Journal of Research in Medical and Dental Science
2023, Volume 11, Issue 08, Page No: 078-082

Available Online at: www.jrmds.in
eISSN No.2347-2367: pISSN No.2347-2545

Assessment of Gingival Crevicular Fluid Levels of "Vitamin-D Binding Protein" in Periodontitis Patients with Stage II-IV

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ABSTRACT

Background: Vitamin D Binding Protein (DBP) is the major transporter protein of vitamin D in the bloodstream. In addition, it plays an essential role in immunomodulatory and anti-inflammatory functions. There are limited studies regarding the relationship of this protein with periodontitis, which measured its level in saliva, serum, and gingival crevicular fluid and found a link between periodontal health and periodontist with the level of DBP.
Aims: To assess DBP level in the gingival crevicular fluid of periodontitis patients with (stages II, III, and IV) compared to healthy controls, also to measure clinical parameters and correlate them with the level of DBP in periodontitis patients.
Materials and methods: A total of 49 subjects participated in the study; the Probing Pocket Depth (PPD) and the Clinical Attachment Level (CAL) were measured clinically. Gingival crevicular fluid was collected from each individual. An Enzyme-Linked Immunosorbent Assay (ELISA) was utilized to detect DBP levels.
Results: The level of DBP was found to be decreased in periodontitis groups compared with a healthy control group (p-value <0.001). A significant difference was reported between the healthy control groups and stage III and stage IV. No significant correlation was found between periodontal parameters and DBP level in the gingival crevicular fluid.
Conclusion: According to this study, reduced DBP level was noticed in the gingival crevicular fluid of periodontitis patients in comparison with healthy controls. This suggests that DBP is involved in both periodontal health and the pathogenesis of periodontitis.

Key words: Periodontitis, Gingival crevicular fluid, Vitamin D binding protien, Macrophage activating factor, Actin

HOW TO CITE THIS ARTICLE: Mariam H Hasson, Ayser N Mohammed. Assessment of Gingival Crevicular Fluid Levels of "Vitamin-D Binding Protein" in Periodontitis Patients with Stage II-IV, J Res Med Dent Sci, 2023, 11 (08): 078-082.

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E-mail: marmar.marmar533@gmail.com
Received: 28-May-2022, Manuscript No. JRMDS-22-66423;
Editor assigned: 30-May-2022, PreQC No. JRMDS-22-66423 (PQ);
Reviewed: 10-Jun-2022, QC No. JRMDS-22-66423;
Revised: 03-Aug-2023, Manuscript No. JRMDS-22-66423 (R);
Published: 10-Aug-2023

INTRODUCTION

Periodontal diseases refer to chronic inflammatory conditions affecting the surrounding tissues that support teeth. Periodontitis is characterized by periodontal tissue support loss, which is manifested as the loss of clinical attachment, gingival bleeding, periodontal pockets, and alveolar bone loss recognized radiographically [1].

Vitamin D Binding Protein (DBP), also referred to as Gc-globulin, is a plasma glycoprotein produced mainly by parenchymal cells of the liver and expressed in numerous tissues, including liver, gonads, kidney, neutrophils, and fat. The principal role of DBP is to carry vitamin D and its metabolites and regulation their bioavailability [2].

In addition, DBP also has immunomodulatory and anti-inflammatory functions other than vitamin D carriage, including binding of actin monomers and improved scavenges of fibrillar actin. DBP increases neutrophil and monocyte chemotaxis to inflammatory sites by initiating the C5a mediated signalling. Furthermore, DBP carries saturated and unsaturated free fatty acids; therefore, it may have a role in lipid metabolism. Also, bone modulation is affected by DBP [3]. The different functions of DBP show its potential importance in susceptibility or resistance to various chronic conditions, such as type 1 and type 2 diabetes mellitus [4], osteoporosis [5], inflammatory bowel disease [6], chronic obstructive lung disease [7], and thyroid autoimmunity [8]. An extensive discussion of DBP's role in these diseases is not fully understood.

DBP levels have been studied in relation to periodontitis in only a few previous studies. Based on a relatively small analysis, DBP levels were found to be raised in the whole saliva of periodontitis patients compared to edentulous or dentulous controls and correlated positively with gingival index [9]. A study in the proteomic profile of whole un-stimulated saliva in generalized aggressive periodontitis GAgP confirmed elevated saliva DBP levels in GAgP pateints [10]. Zhang, et al. compared the level of DBP in plasma of GAgP patients with healthy controls and concluded that raised plasma DBP levels were related to

GAgP [11]. Zhang, et al. also measured DBP concentrations in Gingival Crevicular Fluid GCF and plasma of patients with GAgP and found that plasma levels of DBP were higher. Still, GCF levels of DBP were lower, which decreased with increased periodontal damage [12]. Thus, the current study aimed to measure DBP levels in GCF of patients with periodontitis stage II-IV compared to healthy controls and finding an association between periodontitis and DBP.

