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ENDOCAN: A NOVEL BIOMARKER IN GINGIVAL CREVICULAR FLUID IN PERIODONTITIS PATIENTS WITH OR WITHOUT TYPE 2 DIABETES MELLITUS [A CROSS-SECTIONAL STUDY]

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ABSTRACT

BACKGROUND: Clinical parameters are used to diagnose periodontal disease. However, new technical advances in diagnostics for assessing periodontal status are utilizing objective biomarkers. Recently, a number of biomarkers have been identified to predict periodontitis status. Endocan is a novel pro-inflammatory biomarker that is thought to give an insight on periodontal disease status.
Objective: Evaluation of Endocan level in gingival crevicular fluid (GCF) of stage 1 and 2 periodontitis patients with or without type 2 diabetes mellitus.
MATERIALS AND METHODS: 42 patients with stage 1 and 2 periodontitis were divided into two groups: 23 patients with controlled type 2 diabetes mellitus (CT2DM-P) and 19 systemically healthy periodontitis patients (H-P). Endocan level in GCF, periodontal parameters, and Hemoglobin A1c (HbA1c) value were measured. Biochemical analysis of Endocan level was performed by enzyme-linked immunosorbent assay (ELISA).
RESULTS: GCF Endocan level was significantly higher in the CT2DM-P group than in the H-P group. Mean pocket probing depth (PPD), clinical attachment loss (CAL), and bleeding on probing percentage (BOP%) were higher in the CT2DM-P group. Endocan was positively correlated to CAL, BOP%, and HbA1c in both groups, with Endocan and BOP% having a moderate correlation in the CT2DM-P group and Endocan and HbA1c having a strong correlation in the H-P group.
CONCLUSION: Endocan levels, PPD, CAL, and BOP% in CT2DM-P group were higher than H-P group. Positive correlations between BOP%, Glycated hemoglobin and Endocan were also observed.
RUNNING TITLE: Evaluation of Endocan biomarker in periodontitis patients with or without type 2 diabetes mellitus.

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INTRODUCTION

Periodontal disease is a complex immune-inflammatory condition characterized by periodontal tissue destruction, that may develop when the host comes into contact with a periodontal pathogenic microbe.(1) It is linked to altered vascular endothelial function, which alters the protective balance and permeability of the endothelium. It may increase the likelihood of vascular malfunction and systemic inflammation in conditions like cardiovascular disease and diabetes, which would then activate an inflammatory cascade mediated by dominant cytokines.(2)
Increased capillary permeability and vascular dilatation are also symptoms of inflammation. Endothelial cells are in charge of attracting leukocytes in an instant reaction to a microbial biofilm. (3)
Inflammatory mediator concentrations in gingival crevicular fluid (GCF), which flow increases with increased microvasculature permeability, might indicate the degree of inflammation.(4) As a result, GCF is utilized to assess the pathogenicity of periodontal disease as a diagnostic and predictive approach.(4)
Clinical periodontitis indicators including probing pocket depth, clinical attachment level, and bleeding on probing have shortcomings in how well they may give clinicians a real-time assessment of the disease state. Additionally, the future course of

periodontal disease is poorly predicted by these clinical parameters.(5)

A study found that combining saliva-based biomarkers with periodontal biofilm bacteria has potential diagnostic usefulness for determining periodontal disease status.(6) Furthermore another longitudinal study by **Kinney et al 2011**(7) revealed that saliva-derived biomarkers combined with periodontal bacteria can predict periodontal disease progression.

Recognizing how inflammatory mediators and endothelial dysfunction function could shed light on the pathogenic processes between type 2 diabetes (T2DM) and periodontitis.(8)

Endothelial cell-specific molecule-1 (ESM-1), newly referred to as *Endocan*, was first identified in human endothelial cells by **Lassalle et al. 1996**(9). It is a soluble proteoglycan, its function is thought to be controlled by tumor necrosis factor (TNF-α) through modulation of ESM-1 mRNA gene expression. They claimed a possible relationship between *Endocan* and local inflammation.

