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Research Article

Estimation of Levels of Caveolin-1 And Azurocidin In Gingival Crevicular Fluid of Diabetic and Non-Diabetic Patients with Chronic Periodontitis: A Cross Sectional Study

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Abstract

Background: *Periodontitis occurs as a result of complex interactions between bacterial plaques and the host immune system. Diabetes mellitus is a risk factor for periodontitis and leads to inflammatory changes within periodontal tissue. Caveolin-1 is an integral membrane protein that has diverse functions, such as regulating inflammatory mediators and insulin signaling. Azurocidin is a microbicide protein present in the azurophilic granules of neutrophils. It plays a role in innate immunity and is secreted during inflammatory conditions, and its functions are influenced by the glycaemic state. Hence, this study aimed to estimate the levels of caveolin-1 and azurocidin in the GCF of individuals with newly diagnosed type 2 diabetes and nondiabetic individuals with chronic periodontitis.*

Method: *A total of 45 subjects were divided into three groups of 15 each. Group I included periodontally healthy subjects, Group II included chronic periodontitis patients, and Group III included chronic periodontitis patients who were newly diagnosed with type 2 diabetes mellitus. Periodontal parameters such as the PI, BI, GI, PD and CAL were recorded, and GCF samples were collected. Statistical analysis was performed using one-way ANOVA, Pearson's correlation and multiple regression.*

Results: *The GCF levels of caveolin-1 and azurocidin were significantly greater in Group II and Group III than in Group I ($p \leq 0.001$). Caveolin-1 and azurocidin were significantly positively correlated with various periodontal parameters and GCF values ($p \leq 0.001$).*

Conclusion: *The GCF levels of caveolin-1 and azurocidin were greater in chronic periodontitis patients with and without type 2 diabetes mellitus than in periodontally healthy subjects. Hence, both of these molecules may be considered as biomarkers for periodontitis and diabetes mellitus.*

Keywords: Periodontitis, Diabetes mellitus, Caveolin-1, Azurocidin

1. Introduction

Periodontitis is a chronic, polymicrobial and concealed infectious disease of multifactorial origin involving supporting structures of the teeth that triggers an array of events involving immunity as well as the inflammatory response in the host [1]. All of these events are influenced by disease modifiers or risk factors that can be both genetic and environmental, such as diabetes mellitus, smoking, genetic factors, stress and obesity [2]. Epidemiological data confirm that diabetes is a major risk factor for periodontitis and that susceptibility to periodontitis is increased by approximately threefold in people with a longstanding hyperglycaemic state [3].

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated levels of glucose in the blood and is classified into two types: type I DM and type 2 DM [4]. All the forms of DM are associated with hyperglycemia, hyperlipidemia, and associated complications [5]. Frequently, the first recognizable abnormality detected in individuals who are likely to develop type II diabetes (T2D) is insulin resistance (IR). Dysfunction of insulin-responsive

molecules can lead to impaired insulin responsiveness in peripheral tissues and, thus, the development of type II diabetes. Recently, caveolin-1 and caveolae microdomains have emerged as potentially important regulatory elements in the control of insulin [6].

Caveolae are invaginations in the plasma membrane that have three isoforms (caveolin 1, 2, 3). Caveolin-1 (Cav-1) is the principal constituent and anchoring protein of caveoles in the plasma membrane [7]. The functions of caveolin-1 include caveolae formation, insulin signaling, cholesterol transport, G protein subunit regulation, oncogenic transformation, and endothelial nitric oxide synthase (eNOS) regulation [8]. In addition, it is also specifically associated with glycosylated extracellular matrix metalloproteinase (EMMPRIN) and influences its ability to induce matrix metalloproteinase (MMP) production, which in turn leads to collagen destruction, as in diseases such as periodontitis [9].

Furthermore, the reported mechanisms in the literature show that inflammation is a central feature of the pathogenesis of periodontitis and diabetes. The elevated inflammatory state in diabetes patients contributes to a compromised immune response associated with patient vulnerability to bacterial infections. Neutrophils play an important role in the host immune response to bacterial infection [10]. During inflammation, activated neutrophils release proteases, antibiotic proteins, and other granular contents to eliminate phagocytosed microorganisms. There is increasing evidence that one such protein is a cationic antimicrobial protein with a molecular mass of 37 kDa (CAP37), which is also known as azurocidin (AZD) or heparin-binding protein (HBP) [11].

Azurocidin (AZD) is an antimicrobial protein included in the serprocidin subgroup of chymotrypsin-like proteases. It is stowed in azurophilic granules and secretory vesicles of neutrophils. The biological functions of azurocidin include antimicrobial activity, induction of monocyte recruitment to the site of inflammation, augmentation of macrophage phagocytosis, and increases in endothelial permeability and macromolecular efflux through disruption of the cellular barrier [12]. Chronic diseases (such as type 2 diabetes), age-associated insulin resistance, nutritional status, and lifestyle factors have significant effects on neutrophil function. The main factor in the inability of neutrophils to downregulate and exocytose primary granules, such as azurocidin, is a hyperglycemic state, which leads to impaired neutrophils [13]. Metabolic routes by which hyperglycemia is linked to neutrophil dysfunction include the advanced protein glycosylation reaction [14]. Increased glucose concentrations increase protein glycation, leading to early glycation products and advanced glycation end products (AGEs)which are the main factors responsible for the complications observed in diabetic patients [15].

