Journal of
Babol University of Medical Sciences
JBUMS
e-ISSN: 2251-7170 p-ISSN: 1561-4107

The Association between Changes in Serum Biomarkers of Inflammatory Mediators and Periodontal Disease

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Article Type

Research Paper

Received:
Mar 25th 2023
Revised:
May 3rd 2023
Accepted:
May 31st 2023

ABSTRACT

Background and Objective: Periodontal diseases are chronic inflammatory diseases leading to damage to soft and hard tissue. Due to the importance of periodontal health in people, this study was designed to investigate the association between changes in the inflammatory mediators with the development of periodontal disease.

Methods: The case-control study was conducted on 120 individuals who referred to University of Baghdad, Dentistry School, Department of Periodontics in three groups: healthy group, periodontitis group, and gingivitis group, every group containing 40 individuals (20 men and 20 women). The demographic data of all individuals were recorded in the information form. Periodontal parameters, including bleeding on probing (BOP), plaque index (PI), clinical attachment loss (CAL), probing pocket depth (pd) and the serum levels of Interleukin-6 (Il-6), Immunoglobulin G (IgG) by ELISA technique, C-reactive protein (CRP) were evaluated using colorimetric technique.

Findings: The mean range of IL-6 was 17.3940±1.509, 17.1432±2.214, 11.3846±1.119, for periodontitis, gingivitis, and healthy groups, the mean range of CRP was 5.4477±3.771, 3.5853±3.483, 2.3813±1.134 for periodontitis, gingivitis, healthy groups, and the mean range of IgG was 12.3875±2.073, 15.0109±2.380, 9.4851±1.081 for periodontitis, gingivitis, healthy groups, respectively. Biochemical and periodontal parameters showed highly significant increase in periodontitis and gingivitis groups compared to healthy group ($p<0.05$).

Conclusion: According to the results of this study, the high levels of inflammatory mediators are associated with periodontal disease, and increase with the progression of periodontal disease, and the transition from gingivitis to periodontitis, and the study's cutoff values can be applied to estimate healthy cases or patients with periodontal disease.

Keywords: *Periodontal Diseases, Interleukin-6, C-Reactive Protein, Immunoglobulin G.*

Cite this article: Salam Ali S, Ali MS, al-Gebori A, al-Jumaily HAH. The Association between Changes in Serum Biomarkers of Inflammatory Mediators and Periodontal Disease. *Journal of Babol University of Medical Sciences*. 2023; 25(1): 409-16.

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Publisher: Babol University of Medical Sciences

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[DOI: 10.22088/jbums.25.1.409]

Introduction

Gingivitis is an inflammatory condition that affects the soft tissues of the periodontium without destroying the hard structures (1). Periodontitis is a chronic inflammatory disease of the tooth-supporting tissues as a result of the deposition of bacterial biofilm on the tooth's exterior surface, gingival tissues, periodontal ligament, cementum, and alveolar bone are all damaged, and if left untreated, leads to tooth loss (2). Periodontal disease impacts 20% to 50% of the world's population (3).

Interleukin-6 (IL-6) is a small glycoprotein (4), and glycoprotein cytokine secretion because of many diseases like osteoporosis (5). IL-6 like interleukin-17A (IL-17A) is a cytokine from both pro-inflammatory and anti-inflammatory cytokines (6, 7). It can also stimulate B-cell proliferation, as well as the development of plasmacytoma (4). IL-6 as in tumor necrosis factor (TNF) is expressed in macrophages, NK cells, monocytes, and T cells (8, 9). IL-6 stimulates the development of monocytes, activation of cytotoxic T cells, and improved NK cell activity (10). It plays a key role during an inflammatory response, in which serum IL-6 levels are significantly elevated (10). IL-6 affects innate immunity by regulating innate immune cell activity, neutrophil recruitment, adhesion, activation, differentiation (11).

