



# Assessment of Serum Interleukin-1 $\beta$ and Interleukin-6 Levels in Patients with Chronic Periodontitis and Coronary Heart Disease

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## Abstract

**Objective** The aim of this study was to assess whether serum cytokine levels correlate with clinical periodontal parameters in health or disease.

**Materials and Methods** Male subjects (40–60 years) with CP ( $n = 30$ ), CP + CHD ( $n = 30$ ), and healthy controls ( $n = 20$ ) had plaque index (PLI), gingival index (GI), bleeding on probing, probing pocket depth (PPD), and clinical attachment level (CAL) evaluated. Serum IL-1 $\beta$  and IL-6 levels were quantified using enzyme-linked immunosorbent assay.

**Results** PLI, GI, PPD, and CAL were significantly higher in patients with CP + CHD compared to those with CP. Serum levels of IL-1 $\beta$  and IL-6 were also significantly higher in CP + CHD compared to those with CP, with both groups also being significantly higher than controls. There was a strong correlation between IL-1 $\beta$  and PPD and CAL and between IL-6 and GI and CAL in the CP group and between IL-6 and GI and PPD in the CP + CHD group.

**Conclusion** The results provide further evidence that periodontitis triggers systemic inflammation. Cytokine levels in patients with periodontitis may represent a useful screening tool to identify those at greater risk of cardiovascular events.

## Keywords

- ▶ interleukin-1 $\beta$
- ▶ interleukin-6
- ▶ periodontitis
- ▶ coronary heart disease

## Introduction

Coronary artery disease arises as an outcome of hardening and lumen reduction of the arteries that supply the heart due to atherosclerotic plaque buildup.<sup>1</sup> It is characterized by local and systemic host responses, in which cells such as

B and T lymphocytes and macrophages have an important role in the pathogenesis of this disease by releasing of cytokines and other inflammatory mediators.<sup>2</sup> For many years, the coronary heart disease (CHD) is recognized as one of the most common causes of mortality and morbidity.<sup>3</sup>

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Chronic periodontitis (CP) is a destructive inflammatory disease affecting the supporting tissues of the tooth including the periodontal ligament and alveolar bone.<sup>4</sup> A large body of work has been focused to the relationship between CHD and CP. These diseases not only share several common risk factors such as smoking, but also share potential mechanistic links through the presence of chronic inflammation.<sup>5</sup>

A large number of studies have indicated the presence of a positive correlation of clinical signs and inflammatory changes in CP with atherosclerosis and CHD. CP may amplify systemic inflammatory mediator levels as a consequence of persistent infection which may affect other organs and systems, including the vascular endothelium, thus potentially contributing to inflammation-associated atherosclerotic disease.<sup>6</sup> In serologic studies, high titers of antibodies to periodontal bacteria are found in patients with atherosclerosis and CHD disease, and some viable bacteria can be isolated from atherosclerotic plaques.<sup>7</sup>

Through their effects on inflammation and the immune system, interleukin-1 $\beta$  (IL-1 $\beta$ ) and interleukin-6 (IL-6) are considered important mediators of inflammation in both periodontitis and atherosclerosis.<sup>8</sup> In periodontal tissues, IL-1 $\beta$  can stimulate bone resorption,<sup>9</sup> neutrophil and monocyte migration from the vasculature into the periodontal tissue,<sup>10</sup> and expression of proatherogenic members of the tumor necrosis factor super family.<sup>11</sup> IL-6 may also contribute to the relationship between CP and CHD through stimulation of monocyte differentiation into osteoclasts resulting in bone destruction<sup>12</sup> and exacerbation atherosclerotic plaque development and destabilization through a variety of mechanisms such as the release of other proinflammatory cytokines, activation of metalloproteinases (MMPs), and increased acute phase protein secretion.<sup>13</sup>

The aim of this study was to investigate the relationship between serum levels of IL-1 $\beta$  and IL-6 with periodontitis and CHD. We have shown that clinical disease parameters are further raised in periodontitis patients with CHD and that serum IL-1 $\beta$  and IL-6 levels represent potential markers of this process. The presence of these interrelated diseases must be taken into consideration as guidelines suggest patients with moderate to severe periodontitis are notified of the increased risk of cardiovascular disease, which may necessitate further evaluation.<sup>14</sup>