Endocan is linked to systemic disorders like cardiovascular disease; it is strongly expressed by the vascular endothelium during the inflammatory and endothelial activation processes.(10) *Endocan* activity and production are also influenced by vascular endothelial growth factor-A (VEGF-A) and other cytokines, including TNF-α and Interleukin-1 (IL-1).(11)

Tu¨rer et al. 2017(12) reported that *Endocan* levels, VEGF-A and TNF-α may be elevated in periodontitis and lowered following non-surgical periodontal treatment (NSPT). Thus, the presence of *Endocan* in periodontal tissues raises the possibility that it could serve as a diagnostic and predictive biomarker for periodontal disease as well as an inflammatory biomarker.(12)

In **Tayman et al 2020**(13) study, they investigated *Endocan* and other biomarkers levels in GCF of systemically healthy stage III periodontitis patients. They found that *Endocan* GCF level was significantly increased in the periodontitis group with statistically significant correlation between *Endocan* level and PPD, CAL and BOP.

Kumar et al. 2020(14) investigated *Endocan* and TNF-α levels in GCF of T2DM patients with chronic periodontitis. For the influence of endothelial activation in chronic inflammatory conditions including diabetes and chronic periodontitis, *Endocan* has been demonstrated to be a possible prognostic marker as well as an early diagnostic indicator.(14)

Endocan is expressed in periodontitis patients who are otherwise healthy. However, given the enhanced inflammatory state and vascular endothelial dysfunction as two pathogenic processes relating DM and periodontitis, evaluating *Endocan* role in T2DM periodontitis patients seems to be significant.(14)

The utilization of the GCF *Endocan* biomarker as a diagnostic indicator of periodontitis and its association to T2DM has received relatively limited research. In this investigation, the level of the *Endocan* biomarker in GCF was evaluated in patients with stage 1 and 2 periodontitis, with or without controlled T2DM.

The null hypothesis stated that patients with stage 1 or stage 2 periodontitis with or without CT2DM did not have significantly different *Endocan* levels in GCF.

MATERIALS AND METHODS

Ethical approval: Research Ethics Committee of the Faculty of Dentistry at Alexandria University where the study was carried out; granted the appropriate ethical clearance. Approval Number: 0256-06/2021(IRB 00010556). Each patient was informed about the study and provided a signed consent. This study was registered at ClinicalTrials.gov (NCT05667051).

Study design

A cross sectional study following STROBE guidelines.(15)

Sample size and setting

Forty-two periodontitis patients with stage 1 or 2 periodontitis were enrolled; 23 controlled Type 2 diabetic (CT2DM-P) and 19 systemically healthy (H-P). They were chosen before receiving periodontal treatment from patients presented to the outpatient clinics of Department of Periodontology, Faculty of Dentistry, Alexandria University. The study started on April 2022.

The distribution of the study groups (CT2DM-P group = 23; H-P group = 19) is shown in the flow chart (Figure 1).

Sample size calculation

Alpha error was assumed to be 5% and research power to be 80% when estimating sample size. **Kumar et al 2020**(14) reported mean ± SD *Endocan* level= 44.63 ± 7.9 in healthy non-diabetic patients and 56.47 ± 13.0 in well- controlled diabetic patients. The sample size was calculated to be 14 per group based on mean comparisons. Total sample size = number of groups x number of individuals per group = 2 x 14 = 28.(16)

Participants:

Enrolled patients were selected after fulfilling the criteria of:

Inclusion criteria:

(1) Age 40 to 65 years.
(2) Probing pocket depths (PPD) ≤ 5 mm.(17)
(3) Clinical attachment level (CAL) ≤ 4 mm.(17)
(4) Type 2 DM for the past 1 or more years.(14)
(5) HbA1c ≤ 7%.(18)

Exclusion criteria:

(1) Uncontrolled Diabetes Mellitus (HbA1c > 7%).(18)
(2) Systemic antibiotic or anti-inflammatory medication use in the previous 2 months.

(3) Non-surgical periodontal therapy in the previous 6 months.
(4) Surgical periodontal therapy in the previous 12 months.
(5) Pregnancy.
(6) Smokers.

Both groups included patients with history of periodontitis; PPD ≤ 5 mm and CAL ≤ 4 mm; Stage I and II periodontitis were diagnosed according to the 2018 American Academy of Periodontology classification.(17) Type 2 DM was diagnosed according to The 2022 American Diabetes Association (ADA) diabetes mellitus classification.(18)

Intra-examiner reliability: The same trained and calibrated examiner took all of the clinical measurements; intra examiner reliability was measured for PPD, CAL, and POB with an intraclass correlation coefficient >0.82 suggesting very good reliability.(19)

Blinding: Biochemical analysis of *Endocan* levels was performed by a blinded operator.