C-reactive protein (CRP) is a biomarker for the inflammatory response (12), and in the case of inflammation or infection with bacterial and viral agents, induces healthy cells to generate IL-6 (13). It is an acute-phase indicator produced by the liver (14). It is also considered as one of the cytokines generated by wounded tissues and cells that can start a chain of acute-phase reactions. It is currently known to have an early discovery for detecting bacterial or viral infections (15). CRP binds to pathogens and facilitates their clearance by phagocytic cells in response to inflammatory start, acting as the initial line of defense for the host (16). Furthermore, CRP has anti-inflammatory properties by suppressing neutrophil chemotaxis. On the other hand, it induces pro-inflammatory effects by upregulating the production of adhesion molecules as well as pro-inflammatory IL-6 (16). Transcriptional activation of the CRP gene mainly happens in hepatocytes in response to increased levels of inflammatory cytokines, specifically interleukin-6 (IL-6) (17), and excessive levels of IL-6 expression may result in increased Ig production by B cells (18).

Immunoglobulin G (IgG) is a Y-shaped multifunctional glycoprotein made up of four polypeptide chains, linked together by disulfide bonds (19). IgG is the most predominant antibody subclass circulating in human blood (20). It acts as a bridge between antigens and the immune system (21), and it is an immune system component that protects periodontal tissue from pathogens and is present in gingival crevicular fluid (GCF) (21). IgG antibodies against periodontal microorganisms can be used to diagnose periodontal disease because their levels rise after infection and remain elevated for several years (22).

More studies are needed to assess IgM and IgG levels together with periodontal diseases, also to assess IL-6, IgG, and CRP with stage and grad periodontitis and gingivitis, and to assess other interleukins with IgG and CRP together. This study was designed to investigate the association between periodontal disease prognosis with serum biomarkers (IL-6, CRP, and IgG) and its association with the development of periodontal disease.

Methods

This case-control study was approved by the Ethics Committee in the Applied Science Department/ University of Technology and Ministry of Health, Baghdad, Iraq with ethics code (Ref. No. AS 2132-3-11-2021). Additionally, verbal and written consent was obtained from all participants in this research. This case-control study consists of 120 patients separated into three groups, gingivitis, periodontitis, and healthy

[DOI: 10.22088/jbums.25.1.409]

periodontium (control group). Every group included 40 individuals, 20 males and 20 females. The inclusion criteria were moderate and severe gingivitis, as well as generalized periodontitis. The exclusion criteria were having a history of systemic diseases such as diabetes mellitus, cardiovascular diseases, kidney diseases, rheumatoid arthritis, hepatic diseases, etc., having a history of smoking or alcohol drinking, pregnant or lactating mothers, menopausal women, and individuals taking anti-inflammatory medication in the last three months, in addition to any periodontal treatment containing deep scaling and root planning, stable periodontitis cases, patients wearing orthodontic appliances, and mild gingivitis. Sandwich ELISA (Enzyme-Linked Immunosorbent Assay) kits were used to assess serum interleukin-6, and Immunoglobulin G (My BioSource-USA). This technique is utilized to detect antibodies against a specific antigen by strong antibody-antigen interactions, and the C-reactive protein was evaluated by the colorimetric technique (Roche-Germany), and the periodontal parameters were evaluated in all individuals by a specific dentist: bleeding on probing (BOP), plaque index (PI), clinical attachment loss (CAL), probing pocket depth (pd). Data were analyzed using SPSS 26, One-Way ANOVA, and post hoc tests, and ROC curve was used to find cutoff values and to evaluate parameters as diagnostic markers at. $P<0.05$ was considered significant based on parameters between periodontitis, gingivitis, and healthy control groups.

Results

The results of the present study in terms of mean age, and body mass index (BMI), as well as periodontal parameters between periodontitis, gingivitis, and healthy control groups are shown in Table 1.