## Materials and Methods

Eighty male subjects aged between 40 and 60 years were recruited to this study from the Cardiology Clinic of the Baghdad Teaching Hospital and Periodontics Department of the College of Dentistry, University of Baghdad, Baghdad, Iraq. This study was conducted after the approval was granted by the relevant scientific committee (Ref. 122 at 08/11/2015). All the individuals were informed about the purpose of this study and consented to participate in this study. The subjects were divided into study and control groups. Thirty patients were examined and shown to possess CP and CHD (CP + CHD) diagnosed using the catheterization technique

and were receiving clopidogrel medication (Plavix/75 mg). A further 30 patients were examined and shown to have CP as they had equal or less than 30% sites with pocket depths  $\geq 4$  mm with clinical attachment loss of 1 to 2 mm or greater in accordance to the worldwide classification system for periodontal disease (PD).<sup>15</sup>

The control group consisted of 20 patients with no CHD and clinically healthy periodontium with gingival index (GI) scores  $< 0.5$ , no pockets, or clinical attachment loss. However, certain exclusion criteria were applied including the presence of any chronic systemic diseases such as diabetes mellitus, smoking, those using medication such as antimicrobial and anti-inflammatory drugs, or having any periodontal treatment within the last 3 months.

Clinical periodontal examination was performed using a Michigan O periodontal probe on four surfaces (mesial, buccal/labial, distal, and lingual/palatal) of all teeth except third molars. All subjects had at least 20 teeth. The collected clinical data consisted of plaque index (PLI) system,<sup>16</sup> GI system,<sup>17</sup> bleeding on probing (BOP),<sup>18</sup> probing pocket depth (PPD), and clinical attachment level (CAL).<sup>19</sup>

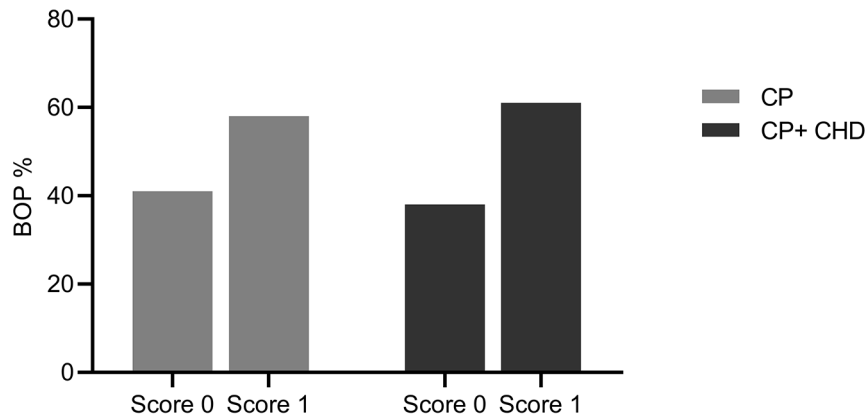
Following examination of the clinical periodontal parameters, 5 mL venous blood was collected from study and control groups. After centrifugation, serum samples were kept frozen at  $-40^{\circ}\text{C}$  and analyzed for IL-1 $\beta$  and IL-6 using enzyme-linked immunosorbent assay (Bioassay Technology Laboratory, Shanghai Korain Biotech Co.).

Data were processed and analyzed for both descriptive and inferential analyses using SPSS (version 23, IBM, United States) and Microsoft Excel (version 2010, Microsoft Corporation, United States). The statistical analysis for the determination of serum IL-1 $\beta$  and IL-6 was assessed using *t*-test, analysis of variance test followed by Tukey's post hoc test, and Pearson's coefficient of correlation.