According to the literature, periodontitis and diabetes mellitus share a cyclic relationship at the heart of their pathogenesis. Additionally, they interact with caveolin-1 and azurocidin to parse their pathophysiology and complications. To the best of our knowledge, no studies have investigated the levels of caveolin 1 and azurocidin in chronic periodontitis patients with and without diabetes. Therefore, the aim of this study was to estimate the levels of caveolin-1 and azurocidin in gingival crevicular fluid (GCF) from diabetic and nondiabetic patients with chronic periodontitis.

2. Methodology

2.1. Study Population:

The study population consisted of 45 subjects selected from the outpatient department of M.R. Ambedkar Dental College and Hospital, Bangalore, Karnataka, India. Approval from the ethical committee of M.R. Ambedkar Dental College and Hospital was obtained (Ethical Reference Number-EC657). The nature and purpose of the study were explained to the subjects, and written consent was obtained. The subjects were allocated to three groups depending on their periodontal status, and GCF samples were obtained from the deepest probing site of each of the subjects by placing calibrated volumetric microcapillary pipettes with a 0-5 µL range.

The duration of the study was one year from August 2020 to August 2021.

2.1.1. Selection Criteria:

Out of the 60 patients screened, 52 patients agreed to participate, and 45 patients fulfilled the inclusion criteria and were divided into three groups.

- Group I: 15 periodontally healthy subjects
- Group II: 15 chronic periodontitis patients without diabetes mellitus.
- Group III: 15 chronic periodontitis patients who were newly diagnosed with type 2 diabetes mellitus.

2.1.2. Inclusion Criteria:

1. Subjects were aged 30-45 years.
2. Subjects with ≥20 teeth excluding the third molars.

- Group I: Subjects with clinically healthy gingiva, no history of diabetes, bleeding on probing (BOP) ≤ 5% of sites examined, no evidence of attachment loss and probing depth (PD) ≤ 3 mm
- Group II: No history of diabetes, presence of bleeding on probing, probing depth ≥5 mm or clinical attachment loss (CAL) ≥3 mm
- Group III: Subjects were newly diagnosed type 2 diabetic individuals with an HbA1c ≥ 6.5, presence of bleeding on probing, probing depth ≥5 mm and clinical attachment loss≥3 mm.

The exclusion criteria were as follows: no history of tobacco consumption, periodontal treatment in the past 6 months, chemotherapeutic regimen during the past 6 months, or medication for diabetes mellitus or any other systemic disease.

2.2. Statistical Analysis

For all the patients, periodontal clinical parameters such as the PI, GI, BI, PD and CAL were recorded. The significance of the difference in the mean values was tested using one-way ANOVA. Pearson's correlation coefficient and multiple

regression analysis were used to determine the associations between clinical periodontal parameters and GCF parameters in all three groups for azurocidin and caveolin-1.

3. Results

The means of all the parameters were compared among all three groups. The mean ages were 36.67±5.49 years in Group I, 42.06±2.74 years in Group II and 41.93± 2.68 years in Group III. The difference in the mean value was statistically significant ($p<0.05$). There were 46.66% (n=7) males and 53.33% (n=8) females in Group I; 53.33% (n=8) males and 46.66% (n=7) females in Group II; and 60% (n=9) male subjects and 40% (n=6) females in Group III.

The mean values of the PI, GI, BI, PD and CAL were highly significantly different among all three groups ($p<0.001$) as shown in Table 1. The mean concentration of GCF caveolin-1 in group I was 5.65±2.76, that in group II was 20.39±6.51, and that in group III was 20.72±7.45. The mean concentration of azurocidin in group I was 0.98±0.53, that in group II was 2.94±1.49, and that in group III was 2.98±2.25. The concentration was significantly greater in group III than in the other groups. ($p<0.001$) (Table 1).

PARAMETER	GROUP I	GROUP II	GROUP III	p value
AGE	36.67+5.49	42.06+2.74	41.93+2.68	0.001*
PI	1.49+0.12	2.10+0.34	2.25+0.44	0.00*
GI	1.18+0.27	1.66+0.23	1.88+0.39	0.00*
BI	13.09+3.92	88.08+5.94	88.47+5.31	0.0001*
PD	2.01+0.27	5.40+0.61	5.43+0.86	0.00*
CAL	0.026+0.032	3.09+0.86	4.10+0.76	0.0001*
GCF CAVEOLIN-1 levels (ng/ml)	5.65+ 2.76	20.39+6.51	20.72+7.45	0.00*
GCF AZUROCIDIN levels (ng/ml)	0.98+0.53	2.94+1.49	2.98+2.25	0.001*

Table 1: Comparison of parameters among the three groups

Pearson's correlation coefficient was used to determine the associations between clinical periodontal parameters and GCF parameters in all three groups. There was a positive correlation between caveolin-1 and PI (r=0.981 in group I, r=0.858 in group II, r=0.673 in group III), GI (r= 0.908 in group I, r=0.974 in group II, r=0.821 in group III), BI (r=0.958 in group I, r=0.948 in group II, r=0.643 in group III), PD (r=0.817 in group I, r=0.928 in group II, r=0.868 in group III) and CAL (r= 0.828 in group I, r=0.934 in group II, r=0.725 in group III). This positive correlation suggested that greater the values of GI, PI, BI, PD and CAL were, the greater the concentration of caveolin-1. (Table 2).