The results of this study include a highly significant elevation ($p<0.001$) in the serum levels of IL-6 in the periodontitis group as compared to the healthy control group, as well as in the gingivitis group compared with the healthy control group, but they found was no significant difference when periodontitis group was compared with gingivitis group as shown in Table 2. The levels of CRP show a highly significant elevation ($p<0.001$) between periodontitis groups and the healthy control group, but show no significant difference between the gingivitis group and the healthy control group, also between periodontitis group and the gingivitis group as shown in Table 2. Levels of IgG showed a highly significant elevation ($p<0.001$) between the periodontitis group and the healthy control group, as well as between the gingivitis group and the healthy control group, and showing a highly significant decrease between periodontitis group and the gingivitis group as shown in Table 2.

Table 1. Classification and mean ages, BMI, and periodontal parameters of the study groups

Variable	Periodontitis Mean±SD	Gingivitis Mean±SD	Healthy (control) Mean±SD	p-value*
Age (years)	35.8±9.06	31.8±7.01	32.13±7.21	0.95
BMI (Kg/m^2)	25.01±4.07	25.00± 3.32	23.63±2.02	0.17
BOP**	30.15±11.68	37.07±10.93	4.99±2.00	0.001
PLI***	2.50±0.68	1.50±0.50	0.88±0. 30	0.001
CAL****	5.45±0.90	-	-	0.001
PPD*****	4.83±0.84	-	-	0.001

*ANOVA -test, **Bleeding on Probing, ***Plaque Index, ****Clinical Attachment Loss, *****Probing Pocket Depth.

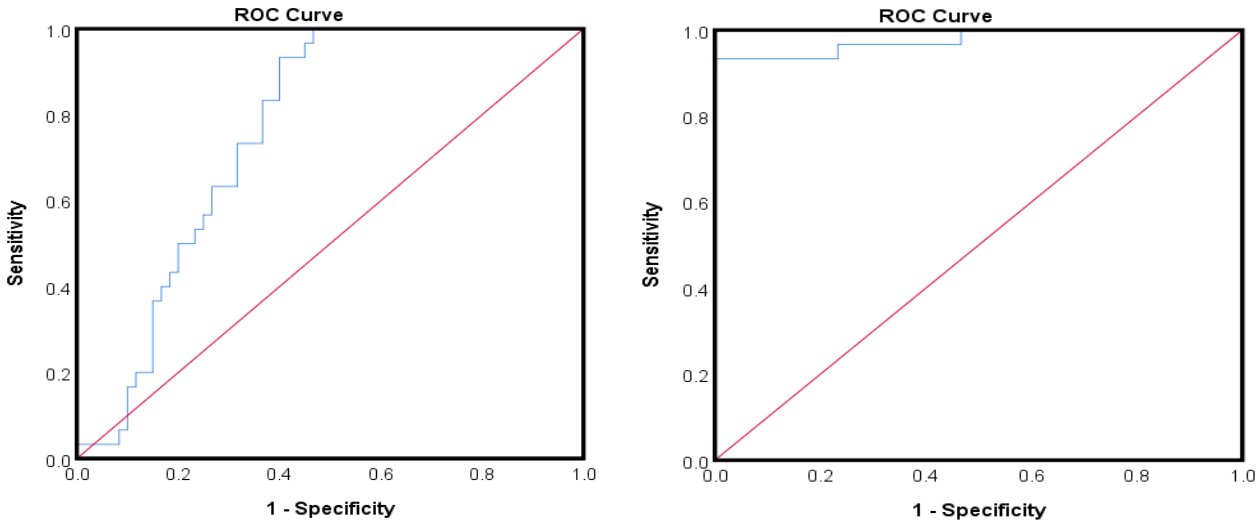
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Table 2. Comparison of the mean difference in the serum levels of IL-6, CRP, and IgG measured in patients and controls

Variable	Healthy (control) Mean±SD	Periodontitis Mean±SD	Gingivitis Mean±SD	p-value* (Comparison between periodontitis with healthy controls)	p-value* (Comparison between gingivitis with healthy controls)	p-value* (Comparison between periodontitis with gingivitis)
IL-6** (pg/mL)	11.38±1.11[a]	17.39±1.50[b]	17.14±2.21[b]	0.001	0.001	0.84
CRP***(ng/mL)	2.38±1.13[a]	5.44±3.77[b]	3.58±3.48[a]	0.001	0.31	0.05
IgG**** (ng/mL)	9.48±1.08[a]	12.38±2.07[b]	15.01±2.38[c]	0.001	0.001	0.001

*ANOVA -test, **Interleukin-6, ***C-reactive protein, ****Immunoglobulin G.

