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Assessment of Serum Levels of Soluble Urokinase Plasminogen Activator Receptor (suPAR) in Patients with Periodontitis and Atherosclerotic Cardiovascular Disease

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ABSTRACT

Background: *Soluble urokinase plasminogen activator receptor (suPAR) has been identified as a biomarker of inflammation and immune activation. SuPAR has been employed as a marker of inflammation in the assessment of patients with cardiac disorders in recent years, either alone or in conjunction with other biomarkers of inflammation. Earlier studies have shown that suPAR is involved in the homeostasis of leukocyte and endothelial cells and hence in coronary heart disease (CHD) and periodontitis development.*

Subjects, materials and methods: *This study recruited 88 subjects, both sexes, ages ranged from 36 to 66 years old, and were arranged into 4 groups: group A, 25 patients with atherosclerotic cardiovascular disease (ASCVD) but no periodontal disease, group B, 25 patients with periodontitis but no systemic disease, group C, 25 patients with both ASCVD and periodontitis, and group D, 13 subjects with no systemic disease and good oral hygiene. Periodontitis was assessed using clinical periodontal parameters probing pocket depth (PPD) and clinical attachment level (CAL). Following clinical evaluation, 5ml of venous blood was taken from each participant. The enzyme-linked immunosorbent assay (ELISA) was then used to evaluate suPAR levels in the blood.*

Results: *The mean values of PPD and CAL were higher in group C than in B. For the serum level of suPAR, there was a significant difference between group C and group D at ($p<0.05$) with large effect size (0.152). Regarding the correlations between suPAR and clinical periodontal parameters, there was a significant positive correlation with CAL in group B and group C at ($p<0.05$).*

Conclusion: *The progressive increase in serum suPAR level in the study groups compared to the control group could reflect the role of periodontitis in atherosclerotic cardiovascular disease.*

Key words: Periodontitis, Atherosclerosis, Urokinase, Serum, Plasminogen activator receptor

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INTRODUCTION

Periodontal diseases (PDs) are inflammatory conditions that damage the supporting tissues of the teeth. Periodontitis is described as the loss of supportive periodontal tissue, as evidenced by clinical loss of attachment, gingival bleeding, and pocketing, as well as bone loss as detected by radiograph [1].

Atherosclerosis is the major cause of heart disease, as it is a chronic inflammatory disease of the arteries caused by lipid buildup and other physiological variables. The main cause of death worldwide is cardiovascular disease (CVD). CVD is estimated to kill about seventeen million people each year, which is about thirty percent of all deaths, and this number is supposed to rise to over twenty-three million by 2030 as the population ages and obesity becomes more prevalent [2].

There has been conjecture for over a century that there is a link between oral health and cardiovascular disease. The search for a relationship between periodontitis and atherosclerosis has escalated, with many associations and causalities being investigated [3,4]. The scientific evidence for these correlations was examined in two publications: one looked at the role of invading bacteria [5], while the other one focused on the process of inflammation [6]. This scholarly research currently implies that periodontitis causes bacteria or their products to enter the circulation, causing the host's inflammatory response to increase through multiple pathways, enhancing the creation and aggravation of atheromatous lesions [7].

Soluble urokinase-type plasminogen activator receptor (suPAR) is a mediator of multiple inflammatory diseases and is an indicator of systemic immune activation, inflammation, and thrombogenesis [8]. SuPAR is released as a soluble form in the blood and other body fluids due to cleavage of the urokinase plasminogen activator receptor (uPAR) which is a membrane-bound receptor that is mainly presented on immune cells and endothelial cells

such as lymphocytes, neutrophils, monocytes, macrophages, endothelial and tumor cells during immune activation and inflammation [8]. The association between increased suPAR and CVD incidence has been documented in recent studies [9-11]. In addition, several studies have shown that suPAR in saliva may be used as an oral health biomarker [12]. Particularly, a positive association between gingival inflammation and high salivary suPAR levels has been shown in pilot studies [13].