PARAMETERS	CORELATION	GROUP I	GROUP II	GROUP III
PI	r value	0.981	0.858	0.673
	p value	0*	0.000*	0.002*
GI	r value	0.908	0.974	0.821
	p value	0.000*	0.000*	0.000*
BI	r value	0.958	0.948	0.643
	p value	0.000*	0.000*	0.004*
PD	r value	0.817	0.928	0.868
	p value	0.000*	0.000*	0.000*
CAL	r value	0.828	0.934	0.725
	p value	0.000*	0.000*	0.001*

Table 2: Correlations of the GCF levels of caveolin-1 with clinical parameters in the three groups

Similarly, Pearson's correlation between the GCF concentration of azurocidin in all three groups and all clinical parameters was positive which suggested that the greater the clinical parameters were, the greater the level of azurocidin (Table 3).

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PARAMETERS	CORELATION	GROUP I	GROUP II	GROUP III
PI	r value	0.847586	0.785222	0.808388
	p value	0.00003*	0.00026*	0.00013*
GI	r value	0.802107	0.933468	0.937121
	p value	0.00016*	0.0000001*	0.00000013*
BI	r value	0.920689	0.860205	0.821363
	p value	0.0000005*	0.001946*	0.000086*
PD	r value	0.850351	0.907146	0.983257
	p value	0.000029*	0.0000015*	0.00*
CAL	r value	0.927749	0.98965	0.882588
	p value	0.0000032*	0.0000012*	0.000003*

Table 3: Correlations of the GCF levels of azurocidin with clinical parameters in the three groups

Multiple linear regression was performed among the groups, and CAL was taken as the dependent variable. There was a positive effect among all the parameters in all groups; however, no statistically significant effect was detected in Group I (f test = 11.29 and p value =0.002) (Table 4). In Group II, only Caveolin-1 (p< 0.03) and Azurocidin (p< 0.01) had statistically significant effects, indicating that an increase in any of these values would lead to an increase in CAL (Table 5). In Group III, the PI had a statistically significant effect (p<0.04), indicating that the greater the PI was, the greater the CAL (Table 6).

Variable	β coefficient	S.D.	T-STAT	p value
PI	-0.3821	0.3695	-1.03E+00	0.1678
GI	0.07768	0.04429	1.75E+00	0.06146
BI	0.000932	0.008235	1.13E-01	0.4565
PD	-0.008094	0.01872	-4.32E-01	0.3392
CAVEOLIN-1	0.01151	0.01024	1.12E+00	0.149
AZUROCIDIN	0.04433	0.02953	1.50E+00	0.08849

Multiple R	0.9584
R square	0.9186
Adjusted R Squared	0.8372
F-TEST (value)	11.29
p value	0.002*

Table 4: Multiple linear regression analysis of the clinical parameters and the GCF levels of caveolin-1 and azurocidin with the CAL as the dependent variable in the chronic gingivitis group

Variable	β coefficient	S.D.	T-STAT	p value
PI	-0.2061	0.3202	-6.44E-01	0.2701
GI	0.5961	0.6583	9.06E-01	0.1976
BI	0.03831	0.03105	1.23E+00	0.1285
PD	0.2341	0.2902	8.07E-01	0.2232
CAVEOLIN-1	-0.04631	0.02128	-2.18E+00	0.03302*
AZUROCIDIN	0.4508	0.08372	5.38E+00	0.000513*

Multiple R	0.9972
R square	0.9944
Adjusted R Squared	0.9887
F-TEST (value)	176.3
p value	<0.001*

Table 5: Multiple linear regression analysis of the clinical parameters and the GCF levels of caveolin-1 and azurocidin with CAL as the dependent variable in the CP group

Variable	β coefficient	S.D.	T-STAT	p value
PI	1.051	0.5513	1.91E+00	0.04916*
GI	0.3983	0.8148	4.89E-01	0.32
BI	-0.0852	0.06708	-1.27E+00	0.1223
PD	0.4996	0.4486	1.11E+00	0.1511
CAVEOLIN-1	0.09386	0.07664	1.23E+00	0.1301
AZUROCIDIN	-0.2622	0.2168	-1.21E+00	0.1329

Multiple R	0.9884
R square	0.9769
Adjusted R Squared	0.9539
F-TEST (value)	42.35
p value	<0.001**

Table 6: Multiple linear regression analysis of the clinical parameters and the GCF levels of caveolin-1 and azurocidin with CAL as the dependent variable in chronic periodontitis patients in the newly diagnosed diabetic group

4. Discussion

Periodontitis is a common, complex and chronic inflammatory disease in which disease progression involves intricate interactions between biofilms and the host immune system, leading to destruction of the supporting structures of the teeth. A significant interrelationship between periodontal disease and systemic health has been established and may predispose, accelerate or increase the progression of periodontitis.

