Circulation levels of YKL-40 and Hs-CRP in stage 3 Molar-incisor pattern of periodontitis

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Abstract
Aim: To evaluate the novel acute phase protein YKL-40 and high-sensitive C-reactive protein (Hs-CRP) in serum of patients with molar-incisor pattern of periodontitis (Stage 3, Grade C).

Material and Methods: Twenty seven patients with molar-incisor pattern of periodontitis and 27 periodontally healthy individuals were included into the study. Serum levels of YKL-40 and Hs-CRP were measured by enzyme-linked immunosorbent assay.

Results: Serum YKL-40 levels were significantly increased in molar-incisor pattern of periodontitis patients compared to healthy controls (P<0.05). Similarly, significantly higher serum Hs-CRP levels were found in the patients with molar-incisor pattern of periodontitis than those of healthy ones (P<0.05). There were positive correlations between serum YKL-40 and Hs-CRP and BOP in healthy control group (P<0.05 for all). Moreover, serum YKL-40 showed positively strong relationships with Hs-CRP and also clinical periodontal parameters (CAL and BOP) in the periodontitis group (P<0.001 for all).

Conclusion: The present data suggest that YKL-40 might be a candidate biomarker for the diagnosis of localized to molar-incisor pattern of periodontitis and may also have the potential to predict the future risk of systemic diseases for this distinct form of periodontitis.

Keywords: Acute-phase proteins; C-reactive protein; human; periodontal diseases; serum; YKL 40 protein

INTRODUCTION

Periodontitis is an inflammatory disease of tooth supporting hard and soft tissues leading to progressive destruction of connective tissue attachment and alveolar bone and finally result in tooth loss (1). A distinct form of periodontitis which is recognized as localized aggressive periodontitis according to the 1999 classification of periodontal diseases, is localized to the first molars and incisors characterized by early onset and rapid progression. It has also familial aggregation and involves systemically healthy individuals differently from the common forms of periodontitis (2-4). To date, numerous of studies revealed that clinical, epidemiological, genetic, microbiological and immunologic features of localized aggressive periodontitis are different from the common forms of periodontitis; and the disease is named as molar-incisor pattern of periodontitis in the new classification of periodontal diseases in 2017 (4-7).

Periodontal tissue destruction is a consequence of unbalanced immune response of the body to the subgingival microbial biofilm. Molar-incisor pattern of periodontitis shows a localized rapid and severe inflammatory reaction by an infiltration of immune cells into the gingival tissue (8). Previous researches reported abnormal host immune response in the disease that commonly includes stimulation of toll-like receptors (TLRs) which activates the expression of inflammatory cytokines and other molecules (8,9). Most of the studies have demonstrated that the pathogenesis of the disease is related to hyper-responsiveness of polymorphonuclear neutrophils (PMNs). “This hyper-inflammatory response” was shown to be resulted in elevated levels of inflammatory mediators such as proinflammatory cytokines and acute phase reactants (APRs) in the circulation of patients (10-14).

APRs, are the earliest and highly complex response of the body to various local or systemic infections or injuries (15,16). The primary host reaction to infection is critical for the integrity of host defense mechanism and acute phase response has a pivotal role in host defense, accordingly (15,16). This systemic response consists of the synthesis...
and the secretion of the acute phase proteins and levels of these proteins in circulation are altered in inflammatory conditions (16).

C-reactive protein (CRP) is an acute phase reactant that plays a key role in innate immune response and considered as a biomarker of systemic inflammation in various inflammatory diseases including periodontal diseases (13,17,18). Clinical studies have demonstrated a relationship between periodontitis and elevated CRP levels (12,18-20). In addition, recent researches have shown that patients with aggressive form of periodontitis had significant elevations in serum CRP levels than individuals with healthy periodontium (13,14,18,21,22).