MATERIALS AND METHODS

Study design

From May to November 2021, 49 subjects (40 males, 9 females) were picked according to inclusion and exclusion criteria. Participants were given detailed information about the study, and they were asked to complete a questionnaire detailing their background information, medical history, and dental history. Body Mass Index (BMI) was considered. Periodontal clinical parameters were assessed, involving periodontal pocket depth PPD and clinical attachment loss CAL. A sample of gingival crevicular fluid was collected from every participant. The ethical committee of the college of the dentistry/University of Baghdad follows the guidelines of Helsinki and Tokyo for humans (reference no.323 on 24/3/2021) that approved the study's protocol. All individuals were distributed into two groups; a control group (7 periodontal healthy subjects) and a study group; (14 periodontitis patients with stage II), (14 periodontitis patients with stage III), (and 14 periodontitis patients with stage IV). Periodontitis groups were defined as interdental CAL equal to or greater than two in nonadjacent teeth or buccal/oral CAL ≥ 3 mm with pocketing >3 mm detected at ≥ 2 teeth. Periodontal health was defined as PPD ≤ 3 mm, BOP <10%, and intact periodontium (no probing attachment loss) [13]. Patients with systemic, chronic conditions with a recognized connection to periodontitis, such as diabetes mellitus, liver diseases, smokers and alcoholic patients, pregnant women, and menopausal women, were excluded.

Clinical assessment

An examination of the entire mouth was achieved using a UNC-15 periodontal probe. PD and CAL were calculated at six sites per tooth for each patient. The CAL was considered the distance from the cementoenamel junction to the pocket's bottom. To confirm staging, each patient had a periapical radiograph taken for the worst periodontitis site. In addition, 3 ml of venous blood was taken from the cubital fossa to analyze liver enzymes: Aspartate Transaminase (AST), and Alanine Transaminase (ALT) to exclude patients with high liver enzymes.

Examiner alignment

The researcher and the experienced practitioner measured clinical parameters (PPD, CAL) of 10 subjects simultaneously. The Intraclass Correlation Coefficient (ICC) was used to evaluate the inter-examiner calibration, and both parameters showed a substantial level of agreement, 0.753 and 0.760, correspondingly. For intra-examiner calibration, the researcher double-measured the similar periodontal parameters of 10 subjects. For both parameters, the level of agreement was almost perfect, 0.933 and 0.855, correspondingly [14].

Sample collection

GCF was collected at the four worst sites affected by periodontitis in the study groups and four sites from mesiobuccal and distobuccal areas of upper incisors in the control group. Before the sampling, each Eppendorf tube (1.5 ml) with 300 µl added Phosphate Buffer Saline (PBS) was weighted by an electronic scale (WNQ-176-1, Hanchen, Germany), and the first weight was (Eppendorf tube, PBS, and added paper strips weight) [15]. After dryness, a cotton roll was used to isolate the test site, and without touching the marginal gingiva, supragingival plaque removal was done. The paper strip (Oraflow Inc.® New York, America) was introduced into the sulcus/ pocket till slight resistance was detected and was reminded in the site for 30 seconds. Blood-contaminated strips were discarded [16]. Following GCF collection, the strips were loaded into the same tube and weighed again within a half-hour of collection. Differential weighting was used to calculate the GCF's volume [17]. Samples were centrifuged at 400 xg (2000 RPM) for 4 min and preserved frozen at −80°C till the phase of the DBP assay.

DBP measurement

An enzyme-linked immunosorbent assay kit (BioSource Systems, Invitrogen, NY, USA) was used to detect DBP concentration in the GCF supernatant. The above presented kit reported an assay sensitivity of<0.074 ng/ml. The assays were carried out as directed by the manufacturer. A total level of GCF DBP was estimated based on the concentration of DBP measured.

Statistical analysis

Statistical Package for Social Science (SPSS version 21) (Chicago, USA, Illinois) was utilized for data description, presentation, and analysis. For nominal variables, Standard Deviation (SD) and mean were used. Additionally, Interclass Correlation Coefficient (ICC), pearson correlation (r), and Levene test were used for inferential statistics. Regarding the normality distribution of quantitative variables, the Shapiro-Wik test was used. One Way Analysis Of Variance (ANOVA) with Games-Howell posthoc test was also achieved.

RESULTS

Table 1 show all studied variables, including demographic variables, periodontal parameters that were obtained from the site of GCF collection, the levels of AST and ALT, and GCF level of DBP, which were normally distributed by use of the Shapiro-Wilk test at (P-value>0.05).

Table 1: Normality test of studied variables.

Test		Groups							
		Control		Stage II		Stage III		Stage IV	
		Statistic	P-value	Statistic	P-value	Statistic	P-value	Statistic	P-value
Shapiro-WILK	**Age**	0.994	0.998	0.918	0.207	0.935	0.358	0.931	0.314
	BMI	0.956	0.786	0.91	0.159	0.885	0.068	0.933	0.341
	AST	0.936	0.603	0.936	0.375	0.881	0.06	0.893	0.091
	ALT	0.81	0.051	0.925	0.257	0.883	0.064	0.891	0.085
	DBP	0.87	0.184	0.89	0.08	0.881	0.06	0.923	0.24
	PPD			0.885	0.068	0.907	0.142	0.974	0.921
	CAL			0.935	0.364	0.944	0.465	0.933	0.34

Periodontitis groups had significantly lower GCF DBP concentrations than the control group, with a significant difference observed between the studied groups (P-value<0.001), as presented in Table 2.

