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PROGNOSTIC VALUE OF SERUM HIGH SENSITIVITY C-REACTIVE PROTEIN IN PATIENTS WITH PERIODONTITIS AND DIABETES MELLITUS TYPE 2 AFTER NON-SURGICAL PERIODONTAL TREATMENT

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ABSTRACT

Objectives: Our aim in the current study is to evaluate the level of high sensitivity C-reactive protein in serum of patients with periodontitis and type 2 diabetes before and after non-surgical periodontal treatment.

Subjects and methods: 60 individuals in total with stage II grade A generalized periodontitis (n=20) and stage II grade B generalized periodontitis with type 2 diabetes (n=20) along with healthy control subjects (n=20) were enrolled in this study. Serum samples from all participants were obtained. Two serum samples, before & after non-surgical periodontal treatment, were gathered from patients with periodontitis and periodontitis with type 2 diabetes. Only one serum sample was collected from healthy controls. Enzyme linked immunosorbent assay technique (ELISA) was used to measure serum levels of high sensitivity C-reactive protein (hs-CRP).

Results: The highest concentration of serum hs-CRP was detected in patients with periodontitis & type 2 diabetes. This is followed by periodontitis group and then controls subjects. A statistical significant reduction in the hs-CRP serum levels after non-surgical periodontal treatment was detected in periodontitis with diabetes group while the periodontitis group showed similar reduction but without statistical significance. High diagnostic accuracy of serum hs-CRP was revealed by the Receiver Operating Characteristic (ROC) curve analysis, especially when comparing periodontitis patients, with or without diabetes, with the control.

Conclusions: Serum hs-CRP could be utilized as a prognostic marker in patients with periodontitis and type 2 diabetes.

KEYWORDS: Periodontitis, type 2 diabetes, serum high sensitivity C-reactive protein, non-surgical periodontal therapy.

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INTRODUCTION

Periodontitis is an inflammatory disease in which there is damage of the teeth supporting apparatus. Periodontal infection stimulates a cascade of events involving the host innate & adaptive immunity[1,2]. This inflammatory reaction is considered the principle key for periodontal destruction [3]. Diabetes mellitus (DM) is an endocrinal disorder caused by disruption in insulin formation. Consequently a state of hyperglycemia occurs, leading to atypical metabolism of lipid, carbohydrates and protein causing many complications systemically [4]. Periodontitis and DM are chronic disorders with a two-way correlation as they can both stimulate an inflammatory response with subsequent formation of inflammatory mediators & cytokines. These include interleukin-6, interleukin-1, tumor necrosis factor (TNF) & C-reactive protein (CRP) [5, 6]. Several studies showed that DM type 2 is linked to elevated severity & prevalence of periodontal disease. Moreover, current evidence showed that periodontitis is the sixth complication of DM [7].

Acute phase proteins are those proteins whose serum levels are changed due to inflammation by at least 25% [8]. One of them is CRP, a plasma marker of inflammation participating in the systemic reaction to inflammation. It is a non-specific sensitive protein, formed as a result to various types of injury (infection, trauma and hypoxia) [9, 10]. The normal levels of CRP vary from 1.0 to 3.0 mg/L among different populations. Moreover, by ultrasensitive techniques, it can be registered at low levels less than 1.0 mg/L [11]. The high-sensitivity CRP (hs-CRP) can be measured by immune-fluorescent assay or enzyme-linked immunosorbent assay (ELISA) [12]. An increased level of hs-CRP was found in association with various diseases including cardiovascular diseases such as heart strokes and myocardial infarctions [13-15]. It is also associated with DM and periodontal diseases [16, 17]. In fact, CRP was described as a noteworthy risk indicator for the progress of DM [18].

Patients having periodontitis showed higher CRP concentrations when compared to healthy subjects, but only few studies controlled the possible factors that can affect CRP levels [19, 20]. Precisely, for the CRP level to act as an inflammatory non-specific maker in periodontitis, possible CRP associated factors like hypertension, smoking, obesity and chronic inflammation, should be excluded [21]. Consequently, the aim of our investigation is to assess the levels of serum hs-CRP in periodontitis patients, with and without diabetes mellitus type 2, in a trial to analyze the influence of non-surgical periodontal therapy upon its concentration.