Clinical assessments:

Evaluation of periodontal parameters

Periodontal parameters were assessed. Clinical parameters recorded were PPD(17), CAL(17), and BOP %(20) using Williams (Nordent Inc., Illinois, USA) calibrated periodontal probe.(20) PPD and CAL recordings were made to the nearest mm; observations close to 0.5 mm were rounded to the upper whole mm.

BOP%(20) was assessed on 4 points per tooth: Distal, Mesial, Facial, and Lingual/Palatal points. Percentage was calculated as number of bleeding points divided by total numbers of teeth points then multiplied by 100.

Glycemic control evaluation:

Glycated hemoglobin (HbA1c)(18) was determined for all participants at the beginning of the study.

Gingival crevicular fluid (GCF) collection and sampling:

Patients were asked to rinse with water before having GCF samples taken from the deepest pockets. After isolation using cotton and suction and sulcus drying gently by air; absorbent paper points were inserted till resistance was felt and then kept for 30 sec at least. Then they were inspected and blood contaminated points were discarded. Samples were collected in polypropylene tubes "DNA, RNA free Eppendorf's (Eppendorf Co., Hamburg, Germany)" containing phosphate buffer solution (PBS), samples were centrifuged then stored at ultra-freeze until biochemical analysis.(21)

Biochemical analysis:

Endocan level was assessed in GCF samples by commercially available BT LAB Biotech (Biotech Co.Ltd., Endocan ELISA Kit, Zhejiang, China) ELISA kit. The assay was carried out in accordance with the manufacturer's instructions.

Statistical analysis

All quantitative variables were verified for normality using descriptive statistics, plots (histograms, Q-Q plots, and boxplots), and normality tests. Because all variables had a normal distribution, means and standard deviations (SD) were computed. The independent samples t-test with mean difference calculation and 95% confidence intervals (CI) was used to compare quantitative variables between the two research groups. The chi-square and Fisher's exact tests were used to compare qualitative variables between the two research groups. Pearson correlation was used to examine the relationship between *Endocan* levels and different parameters. The significance level was set at p value <0.05. Data was analyzed using IBM (IBM Corp., Armonk, New York, USA) SPSS for Windows (Version 23.0).

RESULTS

The demographic variables between the two studied groups. 31.6% of the H-P group participants were males, while 68.4% were females. 21.7% of the CT2DM-P group participants were males, while 78.3% were females. The mean age of the H-P group was 46.53, and the CT2DM-P group was 50,65. (Table 1)

Clinical results:

Comparing the mean PPD and mean CAL in the two groups. The CT2DM-P group had significantly higher mean PPD than the H-P group. The mean (SD) PPD was= 4.83 (0.49) in the CT2DM-P group and 4.32 (0.58) in the H-P group [mean (SD) difference= -0.51 (1.08), p= 0.004]. (Table 2, Figure 2)

Furthermore, the CT2DM-P group had significantly higher mean CAL than the H-P group. The mean (SD) CAL was= 3.39 (0.72) in the CT2DM-P group and 2.74 (0.73) in the H-P group [mean (SD) difference= -0.65 (1.46), p= 0.006]. (Table 2, Figure 2)

As for BOP%; The CT2DM-P group had significantly higher mean BOP% than the H-P group. The mean (SD) BOP% was= 54.70 (15.82) in the CT2DM-P group and 35.47 (14.60) in the H-P group [mean (SD) difference= -19.22 (30.70), p= 0.001]. (Table 2, Figure 3)

HbA1c mean value was significantly higher in CT2DM-P group than the H-P group. The mean (SD) HbA1c was= 6.91 (0.10) in the CT2DM-P group and 5.36 (0.50) in the H-P group [mean (SD) difference= -1.55 (0.76), p= 0.001]. (Table 2, Figure 4)

Biochemical results:

The CT2DM-P group had significantly higher mean *Endocan* level than the H-P group. The mean (SD) *Endocan* level was= 1.88 (0.12) in the CT2DM-P group and 1.63 (0.11) in the H-P group [mean (SD) difference= -0.25 (2.34), p= 0.001]. (Table 2, Figure 5)