An emerging biomarker of inflammation YKL-40, also known as chitinase 3-like 1 protein (CHI3L1), is an acute phase protein with a 40-kDa molecular weight and the glycoprotein is mainly produced by activated neutrophils and macrophages in inflamed tissues (23). Its plasma concentration increases reversibly by more than 25% in patients with inflammatory conditions (24). Moreover, YKL-40 has an established role in angiogenesis, cell migration and differentiation extracellular matrix remodelling and endothelial dysfunction (25). There is an increasing body of evidence that YKL-40 is associated with a variety of inflammatory diseases consisting of rheumatoid arthritis, osteoarthritis, atherosclerosis, cardiovascular diseases and its serum levels reported to have a potential to reflect the degree of inflammation (23-27). It is also important to note that YKL-40 is secreted by locally activated macrophages and neutrophils while CRP is a primarily systemic inflammation marker secreted by hepatocytes (25). Therefore, YKL-40 could serve as a specific marker of tissue inflammation because of its local production. Very few studies conducted on YKL-40 in inflammatory periodontal diseases and these studies have shown elevated levels of YKL-40 in gingival crevicular fluid (28-30) and serum (28) in chronic periodontitis and also gingivitis patients (28). However, no study has been focused on YKL-40 levels in molar-incisor pattern of periodontitis which is the specific and the rapidly progressing form of periodontitis characterized by severe destruction of periodontal attachment at an early age.

Periodontal inflammation seems to be resulted in activation of host immune inflammatory response by the entry of periodontopathogens to the circulation. It has also been reported that localized inflammation could influence the systemic levels of inflammatory mediators (31). Accordingly, changes in systemic circulation can be found in patients with molar incisor pattern of periodontitis patients due to rapidly progressing inflammation of periodontal tissues and hyper-inflammatory host response. At this point, a reliable biomarker is needed to measure the disease activity and also the systemic contribution of the localized periodontal destruction. Therefore, in the present study we aimed to investigate for the first time, serum YKL-40 and high-sensitive CRP (Hs-CRP) levels in stage 3 molar-incisor pattern of periodontitis patients and their correlation with clinical periodontal parameters.

MATERIAL and METHODS

Study Population and Clinical Periodontal Examination
Fifty-four systemically healthy and non-smoker participants with age range 23 to 32 years (25 males and 29 females) enrolled to the present study from the Department of Periodontology, Faculty of Dentistry, Giresun Bulent Ecevit University, Giresun, Turkey between February and November of 2019. The study was approved by the Ethics Committee of Giresun University, Giresun, Turkey with Protocol number: KAEK-75. The study protocol was explained and written informed consent was provided in accordance with the Helsinki Declaration from all individuals before the enrollment into the study.

Medical and dental histories of all participants were evaluated, and the patients were excluded if they had any systemic disorder, a history of smoking, current pregnancy or lactation, received any periodontal treatment in the past 6 months or used antibiotic, anti-inflammatory drugs or any other drugs within the past 6 months.

The full mouth periodontal clinical and radiographic examination has been performed for all participants. Clinical periodontal assessment included the measurements of probing depth (PD), clinical attachment level (CAL), gingival index (GI) (32), plaque index (PI) (33) and bleeding on probing (BOP) (34). Missing teeth were also recorded. All these measurements were recorded at six sites around each tooth (except third molars) with a manual periodontal probe (Hu-Friedy, Williams probe, Chicago, IL) by a pre-calibrated examiner. For calibration of the investigator, 10 individuals were selected randomly prior to study commencement and examined on two separate occasions, 48 hours apart. The investigator’s measurements were considered sufficiently reproducible if those taken at baseline and at 48 hours differed by no more than 10% at the millimeter level.

According to their periodontal conditions, participants were classified as healthy controls (n=27) and stage 3 molar-incisor pattern of periodontitis (n=27). Diagnoses were based on the criteria stated in the consensus report of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions (4,7). Healthy controls (Group 1) consisted of the individuals having BOP < 10% and PD ≤ 3 mm without clinical attachment loss or radiographic sign of alveolar bone destruction. The inclusion criteria for stage 3 molar-incisor pattern of periodontitis patients (Group 2) were interdental CAL ≥ 5 mm and PD ≥ 6 mm on at least two permanent teeth localized in the first molar and incisor teeth, and no more than two teeth other than first molars and incisors. They showed no tooth loss of more than 2 teeth and all were under 35 years of age. Familial aggregation was also evaluated by asking the participants if they had any family member with a history of periodontitis. Additionally, periodontitis grade of patients was calculated by radiographic bone loss/age to determine the progression rate of disease, and all were grade C.
**Serum Sampling**

To obtain serum samples, 5mL of venous blood were drawn from the antecubital vein by a standard venipuncture method. Serum was isolated by centrifugation at 1,500 x g for 15 minutes at 4°C and then immediately frozen and stored at −80ºC until further analysis.