SUBJECTS AND METHODS

This trial had been registered in ClinicalTrials.gov (NCT03908606).

Study population

This study involved 60 subjects categorized as follows; 20 patients diagnosed with stage II grade A generalized periodontitis (group I), 9 females and 11 males with a mean age of 45.1 ± 4.48. 20 patients diagnosed with stage II grade B generalized periodontitis in addition to type 2 diabetes mellitus (group II), 9 females and 11 males with a mean age of 43.5 ± 5.46. Periodontitis diagnosis was based on having >30% of the sites with clinical attachment level (CAL) 3-4 mm according to the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions [22], and 20 periodontally and systemically healthy individuals acting as a control group (group III) ,10 females and 10 males with mean age 42.9 ± 5.11.

Diabetic patients were comprised in this study following the criteria of American Diabetes Association [23]. Type 2 diabetes is defined as untreated hyperglycemia with level of glycated hemoglobin (HbA1c) more than 6.5%. All included individuals were gathered from the diagnostic center, Faculty of Dentistry, Cairo University in the time interval

from April 2018 to January 2019. According to the modified Cornell Medical Index [24], all participants had no systemic disease other than diabetes in group II.

Inclusion criteria

The included individuals' age range was 30-50 years. Before enrollment in this trial by at least 6 months, all patients did not receive any periodontal therapy and had no history of drug administration that may alter the periodontal condition.

Exclusion criteria

Smoking individuals, pregnant or lactating females, cardiovascular diseases, hypertension, any other systemic or immunologic condition, obesity [25] and oral mucosal lesions were the bases for excluding subjects from the current investigation.

The protocol of this study has been accepted by the Research Ethical Committee and followed the Declaration of Helsinki. Before participation in the study, the individuals received full details about the investigation procedures & signed informed consents.

Performed periodontal procedures

Clinical parameters at six sites (mesiobuccal, distobuccal, midbuccal, mesiolingual, distolingual and midlingual) for every tooth, including gingival index (GI) [26], plaque index (PI) [27], probing depth (PD) [28] and clinical attachment level (CAL) [29] were registered at base-line for all groups and at 8 weeks after treatment for group I and II patients. Single examiner using William's periodontal probe performed the periodontal examination for all subjects.

In the first appointment and after clinical examination, diagnosis and categorization were completed, oral hygiene instructions were given along with chlorhexidine mouthwash prescriptions to be used twice daily for one week for the patients included in group I and II. Full mouth scaling and root planning (SRP) has been carried out under local anesthesia to group I and II patients using ultrasonic scalers (NSK nonoptic ultrasonic scaler, Kanuma-shi, Japan) & Gracey curettes (Lustra Gracey periodontal curettes, Dentsply, Surrey, UK) in 2-4 successive sessions and analgesics were given only if needed. Follow ups were scheduled weekly for each patient to stress and ensure that the patients had adhered to the given instructions. Re-evaluation was done after 8 weeks for periodontal parameters recording and for obtaining the second serum sample from group I and II.

Blood samples collection

Under aseptic conditions, three milliliter blood from all subjects at baseline were collected in ethylene-diaminetetraacetic acid (EDTA) tubes by standard venipuncture at the antecubital fossa and again 8 weeks after non-surgical periodontal treatment for the periodontitis patients with or without type 2 diabetes. Tubes were kept in ice and transported to the biochemistry lab at the Biochemistry Department, Faculty of Medicine, Cairo University, where it was stored at -70°C until all samples were collected. All collected samples were given specific serial number.

Determination of hs-CRP in serum samples

Serum samples were centrifuged for 2 minutes at 10.000 xg and the clarified supernatant was filtered through a 0.45 μm low protein binding membrane, separated into 0.5 ml aliquots and frozen at -80°C until use for quantitation of serum hs-CRP by ELISA. Micro-titer strips coated with anti-CRP antibody were incubated with dilated standard sera and patient samples. During this incubation step CRP was bound specifically to the wells. After removal of the unbound serum proteins by a washing procedure, the antigen-antibody complex in each well was detected with specific peroxidase-conjugated antibodies after removal of the unbound conjugate, the strips were

incubated with a chromogen solution containing tetramethylbenzidin and hydrogen peroxide: a blue color developed in proportion to the amount of immune-complex bound to the wells of the strips. The enzymatic reaction was stopped by the addition of 2 N H_2SO_4 and the absorbance values at 450 nm are determined. A standard curve was obtained by plotting the absorbance values versus the corresponding standard values. The concentration of CRP in patient samples was determined by interpolation from the standard curve.