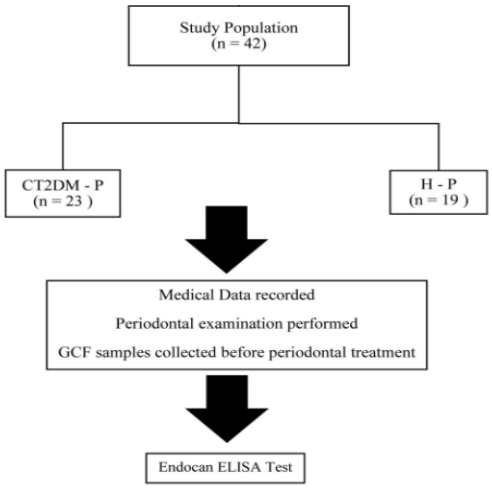

Figure (1): Flowchart of the study

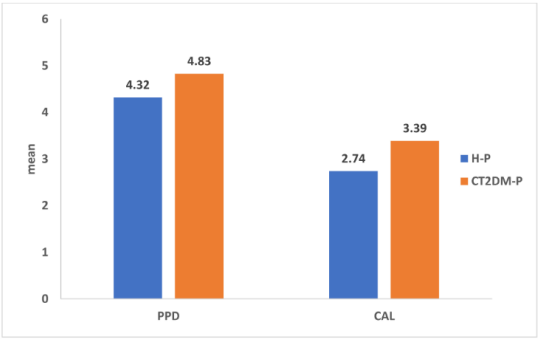

Figure (2): PPD and CAL in the two study groups

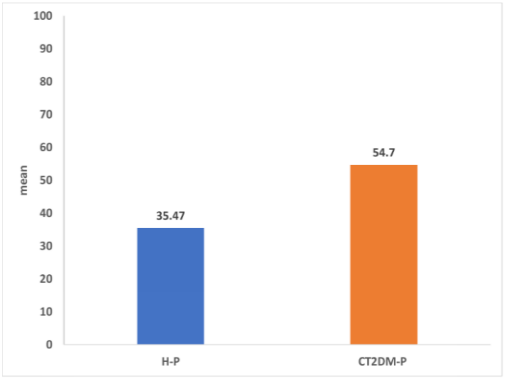

Figure (3): BOP% in the two study groups

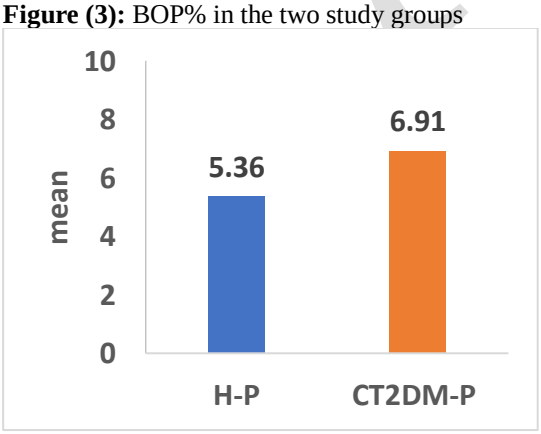

Figure (4): HbA1c in the two study groups

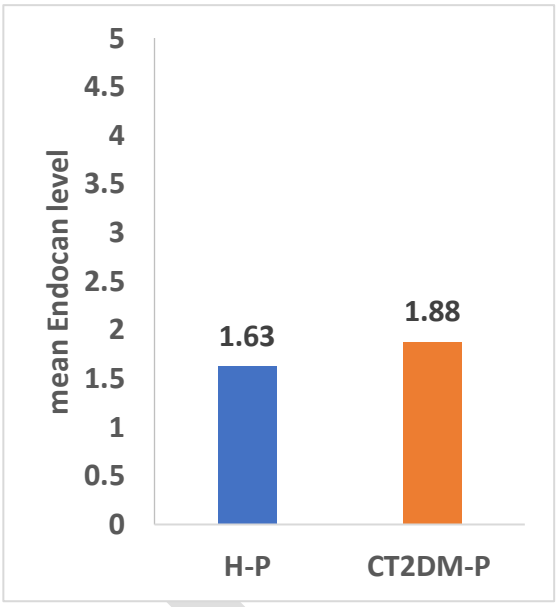

Figure (5): Endocan in the two study groups

Correlation between Endocan and different parameters

PPD was not correlated to *Endocan* in both groups. CAL was weakly and positively correlated to *Endocan* in both groups; however the correlation was not statistically significant (p >0.05). BOP% was positively correlated to *Endocan* in both groups with higher correlation in the CT2DM-P group. The BOP% and *Endocan* were significantly moderately correlated in the CT2DM-P group(r= 0.50, p= 0.02). HbA1c was positively correlated to *Endocan* in both groups; with a strong positive statistically significant correlation in the H-P group.(Table 3)