Diabetes mellitus is a group of complex multisystem metabolic disorders that are a proven risk factor for periodontitis and has been supported by ample evidence. Conversely, there is evidence that periodontitis can adversely affect glycaemic control.

Caveolin-1 is an integral membrane scaffolding protein localized on the cytoplasmic side of the peripheral membrane of caveolae. It is present in several types of cells but is abundant in endothelial cells, fibroblasts, adipocytes and smooth muscle cells [16]. Recently, the colocalization of Cav-1 in hPDLCs and HGFs has been strongly demonstrated [17, 18]. In addition to its diverse cellular presence, Cav-1 is implicated in several cellular processes, such as inflammation. During inflammation, it serves as an immunomodulatory agent by modulating the activity of immune cells such as neutrophils, lymphocytes and APCs [16].

The azurophilic granules of neutrophils contain the antimicrobial protein azurocidin. Azurocidin exhibits a broad spectrum of antimicrobial activity, particularly against gram-negative bacteria. It also has chemotactic effects on monocytes/macrophages and T cells and enhances macrophage phagocytosis. During inflammation, the release of TNF-α and IL-6 from monocytes in response to lipopolysaccharide (LPS) is induced by azurocidin [19].

In light of the current understanding of the role of caveolin-1 and azurocidin in the inflammatory process, the present study was designed to estimate the GCF levels of caveolin-1 and azurocidin in selected study groups to correlate these levels with periodontal clinical parameters and to explore the possibility of using these factors as biomarkers in periodontitis and diabetes mellitus.

The mean PI value of Group III was highest, which is in accordance with the study by Campus et al [20]. This can be attributed to diabetic patients having more dental plaque and poorer oral hygiene than nondiabetic patients, which may be attributed to higher levels of glucose in gingival crevicular fluid and saliva [21].

When the mean GI was compared between groups, it was highest in Group III, similar to the findings of the study by Norma Sznajder et al., hence validating the microvascular changes in the endothelium of diabetic patients [22]. The mean BI was significantly lower in the chronic gingivitis group than in the nondiabetic and diabetic groups, similar to the findings of Tchobroutsky [23]. This may be because excess production of cytokines in diabetic patients plays an important role in both micro- and macrovascular alterations, which promote bleeding on probing [24]. Increased glucose levels in GCF may also result in altered plaque microflora, causing an increase in gingival bleeding [25].

In addition, the diabetic group showed significant increases in the mean PD and CAL, with highly significant p values (≤0.001), similar to the findings of Barnett et al. and Campbell et al. [26, 27]. Chavarry and coworkers carried out a systematic review and meta-analyses that indicated a significantly greater mean CAL and a greater mean periodontal probing depth in individuals with type 2 diabetes mellitus than in control subjects [28]. This can be explained by the fact that during the inflammatory response to the bacterial toxins in periodontal tissue, various cytokines, prostaglandins, and interleukins are released, which play a major role in the amplification of tissue destruction.

Gingival crevicular fluid comprises an assemblage of cellular and biochemical factors that determine the metabolic status of several components of the periodontium. The collection of GCF is a relatively simple and noninvasive procedure.

Therefore, the levels of Caveolin-1 and Azurocidin were estimated using GCF samples in this study.

According to the evaluation of Caveolin-1 levels, Group III had significantly greater caveolin-1 levels ($p<0.001^*$). The caveolin-1 levels were lowest in Group I. However, the caveolin-1 levels were greater in Group II than in the chronic gingivitis group, suggesting that Caveolin-1 plays a role in regulating inflammatory conditions through its effector responses for immune cells, leading to its immunomodulatory function. These findings are in accordance with those of a 2012 study by Takizawa et al., which suggested that secreted caveolin-1 derived from periodontal fibroblastic cells enhances inflammation [29]. The mechanism for this inflammatory pathway was proposed by Yamaguchi et al. in 2008, who suggested that Cav-1 is involved in IL-6, a potent inflammatory mediator, and enhances the production of cathepsin, which causes intracellular proteolysis and extracellular matrix remodelling, leading to tissue destruction in periodontitis [30]. In 2015, Lee et al. reported that Cav-1 inhibition positively regulates osteoblastic differentiation in hPDLCs, cementoblasts and osteoblasts [18].

In addition to its role in periodontal tissue, Cav-1 has been shown to be involved in systemic inflammatory conditions in several studies, as evidenced by Ohnuma et al. in 2006 and Hu et al. in 2007. These authors suggested that Cav-1 expressed in neutrophils plays an important role in the mechanism of PMN activation-mediated inflammation and that Cav-1 interaction with immune cells leads to antigen-specific T-cell activation. Additionally, overexpression of Cav-1 aggravates the inflammatory response [16,30, 31]

Several other animal studies have also shown a positive correlation between Cav-1 and chronic disease conditions [32-34].