The receiving operating characteristic (ROC) curve was used to investigate the possibility of using IL-6 serum levels to diagnose periodontal disease, and the findings show that the area under the curve (AUC) value in the periodontitis group was 0.761, with a 95%-confidence interval (CI) ranging from 0.664 to 0.857 at ($p<0.001$), and the cut off value was determined at maximum sensitivity and specificity (93.3% and 60%, respectively), and the findings show that the area under the curve (AUC) value in the gingivitis group was 0.977. The 95% CI ranged from 0.941 to 1.000 at ($p<0.001$), and the cut off value was established at maximal sensitivity and specificity (93.3% and 100%), as shown in Figure 1.



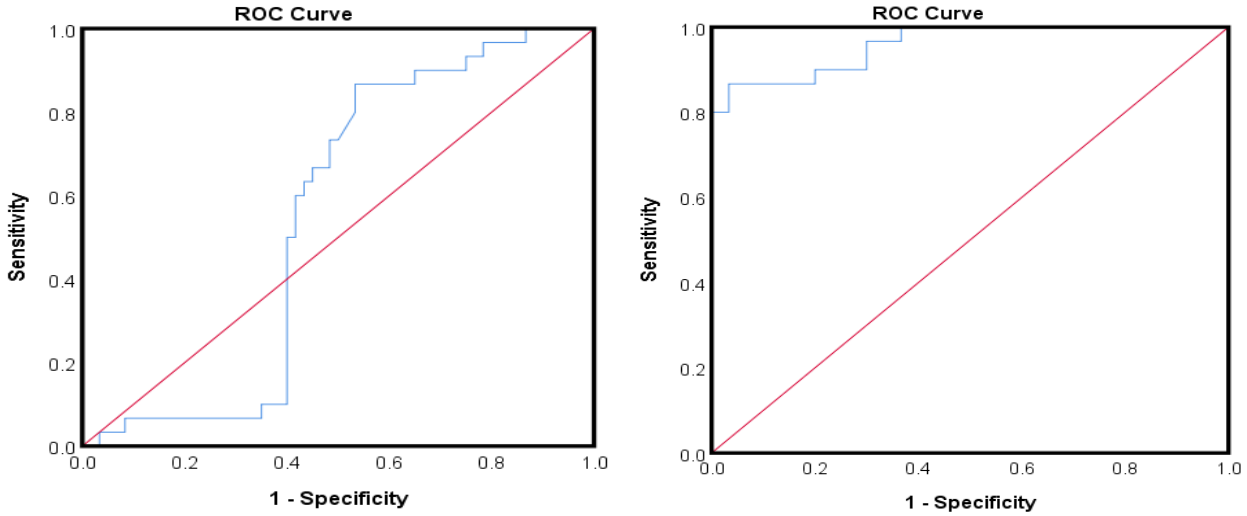
(a) (b)

Figure 1. ROC curve for levels of IL-6, (a) ROC of IL-6 in the periodontitis group, (b) ROC of IL-6 in the gingivitis group

The receiving operating characteristic (ROC) curve was used to investigate the possibility of using IgG levels in the serum to diagnose periodontal disease, and the findings show that in the periodontitis group, the area under the curve (AUC) value was 0.549, 95% CI ranged from 0.430 to 0.669 at ($p=0.446$), and cut off value was 10.4035ng/mL calculated at maximum sensitivity and specificity (86.7% and 46.7%

[DOI: 10.22088/jbums.25.1.409]

respectively), and based on results in the gingivitis group, the area under the curve (AUC) value was 0.959, 95% CI ranged from 0.915 to1.000 at ($p<0.001$), and cut off value was 11.4935ng/mL calculated at maximum sensitivity and specificity (86.7% and 96.7%), as shown in Figure 2.