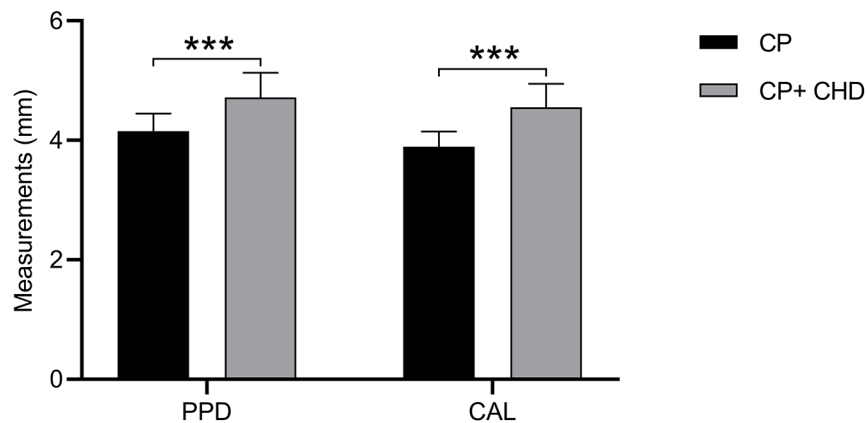
## Results

The results revealed that mean values of PLI, GI, PPD, and CAL were significantly higher in the CP + CHD group compared with the CP and control groups. The percentage of score 1 BOP sites was significantly higher in the CP + CHD group than the CP group. The statistical analyses of clinical periodontal parameters for all groups are summarized in ► **Figs. 1 and 2** and ► **Table 1**.

The mean serum levels of IL-1 $\beta$  and IL-6 were significantly higher in the CP + CHD group followed by the CP group then the control group ( $p < 0.001$ ) (► **Fig. 3A and B**). The correlation analysis shows that there was a weak positive correlation between BOP with serum IL-1 $\beta$  levels in the CP group, while there were moderately significant positive correlations with both PLI and GI ( $p < 0.05$ ), and a significant positive correlation with PPD and CAL ( $p < 0.01$ ). For the CP + CHD group, there were weak positive correlations between serum IL-1 $\beta$  levels with PLI and PPD, while highly significant positive correlations were observed with GI, BOP, and CAL ( $p < 0.05$ ). In the CP group there was moderate positive correlation between serum IL-6 levels and PLI, BOP, and PPD



**Fig. 1** Percentage of bleeding on probing (BOP) in patients with chronic periodontitis (CP) and coronary heart disease patients affected by periodontitis (CP-CHD).



**Fig. 2** Mean probing pocket depth (PPD) and clinical attachment level (CAL) in patients with chronic periodontitis (CP) and coronary heart disease patients affected by periodontitis (CP-CHD). \*\*\*Indicates statistically significant differences at  $p \leq 0.001$ .

**Table 1** Mean plaque (PLI) and gingival index (GI) scores are significantly higher in association with chronic periodontitis (CP) and coronary heart disease patients affected by periodontitis (CP-CHD) when compared with the control group (Con)

Indices	Groups	Mean $\pm$ SD	Comparisons	$p$ -Value*
PLI	Control	0.50 $\pm$ 0.112	Con vs. CP	< 0.001
	CP	1.69 $\pm$ 0.125	Con vs. CHD	< 0.001
	CP + CHD	1.78 $\pm$ 0.136	CP vs. CP + CHD	< 0.05
GI	Control	0.57 $\pm$ 0.119	Con vs. CP	< 0.001
	CP	1.74 $\pm$ 0.121	Con vs. CP + CHD	< 0.001
	CP + CHD	1.83 $\pm$ 0.131	CP vs. CP + CHD	< 0.05