Statistical analysis

All data obtained have been statistically analyzed to compare the means of patient and control groups using Tukey's contrasts for multiple comparisons of means. All statistical work was done using R statistical software (version 3.3.3) [30] using R Commander Package (version 2.5) [31,32].

Receiver Operating Characteristic (ROC) curve has been made to clarify the cut-off values of serum hs-CRP for distinction between different groups. MedCalc Version 11.3 for Windows (MedCalc Software bvba) was used to perform ROC curve analysis.

Sample size calculation:

The primary outcome used for power analysis was the correlation between serum hs-CRP and pocket depth in periodontitis patients with diabetes mellitus. According to **Priyanka et al.,** [33] results, the correlation coefficient was 0.426. Using alpha (α) level of (5%) & Beta (β) level of (20%) at a power of 80%; the anticipated sample size was 40 subjects in total. To compensate for a dropout rate of 25% in addition to 15% for the use of non-parametric tests; sample size was increased to 56 subjects then rounded to a total of 60 subjects allowing for division into three groups (20 individuals in each group). Calculation of sample size has been carried out by G*Power Version 3.1.9.2.

RESULTS

All demographic data of included subjects are shown in table (1). There was no statistical significance found when age was concerned among all the included groups.

Comparing the serum levels of hs-CRP, before treatment, revealed that the highest value falls in group II with statistical significance compared to group I and control group. In addition, periodontitis group showed increased level when compared to control group with statistical significance (Table 2). As regards to serum hs-CRP level after treatment, comparing group I and group II together a statistical significance was registered with the higher hs-CRP level still exists within group II (Table 2).

Serum level of hs-CRP, when compared before & after treatment in group II, showed a decreased value of the mean after treatment with statistical significance. The mean of hs-CRP in group I, after treatment, was also decreased with statistical significant difference. The clinical parameters, represented in GI, PI, PD and CAL, were improved significantly after treatment in both groups compared to the values observed before SRP. Moreover, when the HbA1C was measured in group II after treatment, it was found to be reduced than before treatment with statistical significance (Table 2).

Differentiation between group I & II (Table 3 and Figure 1A) At cut-off value of 7.2μg/mL; hs-CRP showed 72.5% diagnostic accuracy for differentiation between both groups.

Differentiation between group II & III (Table 3 and Figure 1B) At cut-off value of 3.35μg/mL; hs-CRP showed 95% diagnostic accuracy for differentiation between Periodontitis with type 2 Diabetes and Control groups.

Differentiation between group I & III (Table 3 and Figure 1C) At cut-off value of 2.29μg/mL; hsCRP showed 85% diagnostic accuracy for differentiation between Periodontitis and Control groups.

TABLE (1): Demographic statistics of studied groups {mean ± standard deviation (SD)}

	Group I (n = 20)	**Group II (n = 20)**	**Group III (n = 20)**
Age (Years)			
Mean ± SD	45.1±4.48[a]	43.5±5.46[a]	42.9±5.11a
Range	37-51	34-52	35-51
Gender [n (%)]			
Male	11/20 (55%)	11/20 (55%)	10/20 (50%)
Female	9/20 (45%)	9/20 (45%)	10/20 (50%)

In the same row, different superscripts means statistical significant difference

TABLE (2): Comparison between clinical periodontal parameters & values of serum hs-CRP (μg/mL) in all studied groups (mean ± standard deviation)

Clinical parameters	Group I		Group II		Group III
	Before SRP	**After SRP**	**Before SRP**	**After SRP**	
PI	1.51 ± 0.21[a]	0.37 ± 0.15[b]	1.81 ± 0.52[a]	0.83 ± 0.32[b]	0.4 ± 0.08
GI	1.37 ± 0.17[a]	0.39 ± 0.21[b]	1.96 ± 0.48[a]	0.91 ± 0.41[b]	0.3±0.12
PD (mm)	3.58 ± 0.39[a]	2.42 ± 0.39[b]	4.03 ± 0.56[a]	3.21 ± 0.37[b]	1.9 ± 0.41
CAL (mm)	4.02 ± 0.72[a]	3.13 ± 0.74[b]	4.45 ± 0.56[a]	3.53 ± 0.44[b]	--
HbA1c (%)	**-**	**-**	8.03[a]	6.41[b]	**--**
hs-CRP (μg/ml)	6.40 ±2.97[a]*	4.66±2.12[b]	10.84±4.52*[a]	7.46±2.89[b]	1.766± 0.809*

Same Letters in the same group is not statistically significant; * statistically significant at P< 0.05 compared to each other and to control subjects.

