & CAL) were described in (table 2) and there was a highly significant difference between clinical periodontal parameters (PI, GI, CAL, PPD). In comparison, there was a non-significant difference between the BOP(1) and BOP(0) between the two groups.

TABLE 1 Comparison between P and DP groups in PLI and GI

Group	Mean ± SD	
	PLI	GI
P	1.395 ±0.33	1.314 ±0.31
DP	1.880 ±0.39	2.021 ±0.21
T-test	0.186 **	0.137 **
P-value	0.0001	0.0001
	** (P≤0.01).	

TABLE 2 Comparison between P and DP groups in CAL, PPD, BOP(1) and BOP(0)

Group	Mean ± SD			
	CAL	PPD	BOP (1)	BOP (0)
P	2.337 ±0.60	3.403 ±0.34	18.97 ±14.34	81.03 ±14.34
DP	3.356 ±0.56	4.406 ±0.30	18.18 ±8.47	81.92 ±8.40
T-test	0.301 **	0.166 **	6.087 NS	6.074 NS
P-value	0.0001	0.0001	0.797	0.770
** (P≤0.01), NS: Non-Significant.				

The Mean ± SD of ON and OCN were significantly higher in the studied groups as shown in Table 3 compared to with healthy at P-value <0.01.

TABLE 3 Comparison between different groups in ON and OCN

Group	Mean ± SD	
	ON	OCN
Control	1.257 ±0.26 b	12.07 ±3.11 b
DM	1.426 ±0.30 a	17.31 ±3.35 a
DP	1.455 ±0.22 a	16.76 ±3.67 a
P	1.556 ±0.24 a	15.68 ±4.83 a
LSD value	0.133 **	1.945 **
P-value	0.0003	0.0001

Furthermore, the association between biomarkers (ON and ONC) and clinical periodontal parameters were illustrated in Table 4 and 5 as there was a significant association of ON with the clinical attachment loss for the DP group. In contrast, there was a non- significant association with other clinical periodontal parameters (PLI, GI, BOP, PPD), as shown in Table 4.

TABLE 4 Correlation coefficient ON and PLI, GI, CAL, PPD, BOP(1) and BOP(0) in P and DP groups

Parameters	P group		DP group	
	ON- r	P-value	ON- r	P-value
PLI	0.23 NS	0.207	-0.04 NS	0.841
GI	0.25 NS	0.173	-0.03 NS	0.866
CAL	-0.09 NS	0.607	-0.45 **	0.011
PPD	-0.17 NS	0.365	-0.04 NS	0.799
BOP(1)	-0.07 NS	0.365	0.05 NS	0.791
BOP(0)	0.07 NS	0.694	-0.05 NS	0.765

** (P≤0.01), NS: Non-Significant.

In Table 5, the correlations between periodontitis (P) group OCN and clinical periodontal parameters (PPD and CAL) were weak negative non-significant associations. While GI, PLI, and BOP (1) demonstrated significant positive relationships, BOP score(0) demonstrated significant negative correlations. As a result, there were no significant relationships between OCN level and any clinical periodontal markers in the diabetic periodontitis group.

TABLE 5 Correlation coefficient OCN and PLI, GI, CAL, PPD, BOP (1), and BOP(0) in P and DP groups

Parameters	P group		DP group	
	OCN- r	P-value	OCN- r	P-value
PLI	0.52 **	0.0026	0.01 NS	0.959
GI	0.48 **	0.0063	-0.13 NS	0.492
CAL	0.34 NS	0.0617	-0.35 NS	0.057
PPD	0.02 NS	0.889	-0.14 NS	0.433
BOP(1)	0.38 *	0.035	0.14 NS	0.433
BOP(0)	-0.38 *	0.035	-0.15 NS	0.430

* (P≤0.05), ** (P≤0.01), NS: Non-Significant.

Receiver operator characteristics ROC analysis curve

The ROC analysis data demonstrate that osteocalcin and osteonectin have an excellent ability to predict periodontitis in the diabetic and periodontitis groups compared to healthy subjects. This result was achieved based on investigations that included the test's sensitivity and specificity parameters, the area under the curve, and other relevant characteristics, as shown in Table 6 and Figures 1 and 2.

TABLE 6 ROC analysis data of osteocalcin and Osteonectin level of Patient Groups Related to Healthy Group

Diabetic with periodontitis

Variable	AUC	SE [a]	95% CI [b]
OCN	0.988	0.0127	0.918 to 1.000
ON	0.905	0.0421	0.801 to 0.965
Significance level			P = 0.0453

Periodontitis

Variable	AUC	SE [a]	95% CI [b]
OCN	0.930	0.0319	0.834 to 0.980
ON	0.964	0.0274	0.881 to 0.995
Significance level			P = 0.3785