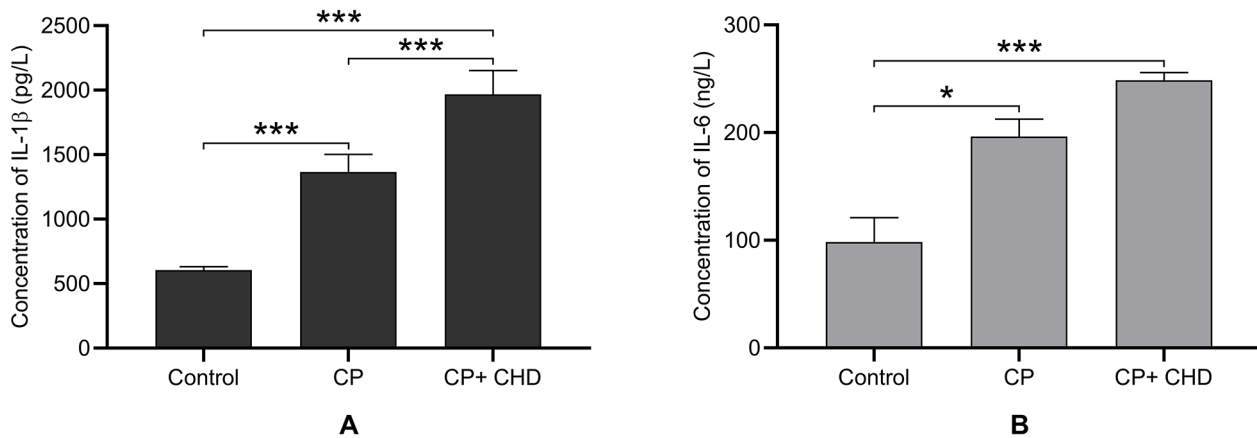
Abbreviation: CP, chronic periodontitis; CHD, coronary heart disease; PLI, plaque index; GI, Gingival index; SD, standard deviation.

\*ANOVA test and post hoc.

( $p < 0.05$ ) as well as strong positive correlations with GI and CAL ( $p > 0.01$ ). In the CP + CHD group significant positive correlations were revealed between serum IL-6 levels with GI and PPD ( $p < 0.01$ ), while the correlation was weak with PLI and BOP and moderate with CAL ( $p < 0.05$ ), as shown in **Table 2**.

## Discussion

CP is a multifactorial inflammatory disease characterized by irreversible progressive loss of the tooth supporting tissues. The progression of this disease is influenced by an imbalance between the microbial dental biofilm and the host immune



**Fig. 3** Mean serum concentration values of interleukin (IL)-1 $\beta$  (A) and IL-6 (B) in patients with chronic periodontitis (CP) and coronary heart disease patients affected by periodontitis (CP-CHD) compared with the control group. \* $p < 0.05$ ; \*\*\* $p < 0.001$ .

**Table 2** Serum levels of IL-1 $\beta$  and IL-6 and clinical periodontal parameters in CP + CHD and CP group

Cytokine	Groups	Statistics	PLI	GI	BOP	PPD	CAL
IL-1 $\beta$	CP	Pearson's <i>r</i>	0.44	0.409	0.29	0.638	0.701
		<i>p</i> -value <sup>§</sup>	0.01	0.02	0.01	<0.001	<0.001
		Significance	*	*	NS	****	****
	CP + CHD	Pearson's <i>r</i>	0.36	0.49	0.433	0.378	0.457
		<i>p</i> -value <sup>§</sup>	0.05	0.005	0.01	0.039	0.011
		Significance	NS	**	*	*	*
IL-6	CP	Pearson's <i>r</i>	0.41	0.63	0.42	0.454	0.619
		<i>p</i> -value <sup>§</sup>	0.02	<0.001	0.01	0.01	<0.001
		Significance	*	****	*	*	****
	CP + CHD	Pearson's <i>r</i>	0.34	0.62	0.365	0.645	0.453
		<i>p</i> -value <sup>§</sup>	0.06	<0.001	0.04	<0.001	0.016
		Significance	NS	****	*	****	*

Abbreviations: BOP, bleeding on probing; CAL, clinical attachment level; CHD, coronary heart disease; CP, chronic periodontitis; GI, gingival index; IL, interleukin; NS, not significant; PLI, plaque index; PPD, probing pocket depth.

<sup>§</sup>Chi-square test; \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ ; \*\*\*\* $P \leq 0.0001$ .

response including enhanced levels of cytokines such as IL-1 $\beta$  and IL-6, which can exacerbate periodontal destruction.<sup>2</sup> The increase in the dental biofilm microbiota may increase the susceptibility to various systemic diseases such as cardiovascular disease, mainly through bacteremia and systemic inflammation. The presence of periodontal pathogens in vascular tissue may directly stimulate atheroma formation and maturation.<sup>19</sup>