Table 2: The DBP (ng/ml) statistical test in GCF between groups using one way ANOVA.

Groups	Mean	± SD	± SE	Minimum	Maximum	P value
Control	1.202	0.812	0.307	0.416	2.808	0.000016 sig
Stage II	0.518	0.396	0.106	0.102	10.392	
Stage III	0.258	0.208	0.056	0.049	0.812	
Stage IV	0.242	0.189	0.051	0.011	0.659	
Note: Levene p value=0.001 Sig.						

Furthermore, after multiple pair-wise comparisons, a significant difference was observed between the healthy control group with Stage III (p-value=0.003) and the healthy control group with stage IV (p-value=0.002); other findings were not significant, as presented in Table 3.

Table 3: Intergroups multiple pair-wise comparisons of the mean values of DBP (ng/ml) in GCF.

Multiple comparisons of DBP (ng/ml) in GCF among groups using Games-Howell			
Groups		**Mean difference**	**P value**
Control	Stage II	0.684	0.234^
	Stage III	0.944	0.003*
	Stage IV	0.959	0.002*
Stage II	Stage III	0.26	0.164^
	Stage IV	0.276	0.122^
Stage III	Stage IV	0.015	0.997^
Note: Not significant at p>0.05, *=significant at p<0.05			

Regarding the correlation between DBP levels and periodontal clinical parameters, no significant correlations were found between Periodontal Clinical Parameters (PPD/CAL) and GCF DBP concentration in periodontitis groups (p-value >0.05); this is presented in Table 4.

Table 4: The groups' correlation between DBP in GCF and periodontal parameters.

Groups	DBP (ng/ml)

		r	P^
Stage II	CAL	0.506	0.065
	PPD	0.042	0.887
Stage III	CAL	-0.431	0.124
	PPD	-0.091	0.757
Stage IV	CAL	0.186	0.524
	PPD	0.154	0.599
Total	PPD	-0.23	0.143
	CAL	-0.244	0.12

Note: Not significant at p>0.05.

DISCUSSION

(DBP) is a plasma glycoprotein considered the primary transporter of vitamin D with other immunomodulatory and anti-inflammatory functions. DBP had been associated with the pathology of different systemic diseases. However, no studies measured the GCF level of DBP in various stages of periodontitis. Therefore, the current study measured DBP concentration in the crevicular fluid of patients with periodontitis stage (II-IV). The present study showed that the mean value of DBP in GCF was higher in the healthy control group. Then it decreased with an increased level of periodontitis, with a significant difference between groups. Results accomplished in the present study were in coordination with the earlier study done by Zhang, et al.

Moreover, Li, Zhu, et al., [18] have reported that DBP is broadly distributed and highly expressed in healthy dental and periodontal tissues, which is in harmony with increased DBP levels in the healthy control group of this study. Nevertheless, there is a disagreeing study reported by Sharmila, et al. [19]. They assessed an increase in GCF DBP levels in chronic periodontitis compared to periodontal health, and they suggested that DBP is involved in the pathogenesis of periodontitis. As observed in our study, the elevation in GCF DBP levels may be due to some reasons. Besides binding vitamin D metabolites, DBP also serves as a binding protein for actin [20]. During cell lysis, vast amounts of actin are released from damaged cells. Actin goes into nucleation and polymerization, leading to disseminated intravascular coagulation and multi-organ failure if not cleaned [21]. Endothelial and microvascular dysfunctions were found to be associated with periodontitis [22]. The high affinity of DBP for G-actin stops the re-polymerization of actin and clears it from the blood. The DBP-actin complexes have a half-life of approximately 30 min in blood, primarily cleared by the liver, spleen, and lungs, and therefore give local protection against actin's damaging effects [23]. This mechanism may partially explain why GCF DBP concentrations were significantly lower in periodontitis groups in our study.

Furthermore, DBP is involved in the host defence and immune system. It has been demonstrated that human DBP can bind and inhibit endotoxin, suggesting it may act as an essential scavenger of endotoxin [24]. Moreover, DBP was associated with the surface of neutrophils, fibroblasts, and B and T cells. Active neutrophils and monocytes increase their DBP binding sites during inflammation, and DBP binds to these sites facilitating C5a induced chemotaxis [25]. Additionally, activated T and B cells during inflammation can convert DBP rapidly into a Macrophage Activating Factor (MAF). As a result, DBP-MAF stimulates macrophages to improve host defenses. When the inflammation is exaggerated, DBP-MAF is able to promote apoptosis in activated macrophages.

In summary, decreased DBP levels in GCF in case of periodontitis may be related to DBP binding to the surface of inflammatory cells and lack of adequate local production due to the destruction of periodontium during periodontitis.

CONCLUSION

Overall, DBP was analyzed in the GCF of periodontitis stages (II-III-IV). Results show that patients with periodontitis had reduced GCF DBP levels than healthy controls. GCF DBP concentration decreased with the increasing stages of periodontitis.

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