TABLE (3) Cut-off value, sensitivity, specificity, predictive values, diagnostic accuracy, Area Under the ROC curve (AUC) & 95% confidence interval (CI) of the (AUC) for hs-CRP to differentiate between different groups

Differentiation	Cut-off value	Sensitivity %	Specificity %	+PV %	-PV %	Diagnostic accuracy %	AUC	95% CI
Periodontitis with DM / Periodontitis	7.2	60	85	80	68	72.5	0.740	0.577-0.866
Periodontitis with DM / control	3.35	90	100	100	90	95	0.958	0.842-0.996
Periodontitis /control	2.29	85	85	85	85	85	0.841	0.691-0.937

+PV: Positive Predictive Value, -PV: Negative Predictive Value

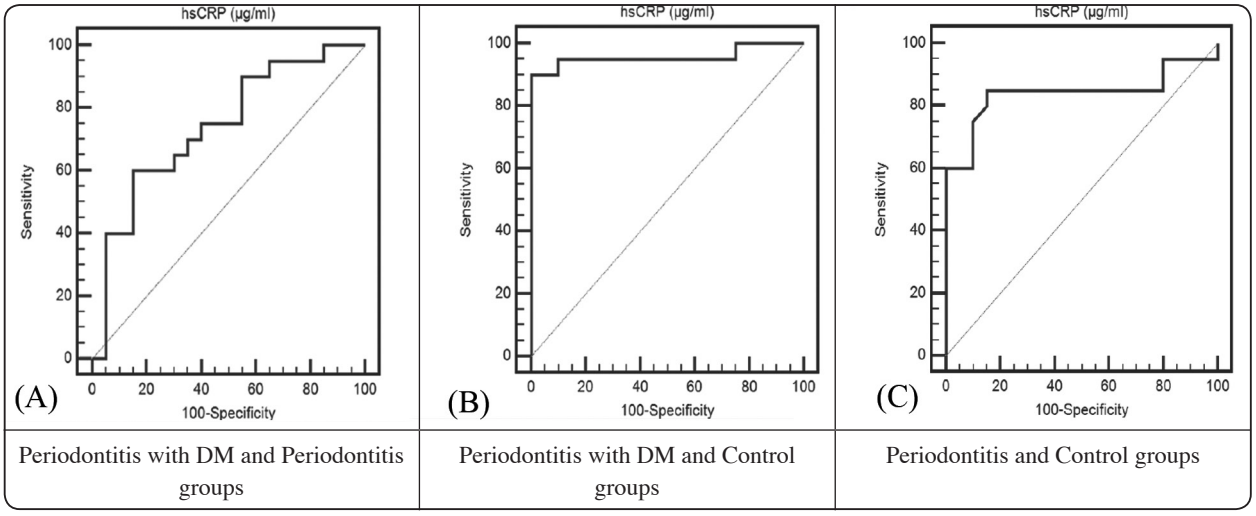

Fig. (1) ROC curves of hs-CRP to differentiate between different study groups

DISCUSSION

Maintenance of oral and dental health has been shown recently to be an essential element to enhance life quality and to prevent the worsening of many systemic disorders [34]. In the current study, we were aiming to investigate hs-CRP serum level as it represents one of the supreme sensitive biomakers utilized to assess the inflammatory condition of a subject that might be a risk factor for other systemic disease, especially in relation to two linked inflammatory diseases like periodontitis and diabetes mellitus type 2.