(a) (b)

Figure 2. ROC curve for levels of IgG, (a) ROC of IgG in the periodontitis group, (b) ROC of IgG in the gingivitis group

Discussion

The current study showed that IL-6, CRP, and IgG levels were significantly elevated in patients with periodontal disease compared to the healthy control group, and the periodontal parameters were significantly elevated in patients with periodontal disease compared to the healthy control group. Therefore, we note from these results that when any inflammation happens, even if it is a minor inflammation, this inflammation will increase inflammatory mediators, and lead to destruction of the tissues of the periodontium.

Al-Taweel et al. showed that the serum levels of IL-6 are significantly higher in chronic periodontitis (CP) with coronary heart disease (CHD) compared to those with chronic periodontitis (CP) without CHD, and this study adds that periodontitis causes systemic inflammation. Cytokine levels in periodontitis patients may be a valuable screening tool for identifying people at higher risk of cardiovascular events (23). Delange et al. compared the levels of IL-6 with mild, moderate and severe periodontitis, and found that severe periodontitis had higher levels of IL-6 (24), in which IL6 levels were linked to inflammatory cases (3).

This study showed a significant connection between periodontitis and serum CRP levels, and found high levels in periodontitis patients (25). A study conducted by Da Venezia et al. found no significant difference in serum level of CRP between gingivitis, periodontitis, and the healthy control group, but showed higher CRP levels in GCF with gingivitis and periodontitis, and found a strong relationship between periodontal disease and CRP levels (26). Several studies showed that CRP serum levels were greater in periodontitis and gingivitis patients compared to healthy controls, indicating the relation and increase with periodontal inflammation (27-29), in which CRP levels were linked to inflammatory cases (3).

[DOI: 10.22088/jbums.25.1.409]

This investigation, which included healthy, gingivitis, and periodontitis, despite differences in the subgingival microbiota, showed no significant differences in plasma antibody levels between patients with healthy control group, gingivitis, and early periodontitis (30). The results of this study showed differences between gingivitis, periodontitis, and healthy control group in IgG serum levels (31). Gadekar et al. found higher levels of mean IgG in plasma and saliva with chronic periodontitis compared to healthy control (32), and the recommendation was that the use of saliva and serum antibody titers for detecting patients with chronic periodontitis could be helpful (32).

Periodontal disease is a multifactorial disease with many contributing factors such as smoking, diet, systemic diseases, and so on. One of the strengths of the present study was the exclusion of these points from this study. Studies in this field with these exclusions are limited, and one of the limitations of the present study is the small sample size. Because the disease is complex and different according to different sampling methodologies, disease diagnosis and measurement methods, and periodontal disease criteria, with the exclusions in this study, it has become difficult to find suitable samples for the study and the study will need longer time to complete.

In this study, the periodontitis group had elevated levels of IL-6, CRP, and IgG in sera in comparison with the healthy control group. The gingivitis group also had highly elevated levels of IL-6, CRP, and IgG in sera. This indicates the stimulation of immune cells to secrete inflammatory mediators, and this result can be used as a diagnostic biomarker for periodontal diseases. Moreover, the study's cutoff values can be applied to estimate the health case or periodontal disease in patients, and these findings are useful in monitoring the progression of periodontal disease, and the transition from gingivitis to periodontitis, and high levels of inflammatory mediators are considered as risk factors for other diseases.

Acknowledgment

We would like to thank Assistant Professor Firas Al-Taweel and Zainab J Raheem from the staff of the Baghdad University/College of Dentistry.

[DOI: 10.22088/jbums.25.1.409]

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[DOI: 10.22088/jbums.25.1.409]