Table (1) :Baseline characteristics of the two study groups

		H-P (n=19)	CT2DM-P (n=23)	P value
Age	**Mean (SD)**	46.53 (7.60)	50.65 (6.69)	T= 1.87 P= 0.07
Gender	**Male**	6 (31.6%)	5 (21.7%)	X^2: 0.52 P_{FE}: 0.50
	Female	13 (68.4%)	18 (78.3%)	

T: Independent samples t-test, X^2: Chi-square test, P_{FE}: Fisher exact p value.

Table (2) : Comparison of different parameters between the two study groups

	H-P (n=19)	CT2DM-P (n=23)	Difference	95% CI	T-test P value
	Mean (SD)				
PPD	4.32 (0.58)	4.83 (0.49)	-0.51 (1.08)	-0.85, -0.18	**T= 3.08 P= 0.004***
CAL	2.74 (0.73)	3.39 (0.72)	-0.65 (1.46)	-1.11, -0.20	**T= 2.90 P= 0.006***
BOP%	35.47 (14.60)	54.70 (15.82)	-19.22 (30.70)	-28.80, -9.65	**T= 4.06 P <0.001***
HbA1c	5.36 (0.50)	6.91 (0.10)	-1.55 (0.76)	-1.79, -1.30	**T= 13.23 P <0.001***
Endocan	1.63 (0.11)	1.88 (0.12)	-0.25 (2.34)	-0.33, -0.18	**T= 7.07 P <0.001***

SD: Standard Deviation, CI: Confidence Interval
*statistically significant at p value <0.05

Table (3) :Correlation between Endocan and different parameters in the two study groups

	H-P	CT2DM-P
PPD	r= -0.07 p= 0.76	r= -0.02 p= 0.94
CAL	r= 0.28 p= 0.25	r= 0.29 p= 0.18
BOP%	r= 0.43 p= 0.06	r= 0.50 **p= 0.02***
HbA1c	r= 0.90 **p <0.001***	r= 0.36 p= 0.10

r= Pearson correlation coefficient
*statistically significant at p value <0.05

DISCUSSION

Endocan is a novel marker of endothelial activation in inflammatory processes.(14) A common finding between periodontal disease and DM is the vascular endothelial alterations influenced by the inflammatory mediators.(14) The goal of the current study is to assess GCF level of *Endocan* in periodontitis patients with or without CT2DM.

The current study included Forty-two individuals with stage I and II periodontitis. The participants were divided into two groups based on their glycemic status: nineteen systemically healthy periodontitis (H-P) and twenty-three controlled Type 2 diabetic (CT2DM-P) (HbA1c ≤ 7).

Biochemical analysis was underwent for both groups by determining the level of *Endocan* in GCF using an ELISA test as a pro-inflammatory marker. Clinical periodontal parameters of PPD, CAL, and BOP% were recorded.

The study subjects ranged in age from 40 to 65 years old. They were medically free other than the CT2DM-P, to prevent the probable influence of systemic diseases on periodontal status and interference in the evaluated biochemical and clinical parameters. Participants who had received antibiotics for two months before to the trial, as well as those with risk factors, such as pregnancy, and smoking were excluded. **Victor D. J. et al 2014**(22) showed that smoking influences ELISA test results of various pro-inflammatory biomarkers in GCF.

Endocan is primarily an inflammatory mediator and an endothelial activation marker.(23) T2DM is related with vascular endothelial alterations, which result in decreased endothelial function. Likewise, in periodontitis; an abnormal rise in pro-inflammatory cytokines may impair endothelial function.(24)

GCF inflammatory-related substances may provide useful information on periodontal disease state.(25) Inflammatory mediator concentrations in GCF have been utilized to predict each patient's vulnerability to periodontitis.(26) Pro-inflammatory VEGF-A biomarker concentrations in the GCF rose with the severity of the disease and decreased after periodontal disease treatment, according to **Pradeep et al. 2011**(27).