MATERIAL AND METHODS

Study design: From March to July 2021, 88 subjects were chosen based on inclusion and exclusion criteria. The goal of the study was explained to everyone. Each participant gave informed consent, and a questionnaire was utilized to record their background information, dental and medical history. A comprehensive assessment of clinical periodontal parameters (PPD, and CAL) was performed, followed by the collection of five ml of venous blood from each individual. The ethical committee of the College of Dentistry/University of Baghdad follows the guidelines of Helsinki and Tokyo for humans (the reference no.249 in 18/2/2021) authorized the study protocol. The subjects were split into four categories: group A, 25 patients have atherosclerosis and healthy periodontium, group B, 25 patients have periodontitis and didn't have any systemic disease. A patient is a periodontitis case in the context of clinical care with Interdental CAL is detectable at ≥2 non-adjacent teeth or buccal or oral CAL ≥3 mm with pocketing >3 mm is detectable at ≥2 teeth [14].

Group C, 25 patients having both atherosclerosis and periodontitis group D, 13 subjects systemically healthy and with healthy periodontium.

Patients having a history of another chronic, systemic condition with a known link to periodontitis, such as diabetes mellitus, rheumatoid arthritis as well as smokers, pregnant women, and contraceptive medications, obesity were also excluded.

Clinical assessment: Assessment of the presence of periodontitis was carried out by recording probing pocket depth and clinical attachment level using a periodontal probe of William's (marking at 1,2,3,5,7,8,9 and 10 mm). PPD was measured from the gingival margin to the base of the pocket while CAL is the distance measured from cemento-enamel junction to the base of the pocket/sulcus at six sites per tooth.

Examiner alignment: For inter examiner calibration; the periodontal parameters (PPD and CAL) for 5 subjects were measured by the researcher and the supervisor at the same time. The measurements were assessed using the intraclass correlation coefficient (ICC), and there was a substantial level of agreement for both parameters 0.760.0.753 respectively.

For intra examiner calibration, the same periodontal parameters for 5 subjects were measured twice by the researcher and there was an almost perfect level of agreement for both parameters 0.855, 0.933 respectively [15].

suPAR measurement: Each participant had 5ml of venous blood drawn from the cubital fossa with a 5ml plastic disposable syringe, which was then transferred to jell separating tubes, centrifuged for 5 minutes at 3000 RPM, and sera separated, the tubes were marked and stored at (-80°C) for later analysis by Enzyme-Linked Immuno-Sorbent Assay (ELISA) for the quantitative determination of serum suPAR.

Statistical analysis: Data description, analysis, and presentation were performed using Statistical Package for Social Science (SPSS version 21) (Chicago, USA, Illinois). Mean, and standard deviation (SD) for nominal variables also Inferential Statistics as, Pearson correlation (r), Levene test, two independent sample T-test, Shapiro Wilk and D'agostino Pearson test for the normality distribution of the quantitative variables, and One Way Analysis of Variance (ANOVA) with Games-Howell posthoc test and Tukey Kramer HSD were performed.

RESULTS

All studied variables were found to be normally distributed using Shapiro-Wilk at ($p>0.05$). In terms of PPD, group C had a greater mean and SD than group B, with a significant difference at P.value < 0.05 and a medium effect size (0.6222) Table 1. For CAL, group C showed a higher mean value and SD of CAL when compared with group B with a significant difference between them at P.value <0.05, and large effect size (1.171) as shown in Table 2.

Table 1: Statistics of PPD among groups using independent sample T test.

Statistics	Groups		T test	P-value	Cohen's D effect size
	B	C			
Mean	4.759	5.169	2.202	0.032* Sig	0.6222
±SD	0.727	0.583			
Minimum	3.66	4			
Maximum	6.61	6.5			

*=significant at p<0.05

Table 2: Statistics of CAL among groups using independent sample T test.

Statistics	Groups		T test	P-value	Cohen's D Effect size
	B	C			
Mean	3.459	4.649	4.143	0.000* Sig	1.171
±SD	1.033	0.999			
Minimum	2	3			
Maximum	5.4	6.68			

*=significant at p<0.05

Primary outcomes

The present study showed that suPAR was higher in group C followed by group A and then group B while lower in group D with a significant difference at P.value <0.05 and large effect size (0.152) Table 3. Furthermore, after doing multiple pairwise comparisons (MPC), when comparing any group with group D, the results were significant as shown in Table 4. Concerning the correlation of suPAR and clinical periodontal parameters in the present study as shown in Table 5. There was a significant strong positive correlation with CAL (r=0.505; p=0.010) in group B, and there was a significant strong positive correlation with CAL (r=0.513; p=0.0087) in group C.

Table 3: Statistical test of suPAR among groups using one way ANOVA.