AUC: area under curve

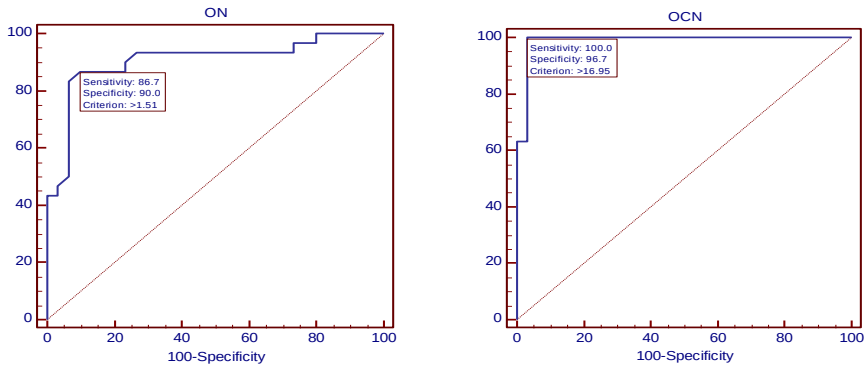

FIGURE 1 ROC curve of osteocalcin and Osteonectin in DM with periodontitis

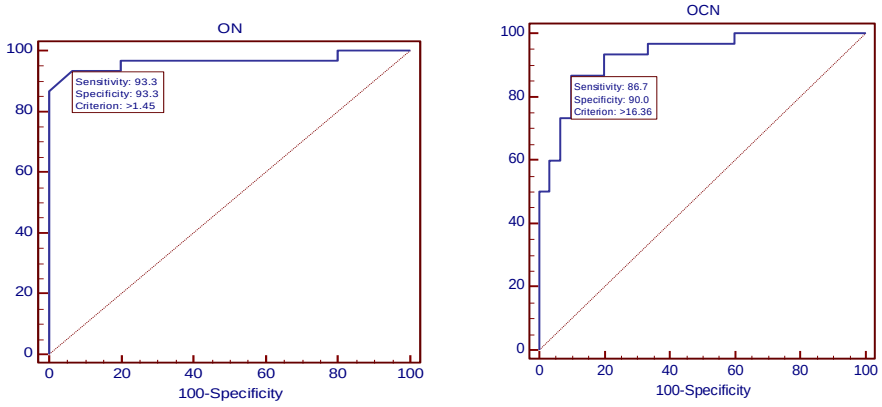

FIGURE 2 ROC curve of osetocalcin and osteonectin in periodontitis

Discussion

This research compared osteocalcin and osteonectin levels in people with diabetes and without periodontitis to healthy controls. The results demonstrated a significant difference in PLI, GI, CAL, and PPD between groups (Diabetic patients with periodontitis) and 2 (periodontitis), but no difference in BOP(0) and BOP(1) between the same groups. These findings were consistent with Nadhia *et al.,* who discovered a significant difference in clinical attachment loss and probing pocket depth between T2 DM patients with periodontitis and systemically healthy periodontitis in 2019 [19]. Furthermore, (Hira *et al.* 2021) discovered that periodontal parameters such as (PI, GI, PPD, and CAL) differed significantly between three groups (periodontitis, DM with periodontitis, and DM without periodontitis) [20]. Our findings show a significant difference in the mean of ON between study groups and controls, with a non-significant association between ON and all periodontal parameters in the periodontitis group, but a significant association with clinical attachment loss in the (DM with periodontitis) group. According to (Niknamet *et al*. 2015) [21], osteonectin improves the repair of degraded alveolar bone through collagen deposition. According to various studies, periodontal disease reduced osteonectin production and caused total collagen to decrease [22, 23]. On the other hand, there was a significant difference between the control and study groups regarding OCN, with a significant association with clinical periodontal parameters in periodontitis groups and a non-significant association in type 2 diabetes patients with periodontitis. These findings were consistent with those of (Joseph *et .al*. 2019, Gursoy *et al.* 2013, and Stanescu, Totan A. 2017) [24, 25, 26], who discovered a significant association between biomarkers and periodon while disagreeing with (Taba *et al.,* 2005) and (Cutando *et al.,* 2013) who discovered that salivary osteocalcin levels were significantly connected in diabetic group periodontitis [27,28].

Osteocalcin was primarily generated by osteoblasts and played a significant function in both bone resorption and mineralization. When resorption and formation are linked, serum osteocalcin is considered a valid marker of bone turnover; when formation and resorption are uncoupled, serum osteocalcin is considered a particular marker of bone formation. It may be involved in recruiting osteoclasts to newly produced bone sites, acting as a negative regulator. It is well-known from Clinical and Experimental notes by Merlotti *et al*., [29], Swhwetz *et al*., [30], that Serum Osteocalcin levels are lower in diabetic patients than in normal persons. Many studies have proved that the osteoblast mass and function are decreased in hyperglycemic states, which suppresses Osteocalcin synthesis and secretion [10]. Furthermore, diabetes was a risk factor for periodontal disease, with diabetic individuals having a higher prevalence, range, and severity of periodontitis and gingivitis when compared to healthy adults.

The ROC analysis data show that osteocalcin and osteonectin have an excellent ability to predict periodontitis in diabetic and periodontitis groups when compared to healthy subjects, and this provides a promising diagnostic marker for early detection of periodontal disease because diagnosis is currently based solely on periodontal probing measurement, which may lead to incorrect diagnosis and inappropriate therapeutic intervention. Diabetes mellitus, for example, may operate as a moderator of disease activity, influencing the onset and course of periodontitis and causing a subsequent shift in biomarkers. And breakthroughs in diagnostic research lead to strategies for identifying and quantifying periodontal risk using objective indicators such as bone biomarkers.

Conclusion

Osteocalcin and osteonectin levels are significantly increased in patients groups compared to the control group. Accordingly, it may play a significant role in the diabetic progression and its association with periodontitis. Thus, it may be employed as a good marker for predicting this disease.

Acknowledgements

The author is very thankful to the College of Science for Women| University of Baghdad, and the Albaladyat specialist center for dentistry for their support to carry out this research.

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How to cite this article: Zainab A. Salman, Kadhim K. Ghudhaib, Raghad Fadhil. Evaluating Osteocalcin and Osteonectin in serum male patients with type 2 Diabetes mellitus and periodontitis. *Eurasian Chemical Communications*, 2022, 4(4), 295-302. **Link:** http://www.echemcom.com/article_144787.html