However, in contrast to previous and present data, a study by Agarwal et al in 2010 revealed the role of Cav-1 as a negative regulator of EMMPRIN in neuroinflammation [35]. A similar result was obtained by Wang et al. in 2013, who demonstrated the colocalization of EMMPRIN in gingival tissues. Their study concluded that Cav-1 impairs EMMPRIN glycosylation, leading to decreased MMP production in periodontal disease [36].

In a study by Bae et al in 2019, Cav-1 expression increased under cytokine influence, leading to beta cell apoptosis and diabetic conditions [37]. This finding strongly explains the elevated levels of Cav-1 in group III in the present study. Various studies conducted by Ortega et al. have suggested that Cav-1 plays a role in the inflammatory response to insulin, confirming the direct contribution of Cav-1 to the maintenance of insulin in the body [38]. Under the influence of chronic inflammatory conditions and long-term glucose exposure, insulin sensitivity is compromised, leading to enhanced Cav-1 expression as an adaptive response [39,40]. In support of this, Haddad et al., in 2020, also reported that continuous exposure to high glucose concentrations increased Cav-1 and IR expression and impaired insulin signaling, leading to insulin insensitivity [41]. The possibility of newly diagnosed type 2 diabetes patients being under long-standing undiagnosed glucose exposure is likely due to elevated Cav-1 levels. In another previously conducted study by Kabayama et al. in 2007, under the influence of TNF-α-induced insulin resistance, IR and Cav-1 dynamically segregated [42]. This can indicate that under inflammatory conditions, Cav-1 tends to be dissociated from IR, leading to an increase in its level and impaired insulin signaling, as has also been suggested by the current study. Furthermore, evidence also suggests that Cav-1 may be involved in diabetes-associated inflammation because of the two-way relationship between diabetes and periodontitis.

Because of its ability to affect numerous cellular pathways, Cav-1 is also involved in various diabetes-associated complications. A 2017 study by Ding et al reported the association of Cav-1 expression with the progression of diabetic peripheral neuropathy, which is considered to be an important cause of ulcers in diabetic patients and can lead to compromised quality of life [43]. A review by Krieken and Krepinsky in 2017 showed that under high glucose conditions, Cav-1-mediated signalling leads to increased ECM accumulation, leading to DN [44]. In addition, in 2019, Bonds et al. reported a correlation between Cav-1 in type 2 diabetes mellitus and its effect on the development of Alzheimer's disease; however, this relationship is still a debated topic [45]. Nonetheless, other studies have shown the association of caveolin with diabetes complications such as diabetic retinopathy. Cardiovascular diseases such as coronary disease, cerebrovascular disease, peripheral artery disease and myocardial impairment [41].

The data of the current study and the aforementioned studies support that Cav-1 is upregulated in chronic disease conditions such as periodontitis and diabetes. Furthermore, its association with diabetes-related complications makes it a strong therapeutic molecular target for improving quality of life.

According to Pearson's correlation analysis of all three groups, caveolin-1 expression correlated significantly with clinical parameters, indicating that an increase in GI, PI, bleeding on probing, probing pocket depth and clinical attachment loss subsequently resulted in an increase in the level of Cav-1 in the GCF, indicating that Cav-1 is a biomarker that is positively associated with inflammation in periodontitis and diabetes mellitus patients.

In this study, the levels of azurocidin were also analysed, and they were significantly ($p<0.001^*$) greater in the periodontitis group than in the gingivitis group. These findings were in agreement with the findings of the first study conducted by Choi et al. in 2011. The authors revealed that azurocidin in the GCF is an upregulated protein in periodontitis patients [46]. Another study by Leppilahti et al. in 2014 revealed elevated levels of azurocidin in the GCF of patients with gingivitis and periodontitis, with higher concentrations in the periodontitis group [47]. In 2018, Guzman et al. conducted proteomic profiling of the GCF of periodontitis

patients and concluded that azurocidin was upregulated at baseline in periodontitis patients and decreased in value after periodontal therapy [48].

Recently, in 2020, Afacan and Atmaca Ilhan studied azurocidin in saliva and reported that there was a greater level of salivary azurocidin in periodontitis patients. Moreover, azurocidin has been reported to be elevated even in plasma during systemic septic conditions [49]. A review by Stock et al. in 2018 illustrated the role of neutrophil granule protein involvement in chronic inflammatory conditions such as Alzheimer's disease [50].

Our study is the first to analyse the levels of azurocidin in the GCF of patients with chronic periodontitis and newly diagnosed type 2 diabetes mellitus. The greatest amount of azurocidin was detected in Group III. These findings imply that azurocidin is elevated in chronic disease conditions such as periodontitis and diabetes. The proposed mechanism is that AZD is stored not only in azurophilic granules but also in other granules, such as secretory granules, and under chronic conditions, AZD is secreted from these granules, leading to an increase in the number and specific regulation of monocyte recruitment and activation. These factors increase cytokine release, cause destruction to the host and perpetuate inflammation. A study carried out by Huang et al. in 2018 showed strong evidence of increased neutrophil counts in newly diagnosed type 2 diabetes patients, which is in accordance with our study [20].