Moreover, the alterations of these biomarkers in relation to HbA1c may support the idea that the diabetic state influences periodontitis and vice versa.(28, 29) HbA1c values were determined in this perception. According to **Arman et al. 2016**(30) increasing glycemic state was associated with increased *Endocan* levels, while improving glycemic status resulted in a decrease in *Endocan* levels.

Endocan level was significantly higher in CT2DM group. In intergroup comparisons, diabetics with adequate glycemic control (HbA1c <7%) may had a considerably larger systemic inflammatory burden. **Singhal et al. 2016**(31) and **Vieira Ribeiro et al. 2011**(32); suggested that this discovery could be explained by the fact that in diabetics, the effect of hyperglycemia further promotes endothelial activation and an increased inflammatory response. According to **Kumar et al 2020**(14), the CT2DM-P group had significantly greater *Endocan* levels than the H-P group, which is consistent with the findings of the current study (p=0.001).

In accordance with the findings of **Kumar et al 2020**(14), the CT2DM-P group had significantly higher mean PPD and CAL in the current study (p=0.006, p=0.006).

Furthermore, The CT2DM-P group demonstrated a significantly higher BOP% than the H-P group, (p= 0.001).

In the present study; we found that PPD was not correlated to *Endocan* in either group; which was in line with **Tu¨rer et al. 2017**(12) study in which they investigated correlations between GCF *Endocan*, VEGF-A, and TNF-α levels and PPD and found that neither *Endocan* nor VEGF-A levels were significantly related to PPD. Moreover, similar correlations results were found in **Kumar et al 2020**(14) clinical trial study, as they found that baseline clinical parameter values did not correlate to baseline *Endocan* and TNF-α biomarker levels. However **Tayman et al 2020(13)** found significant correlation between *Endocan* and PPD.

Our study results revealed that CAL was positively correlated with *Endocan* in both groups, however the correlation was not statistically significant (p >0.05). This observation was similar to the results of **Tu¨rer et al. 2017**(12) study; However, the correlation in this study was statistically significant. **Tayman et al 2020**(13) also found statistically significant correlation between CAL and *Endocan*. On the opposite side, *Endocan* level and CAL were not significantly correlated In **Kumar et al 2020**(14) study.

In the current study BOP% was shown to be positively correlated to *Endocan* in both groups, with a stronger significant moderate correlation in the CT2DM-P group (r= 0.50, p= 0.02). which concurred with the findings of **Tayman et al**

2020(13) study results. This finding may be explained by the influence of *Endocan* on angiogenesis as proposed by **Tu¨rer et al. in 2017**(12) and that *Endocan* leads to pro-inflammatory hyper-permeability responses as found by **Lee et al 2014**.(11) On the contrary, **Kumar et al 2020**(14) observed insignificant correlation between *Endocan* level and BOP%.

HbA1c was positively correlated to *Endocan* in both groups; with a strong positive statistically significant correlation in the H-P group which may be implying that any increase in HbA1c correlated significantly with an increase in *Endocan* level. It might be related to the systematic review by **Teshome et al 2017**(33), which discovered a modest decline in HbA1c of about 0.48 percent following three months of periodontitis therapy.

The length, type of DM management, and severity of periodontal disease may have an impact on these results, according to the literature that is currently available. These factors should be further investigated, along with a larger sample size, to establish a conclusive connection between *Endocan*, T2DM, and periodontal disease.

Limitations of our study may include that it lacked the differentiation between active and passive periodontal pockets. Moreover, the absence of evaluations of other inflammatory cytokines or pro-angiogenic biomarkers.

CONCLUSION

According to the results of the present research, *Endocan* levels, PPD, CAL, and BOP% were significantly greater in patients with periodontitis and CT2DM than in those with systemically healthy periodontitis. Significant positive correlations between BOP%, HbA1c and *Endocan* between the two study groups were also observed.

These data may imply that higher glycemic status in CT2DM-P may aggravate inflammatory processes and increase *Endocan* biomarker levels. *Endocan* is a possible predictive biomarker that shows how endothelial activation plays a part in chronic inflammatory diseases including diabetes and periodontitis. It could also be used as a diagnostic biomarker.

Conflict of Interest: The authors declare that they have no conflict of interest.

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