Our results showed that periodontitis patients presented increased level of serum hs-CRP when compared to periodontally healthy subjects, suggesting that increased level of hs-CRP is associated with periodontal status. It is hypothesized that periodontitis, as other inflammatory diseases, may be responsible for this difference in hs-CRP levels. Our results are in accordance to earlier studies that have reported higher levels of serum CRP in periodontitis patients in comparison to control healthy subjects [19, 21, 35-38]. In contrast to our results, no difference was demonstrated in CRP levels of periodontally healthy subjects & patients with periodontitis in other studies [39, 40], these differences in results may be attributed to the different inclusion criteria of enrolled subjects.

Periodontitis and diabetes are known to be two linked diseases where aggravation of periodontal inflammation can be a result of defective metabolic control in diabetes via the advanced glycation end-products (AGEs) effect [41].AGEs stimulates the release of proinflammatory cytokines as IL-1β in hyperglycemia, inducing low-grade inflammation that has a prime role in development of insulin resistance, the target of type 2 DM [42]. Additionally it is one of the destructive mediators in periodontitis [43]. Likewise, endotoxins secreted by periodontal pathogens could increase secretion of proinflammatory mediators including CRP, IL-6 & tumor necrosis factor alpha [44, 45]. This may be the explanation for the results here in this study where the highest values of hs-CRP in serum have been registered among the diabetic patients having periodontitis in comparison to periodontitis patients or the healthy volunteers.

The present study results are in accordance with results of a study carried out by **Priyanka et al.**[33] who found that hs-CRP serum concentrations among their groups was the highest in diabetic patients with periodontitis followed by periodontitis

group & the least in healthy controls. In the same line, **Mohan et al.** [46] found higher levels of serum hs-CRP in diabetic patients with periodontitis compared to periodontitis group without diabetes at baseline.

Concerning the levels of hs-CRP before and after non-surgical periodontal treatment, the present results are consistent with previous studies which demonstrated decrease in its levels after non-surgical periodontal treatment [21, 33, 37, 47, 48] In contrast to our results, **Escobar et al.**[49] revealed that CRP levels in serum were not statistically significantly different when comparing periodontitis & controls before & following non-surgical periodontal modality. This may be due to post-operative follow up period that was only seven days in their research in contrast to postoperatively 8 weeks interval in our study. In addition, they measured serum CRP while here in this investigation we measured serum hs-CRP which could be detected at much lower levels than the CRP.

Relatively reduced CRP levels & other acute-phase molecules already exist within serum but their concentration could be impressively elevated within 72 hours in reaction to infection & tissue damage. The presence of IL-1β, TNF-α & CRP had been connected to many bacterial infections like periodontitis [50]. However, if periodontal therapy, represented by non-surgical periodontal therapy as the main option for periodontitis management, was effectively performed it could diminish the levels of these mediators [49]. These facts were supported by the findings of the present investigation where the serum hs-CRP levels were reduced after non-surgical treatment in both the periodontitis and the periodontitis with diabetes groups, which could prove that serum hs-CRP might be utilized as a prognostic marker for periodontitis patients with or without diabetes.

Moreover, in the herein investigation, the reduction in serum hs-CRP in diabetic group was accompanied by statistically significant decrease in HbA1c level after treatment. This highlights the concept that periodontal inflammation when reduced and controlled could help improving the diabetic metabolic status.

Furthermore, the highest values of hs-CRP recorded in the periodontitis with diabetes group in comparison to the group of periodontitis alone, indicates the role of local inflammatory condition in worsening the systemic inflammation of diabetes.

The performed ROC curve analysis in this investigation showed high diagnostic accuracy of serum hs-CRP between each two groups with the highest accuracy registered in the periodontitis and diabetes group with the control subjects. This is followed by the periodontitis group with the control group. To the authors' knowledge, this study is the first to perform ROC curve analysis for hs-CRP along with diabetes & periodontitis.

The current study tried to exclude many of the factors that might influence the serum level of hs-CRP like obesity, smoking, cardiovascular diseases, hypertension or other inflammatory diseases in order to narrow the cause of elevated hs-CRP observed to periodontal disease and diabetes mellitus alone without any other contributing element. Furthermore, the present study adds evidence to the facts relating periodontitis & diabetes mellitus to each other and to the increased levels of hs-CRP in comparison to healthy controls. Based upon observations of the current study, we can conclude that serum hs-CRP level might be utilized as a diagnostic and/or prognostic marker for periodontitis with and without diabetes mellitus.

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