Pearson's correlation analysis of the periodontal parameters revealed that AZD was significantly correlated with the clinical parameters, indicating that it is a biomarker that is positively associated with inflammation in periodontitis and diabetes mellitus patients. This finding is well supported by a study conducted by Ipek et al. in 2018 in which the authors investigated the role of azurocidin and reported that azurocidin is a potent marker of inflammation [12].

Multiple linear regression analysis was used to evaluate the joint effect of all the periodontal parameters (PI, GI, BI, PD, Caveolin-1, and Azurocidin) with CAL (as the dependent variable) in all three groups, which implies that there is a significant relationship between the set of predictors and the CAL.

Although there are highly significant data related to the findings of our study, there are still some limitations. One of the major limitations of the study is that a larger number of study participants need to be recruited to overcome the variability in the results. Moreover, the levels of caveolin-1 and azurocidin need to be studied at different stages of the disease to determine the relationship between these two molecules and the disease process. Future studies can be designed to study the correlation of the expression of the molecules within groups with different variants of diabetes status and duration with HbA1c levels, which may clarify the relationship between the initiation and progression of periodontal disease.

5. Conclusion

To the best of our knowledge, the present study is the first to analyse the GCF levels of caveolin-1 and azurocidin in chronic periodontitis patients and chronic periodontitis patients with newly diagnosed type 2 diabetes. Our study revealed a correlation between the GCF levels of caveolin-1 and azurocidin in periodontitis and diabetes mellitus, suggesting that these two factors are potent markers of inflammation and have high diagnostic value. This finding underscores the importance of both molecules in normal and disease states and emphasizes the need for further research in this area.

Reference

1. Khaparde, S., Bhandari, V., Ugale, G., Jawade, R., Ardale, M., & Mundada, K. (2016). Acute phase proteins: journey of pivotal marker in periodontal disease. IOSR J Dental Med Sci, 15(7), 92-99.
2. Page, R. C., & Kornman, K. S. (1997). The pathogenesis of human periodontitis: an introduction. Periodontology 2000, 14(1), 9-11.
3. Preshaw, P. M., Alba, A. L., Herrera, D., Jepsen, S., Konstantinidis, A., Makrilakis, K., & Taylor, R. (2012). Periodontitis and diabetes: a two-way relationship. Diabetologia, 55(1), 21-31.
4. Lamster, I. B., Lalla, E., Borgnakke, W. S., & Taylor, G. W. (2008). The relationship between oral health and diabetes mellitus. J Am Dent Assoc, 139, 19S-24S.
5. Grover, H. S., & Luthra, S. (2013). Molecular mechanisms involved in the bidirectional relationship between diabetes mellitus and periodontal disease. J Indian Soc Periodontol, 17(3), 292-301.
6. Cohen, A. W., Combs, T. P., Scherer, P. E., & Lisanti, M. P. (2003). Role of caveolin and caveolae in insulin signaling and diabetes. Am J Physiol Endocrinol Metab, 285(6), E1151-E1160.
7. Jia, G., & Sowers, J. R. (2015). Caveolin-1 in cardiovascular disease: a double-edged sword. Diabetes, 64(11), 3645.
8. Elçioğlu, K. H., Kabasakal, L., Çetinel, Ş., Conturk, G., Sezen, S. F., & Ayanoğlu-Dülger, G. (2010). Changes in caveolin-1 expression and vasoreactivity in the aorta and corpus cavernosum of fructose and streptozotocin-induced diabetic rats. Eur J Pharmacol, 642(1-3), 113-120.
9. Wang, J., Yang, D., Li, C., Shang, S., & Xiang, J. (2014). Expression of extracellular matrix metalloproteinase inducer glycosylation and c aveolin-1 in healthy and inflamed human gingiva. J Periodontal Res, 49(2), 197-204.
10. Insuela, D., Coutinho, D., & Martins, M. (2020). Neutrophil Function Impairment Is a Host Susceptibility Factor to. Cells of the immune system.
11. Heinzelmann, M., Mercer-Jones, M. A., Flodgaard, H., & Miller, F. N. (1998). Heparin-binding protein (CAP37) is internalized in monocytes and increases LPS-induced monocyte activation. J Immunol, 160(11), 5530-5536.
12. Ipek, E., Yolcu, M., Yildirim, E., Altinkaynak, K., Ozbek Sebin, S., Kalkan, K., ... & Ozturk, M. (2018). A novel marker of inflammation: azurocidin in patients with ST segment elevation myocardial infarction. Int J Mol Sci, 19(12), 3797.