The mean values of PLI, GI, PPD, and CAL were higher in the CP + CHD group than the CP group, which could be explained as hospitalized patients may neglect oral hygiene measures, thus more gingival inflammation could be seen. Plaque biofilm bacteria secrete a variety of byproducts (toxins, enzymes, and H<sub>2</sub>S) that provoke an inflammatory response which can promote periodontal loss, pocket formation, bone resorption, mobility, and tooth loss.<sup>5,20,21</sup>

The serum levels of IL-1 $\beta$  and IL-6 in this study were higher in the study group with CP and atherosclerotic heart disease than other groups with highly significant differences ( $p \leq 0.01$ ). Similar studies are lacking in the literature, while

some have demonstrated similar findings<sup>22</sup> others have shown no baseline differences in IL-6 levels in patients with periodontitis and CHD.<sup>23</sup> In a study of cardiovascular disease patients, IL-6 levels were higher in those with increased CAL but did not appear to specifically distinguish those with CP.<sup>24</sup>

The positive correlation of serum IL-1 $\beta$  levels with clinical periodontal parameters, in both study groups, suggests that IL-1 $\beta$  levels are a potentially sensitive and reliable marker of chronic inflammation activity and resultant tissue destruction. IL-1 $\beta$  is thought to be important in the pathogenesis of PD<sup>25</sup> possibly due to its ability to cause atheromatous plaque instability through the upregulation of MMPs at the site of plaque formation. It also mediates vascular smooth muscle cell proliferation and migration during inflammation, which represents a key step in the development of atherosclerosis.<sup>26</sup> In periodontal tissues, IL-1 $\beta$  is known to stimulate proliferation of keratinocytes, fibroblasts, and endothelial cells and augments fibroblast synthesis of collagenase, hyaluronate, fibronectin, and prostaglandin E2. In addition, it upregulates

MMP and downregulates tissue inhibitor of MMP production leading to bone resorption, an increase in PPD, and loss of attachment.<sup>27</sup>

Serum IL-6 may be upregulated due to systemic exposure to oral bacteria and lipopolysaccharide that enter the circulation from periodontal pocket, which in turn stimulates the liver to release acute phase proteins including C-reactive protein.<sup>28</sup> In atherosclerosis, IL-6 has proinflammatory and procoagulant effects, thus may contribute to the progression of atherosclerosis. Also, the retention of low-density lipoprotein in the intima of a vessel may undergo oxidative changes and consequently may increase secretion of chemokines and proinflammatory cytokines, such as IL-6.<sup>29</sup>

The positive correlation between IL-6 levels and different periodontal parameters may be due to the dental plaque bacteria and bacterial byproducts inducing release of proinflammatory cytokines which in turn gives rise to increased systemic IL-6 levels. IL-6 is also secreted by osteoblasts which may have an effect on differentiation of monocyte into osteoclasts that have key role in alveolar bone resorption which is the hallmark of periodontitis progression.<sup>8</sup> It has also been revealed that in clinically healthy gingiva, inflammatory mediators such as IL-1 $\beta$  and IL-6 are present at low levels<sup>30</sup> and that nonsurgical treatment of periodontal disease can result in reduced IL-6 levels.<sup>23</sup>

## Conclusion

Patients with both CP and CHD have higher levels of the inflammatory markers IL-1 $\beta$  and IL-6 than those with just periodontitis or control subjects. Cytokine levels also correlated with several clinical periodontal parameters. These results may be used to predict patients with periodontitis that are at increased risk of developing atherosclerotic heart disease, and such correlation may be used as a marker for disease activity. Increase awareness to obtain an optimum standard of periodontal health is necessary to minimize the resultant bacteremia, which may influence the health of susceptible organs including those of the cardiovascular system.

### Authors' Contributions

F.B.H.A.-T is the author guarantor and contributed to the concept, design, intellectual content, literature search, clinical study, data acquisition, analysis and statistics, manuscript preparation, and editing of the study. S.S.S. contributed to the concept, design, intellectual content, clinical study, and data acquisition. O.H.A. contributed to the concept, design, intellectual content, clinical study, and data acquisition. S.A.W. contributed to the concept, design, intellectual content, data analysis, manuscript editing, and review. The manuscript has been read and approved by all authors, the requirements for authorship have been met, and each author believes the manuscript represents honest work.

### Conflict of Interest

The authors confirm that they have no conflicts of interest.

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