Groups	Mean	±SD	Minimum	Maximum	F	P value	Effect size
A	112.213	18.668	81.978	148.275	5.501	0.002* Sig	0.152
B	103.23	28.329	53.582	167.743			
C	127.111	60.927	68.815	271.842			
D	77.373	25.865	39.649	120.74			

Levene statistics=8.479, p value=0.000 Sig, *= significant at p<0.05.

Table 4: Inter groups multiple pairwise comparisons of the mean values of suPAR.

Multiple Comparisons			
Dependent Variable: suPAR (pg/ml			
Games-Howell			
(IGroups	(IIGroups	Mean Difference (I-II	Sig.
A	B	8.9826	0.5534 ^
	C	-14.8978	0.6508 ^
	D	34.8403	0.0020 *
B	C	-23.8804	0.3017 ^
	D	25.8577	0.0411 *
C	D	49.7381	0.0064 *

*=significant at p<0.05, ^=not significant at p>0.05

Table 5: Correlation between suPAR and periodontal parameters by each group.

Groups		suPAR (pg/ml)	
		r	p value
B	PPD (mm)	0.179	0.393
	CAL (mm)	0.505	0.010*
C	PPD (mm)	0.153	0.464
	CAL (mm)	0.513	0.0087*

*=significant at p<0.05

DISCUSSION

The current study found that group C's mean PPD values were higher than group B's, with a significant difference between the two groups. These findings agreed with Vražić et al.,2015 [16].

This might be due to the rise in plaque and bacterial invasion with its toxin, which causes more destruction to alveolar bone tissue and sulcular and junctional epithelium, eventually causing a rise in the supply of nutrients required for bacterium proliferation [17].

There are potential explanations for increased periodontal damage when atherosclerosis is present: either a direct impact of atherosclerosis on the immune system, triggering the release of enzymes and pro-inflammatory cytokines like IL-1, IL-6, MMPs, and TNF-, or the impact of plaque accumulation. Periodontal damage has also been connected to endothelial dysfunction [18].

In addition, the CAL values in group III were higher than those in group II. These findings corroborated with Vražić et al and Androsz-Kowalska et al [16,19]. These outcomes could be explained by the fact that non-inflamed tissue cells and cells such as PMNs and monocytes, which played an active role in inflammatory processes and were involved in numerous activities that would increase the amount of damage, produced cytokines in response to the presence of pathogens, leading to collagen and bone destruction. Furthermore, the presence of atherosclerosis would result in increased cytokine release, greater damage, and hence higher CAL levels [20].

The mean serum suPAR levels were significant between groups (C, A) and D, and significant between groups B and D, according to the current data. These findings corroborated previous findings by Isola et al., 2021 [21]. Certain studies have linked the high serum suPAR levels with CVD [22,23] as a possible explanation, suPAR may be suggested as a risk factor for the development of CVD and endothelial dysfunction and through a mechanism controlled by NO and associated free radicals, according to new research. SuPAR levels in the blood have also been associated to initial carotid dysfunction and an increased risk of CHD progression, underscoring suPAR's important inhibitory action in endothelial homeostasis.

Inflammation and leukocyte activation have been linked to the release of SuPAR into numerous body fluids. Also, periodontitis may cause a rise in systemic suPAR levels due to a constant inflammatory impact on gingival tissues, which could be another unfavorable risk factor for the development of endothelial dysfunctions and CHD [24,25]. In groups B and C, there was a positive correlation with CAL. These results matched those of Isola et al., 2021 [21], who found a positive correlation between CAL and suPAR in plasma and saliva.

This could be due to the presence of bacteria in the periodontium which can affect plasminogen activation. Porphyromonas gingivalis, keystone bacteria in periodontal disease, increases plasminogen activation via a macrophage-dependent mechanism. P. gingivalis can also activate the uPA proteolytic pathway, increasing tissue injury and bacterial virulence [26,27].

CONCLUSION

The higher serum suPAR levels in group C may suggest a possible role in atherogenesis and the correlation with CAL in group B and C implying an indirect role in the beginning and progression of periodontal disease.

CONFLICTS OF INTEREST

No conflicts of interest.

ETHICAL CLEARANCE

This research has exemption as it a routine treatment (no new materials were used).

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Conflict of Interest the authors declare that there are no potential conflicts of interest related to the study.

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