13. Moreno-Navarrete, J. M., & Fernández-Real, J. M. (2011). Antimicrobial-sensing proteins in obesity and type 2 diabetes: the buffering efficiency hypothesis. Diabetes Care, 34(Suppl 2), S335.
14. Alba-Loureiro, T. C., Munhoz, C. D., Martins, J. O., Cerchiaro, G. A., Scavone, C., Curi, R., & Sannomiya, P. (2007). Neutrophil function and metabolism in individuals with diabetes mellitus. Braz J Med Biol Res, 40, 1037-1044.
15. de Souza Ferreira, C., Araújo, T. H., Ângelo, M. L., Pennacchi, P. C., Okada, S. S., de Araújo Paula, F. B., ... & Rodrigues, M. R. (2012). Neutrophil dysfunction induced by hyperglycemia: modulation of myeloperoxidase activity. Cell Biochem Funct, 30(7), 604-610.
16. Hu, G., Ye, R. D., Dinauer, M. C., Malik, A. B., & Minshall, R. D. (2008). Neutrophil caveolin-1 expression contributes to mechanism of lung inflammation and injury. Am J Physiol Lung Cell Mol Physiol, 294(2), L178-L186.
17. Yamaguchi, T., Naruishi, K., Arai, H., Nishimura, F., & Takashiba, S. (2008). IL-6/sIL-6R enhances cathepsin B and L production via caveolin-1-mediated JNK-AP-1 pathway in human gingival fibroblasts. J Cell Physiol, 217(2), 423-432.
18. Lee, S. Y., Yi, J. K., Yun, H. M., Bae, C. H., Cho, E. S., Lee, K. S., & Kim, E. C. (2016). Expression of Caveolin-1 in periodontal tissue and its role in osteoblastic and cementoblastic differentiation in vitro. Calcified tissue international, 98(5), 497-510.
19. Afacan, B., & Atmaca İlhan, H. (2020). Salivary Azurocidin and Tumor Necrosis Factor-α Levels in Patients with Stage III-IV, Grade C Periodontitis. Ege Üniversitesi Diş Hekimliği Fakültesi Dergisi, 41(1), 47-54.
20. Campus, G., Salem, A., Uzzau, S., Baldoni, E., & Tonolo, G. (2005). Diabetes and periodontal disease: A case-control study. J Periodontol, 76(3), 418-425.
21. Mohamed, H. G., Idris, S. B., Ahmed, M. F., Bøe, O. E., Mustafa, K., Ibrahim, S. O., & Åstrøm, A. N. (2013). Association between oral health status and type 2 diabetes mellitus among Sudanese adults: a matched case-control study. PloS one, 8(12), e82158.
22. Sznajder, N., Carraro, J. J., Rugna, S., & Sereday, M. (1978). Periodontal findings in diabetic and nondiabetic patients J Periodontol, 49(9), 445-448.
23. Tchobroutsky, G. (1978). Relation of diabetic control to development of microvascular complications. Diabetologia, 15(3), 143-152.
24. Witztum, J. L. (1997). Role of modified lipoproteins in diabetic macroangiopathy. Diabetes, 46(Supplement_2), S112-S114.
25. Ambon, J. J., Reynolds, H., Fisher, J. G., Shlossman, M., Dunford, R., & Genco, R. J. (1988). Microbiological and immunological studies of adult periodontitis in patients with noninsulin-dependent diabetes mellitus. J Periodontol, 59(1), 23-31.
26. Barnett, M. L., Baker, R. L., Yancey, J. M., MacMillan, D. R., & Kotoyan, M. (1984). Absence of periodontitis in a population of insulin-dependent diabetes mellitus (IDDM) patients. J Periodontol, 55(7), 402-405.
27. Campbell, M. J. A. (1972). Epidemiology of periodontal disease in the diabetic and the non-diabetic. Aust Dent J, 17(4), 274-278.
28. Chávarry, N. G., Vettore, M. V., Sansone, C., & Sheiham, A. (2009). The relationship between diabetes mellitus and destructive periodontal disease: a meta-analysis. Oral Health Prev Dent, 7(2), 107-27.
29. Takizawa, N., Sawada, S., Chosa, N., Ishisaki, A., & Naruishi, K. (2013). Secreted caveolin-1 enhances periodontal inflammation by targeting gingival fibroblasts. Biomed Res, 34(1), 1-11.
30. Ohnuma, K., Inoue, H., Uchiyama, M., Yamochi, T., Hosono, O., Dang, N. H., & Morimoto, C. (2006). T-cell activation via CD26 and caveolin-1 in rheumatoid synovium. Mod Rheumatol, 16(1), 3-13.
31. Lv, X. J., Li, Y. Y., Zhang, Y. J., Mao, M., & Qian, G. S. (2010). Over-expression of caveolin-1 aggravate LPS-induced inflammatory response in AT-1 cells via up-regulation of cPLA2/p38 MAPK. Inflamm Res, 59(7), 531-541.
32. Gurley, J. M., Gmyrek, G. B., McClellan, M. E., Hargis, E. A., Hauck, S. M., Dozmorov, M. G., ... & Elliott, M. H. (2020). Neuroretinal-derived caveolin-1 promotes endotoxin-induced inflammation in the murine retina. Invest Ophthalmol Vis Sci, 61(12), 19-19.
33. Li, X., Gu, X., Boyce, T. M., Zheng, M., Reagan, A. M., Qi, H., ... & Elliott, M. H. (2014). Caveolin-1 increases proinflammatory chemoattractants and blood–retinal barrier breakdown but decreases leukocyte recruitment in inflammation. Invest Ophthalmol Vis Sci, 55(10), 6224-6234.
34. Medina, F. A., De Almeida, C. J., Dew, E., Li, J., Bonuccelli, G., Williams, T. M., ... & Lisanti, M. P. (2006). Caveolin-1-deficient mice show defects in innate immunity and inflammatory immune response during Salmonella enterica serovar Typhimurium infection. Infect Immun, 74(12), 6665-6674.
35. Agrawal, S. M., & Yong, V. W. (2011). The many faces of EMMPRIN—roles in neuroinflammation. Biochim Biophys Acta, 1812(2), 213-219.
36. Wang, X. M., Kim, H. P., Song, R., & Choi, A. M. (2006). Caveolin-1 confers antiinflammatory effects in murine macrophages via the MKK3/p38 MAPK pathway. Am J Respir Cell Mol Biol, 34(4), 434-442.
37. Bae, Gong Deuk, Eun-Young Park, Kyong Kim, Se-Eun Jang, Hee-Sook Jun, and Yoon Sin Oh. "Upregulation of caveolin-1 and its colocalization with cytokine receptors contributes to beta cell apoptosis." Sci Rep 9, no. 1 (2019): 16785.
38. Palacios-Ortega, S., Varela-Guruceaga, M., Algarabel, M., Milagro, F. I., Martínez, J. A., & De Miguel, C. (2015). Effect of TNF-alpha on caveolin-1 expression and insulin signaling during adipocyte differentiation and in mature adipocytes. Cell Physiol Biochem, 36(4), 1499-1516.
39. Palacios-Ortega, S., Varela-Guruceaga, M., Milagro, F. I., Martínez, J. A., & De Miguel, C. (2014). Expression of Caveolin 1 is enhanced by DNA demethylation during adipocyte differentiation. status of insulin signaling. PloS one, 9(4), e95100.
40. Palacios-Ortega, S., Varela-Guruceaga, M., Martínez, J. A., de Miguel, C., & Milagro, F. I. (2016). Effects of high glucose on caveolin-1 and insulin signaling in 3T3-L1 adipocytes. Adipocyte, 5(1), 65-80.
41. Haddad, D., Al Madhoun, A., Nizam, R., & Al-Mulla,

F. (2020). Role of caveolin-1 in diabetes and its complications. Oxid Med Cell Longev, 2020(1), 9761539.
42. Kabayama, K., Sato, T., Saito, K., Loberto, N., Prinetti, A., Sonnino, S., ... & Inokuchi, J. I. (2007). Dissociation of the insulin receptor and caveolin-1 complex by ganglioside GM3 in the state of insulin resistance. Proc Natl Acad Sci U S A, 104(34), 13678-13683.
43. Ding, M., Zhu, X., Ye, L., Wang, M., Cui, Y., Xu, J., ... & Wang, P. (2017). Association between Caveolin-1 expression and pathophysiological progression of femoral nerves in diabetic foot amputation patients. Open Life Sciences, 12(1), 308-314.
44. Van Krieken, R., & Krepinsky, J. C. (2017). Caveolin-1 in the pathogenesis of diabetic nephropathy: potential therapeutic target?. Curr Diab Rep, 17(3), 19.
45. Bonds, J. A., Shetti, A., Bheri, A., Chen, Z., Disouky, A., Tai, L., ... & Lazarov, O. (2019). Depletion of caveolin-1 in type 2 diabetes model induces Alzheimer's disease pathology precursors. J Neurosci, 39(43), 8576-8583.
46. Choi, Y. J., Heo, S. H., Lee, J. M., & Cho, J. Y. (2011). Identification of azurocidin as a potential periodontitis biomarker by a proteomic analysis of gingival crevicular fluid. Proteome Sci, 9(1), 42.
47. Leppilahti, J. M., Hernández-Ríos, P. A., Gamonal, J. A., Tervahartiala, T., Brignardello-Petersen, R., Mantyla, P., ... & Hernández, M. (2014). Matrix metalloproteinases and myeloperoxidase in gingival crevicular fluid provide site-specific diagnostic value for chronic periodontitis. J Clin Periodontol, 41(4), 348-356.
48. Guzman, Y. A., Sakellari, D., Papadimitriou, K., & Floudas, C. A. (2018). High-throughput proteomic analysis of candidate biomarker changes in gingival crevicular fluid after treatment of chronic periodontitis. J Periodontal Res, 53(5), 853-860.
49. Yang, Y., Liu, G., He, Q., Shen, J., Xu, L., Zhu, P., & Zhao, M. (2019). A promising candidate: heparin-binding protein steps onto the stage of sepsis prediction. J Immunol Res, 2019(1), 7515346.
50. Dowey, R., Iqbal, A., Heller, S. R., Sabroe, I., & Prince, L. R. (2021). A bittersweet response to infection in diabetes; targeting neutrophils to modify inflammation and improve host immunity. Front Immunol, 12, 678771.