**Determination of YKL-40 and Hs-CRP in Serum**

Serum YKL-40 levels were measured using commercial enzyme-linked immunosorbent assay (ELISA) kit (Human YKL-40/CHI3L1 kit, Sunred Biological Technology, Shanghai, China). Analysis was performed according to the instructions provided by the manufacturer. The concentrations of YKL-40 were then determined by comparing the optic density of the samples to the standard curve. The results were expressed as nanograms per milliliters and the minimum detection limit of YKL-40 was 2 ng/mL.

The concentrations of Hs-CRP in the serum were measured using the CRP latex immunoturbidimetric assay kit (Roche Diagnostics GmbH, Mannheim, Germany). The enzymatic reactions were quantified in a Roche Hitachi Cobas C501 automatic analyser. The levels of Hs-CRP were expressed as milligrams per deciliters and the lower detection limit of Hs-CRP was 0.1 mg/dL.

**Statistical Analysis**

Statistical power calculations were performed and 27 participants were required for each group to detect the differences between groups at 0.85 effect size level with a power of 80% at the P<0.05 level (22).

The distribution of clinical and biochemical data were determined by Shapiro-Wilk normality test. Statistical analysis was performed by non-parametric tests and continuous variables are presented as median and minimum-maximum since the data were not normally distributed. Comparisons of all continuous variables between groups were assessed with Mann-Whitney U test. Sex distribution between groups was tested with chi-square analysis. The Spearman rank correlation test was used to detect the correlations of biochemical parameters with CAL and BOP, and also with each other. All statistical analyses were performed using a commercially available software program (SPSS Version 19.0, Chicago, IL, USA) and significance level was taken as P<0.05 statistically.

**RESULTS**

**Demographic Characteristics and Clinical Periodontal Findings**

The characteristics of the participants are shown in Table 1. There were no significant differences between groups regarding age and sex distribution (P>0.05).

<table>
<thead>
<tr>
<th></th>
<th>Sex* (male:female)</th>
<th>Age* (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>12 : 15</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>(23-32)</td>
<td>(23-32)</td>
</tr>
<tr>
<td>Group 2</td>
<td>13 : 14</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>(23-32)</td>
<td>(23-32)</td>
</tr>
</tbody>
</table>

Data are expressed as the median (min-max)

*No significant difference between groups (P>0.05).

Full mouth clinical periodontal findings of the study groups are outlined in Table 2. All clinical periodontal parameters (PD, CAL, GI, PI and BOP) were significantly worse in molar-incisor pattern of periodontitis group compared to healthy control group (P<0.05). Number of teeth was lower in periodontitis group than healthy ones (P<0.05).

<table>
<thead>
<tr>
<th></th>
<th>PD* (mm)</th>
<th>CAL* (mm)</th>
<th>GI*</th>
<th>PI*</th>
<th>BOP* (%)</th>
<th>Number of Teeth*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>1.72 (1.33 to 2.16)</td>
<td>1.72 (1.33 to 2.16)</td>
<td>0.04</td>
<td>0.33 (0.12 to 0.87)</td>
<td>0.59 (0 to 3.57)</td>
<td>28</td>
</tr>
<tr>
<td>Group 2</td>
<td>2.98 (2.58 to 3.88)</td>
<td>3.62 (3.01 to 4.27)</td>
<td>1.39</td>
<td>1.21 (0.74 to 1.98)</td>
<td>35.3 (17.42 to 64.17)</td>
<td>27</td>
</tr>
</tbody>
</table>

PD: Probing Depth; CAL: Clinical Attachment Level; GI: Gingival Index; PI: Plaque Index; BOP: Bleeding on Probing

Data are expressed as the median (min-max) *Statistically significant difference between groups (P<0.05). Mann–Whitney U test

**Biochemical Findings**

Serum levels of YKL-40 and Hs-CRP are presented in Figure 1 and Figure 2, respectively. YKL-40 concentrations in serum of Group 2 patients were significantly higher than Group 1 (P<0.05). Similarly, significantly higher serum Hs-CRP levels were detected in Group 2 compared to healthy control group (P<0.05).

**Correlations**

Table 3 summarizes the correlations in Group 1, Group 2 and also in all groups. In healthy control group, positive correlations were observed between serum YKL-40 and Hs-CRP and BOP (P<0.05 for all). For periodontitis group, serum YKL-40 was positively correlated with Hs-CRP levels in serum (P<0.001). Moreover, both biomarker levels in serum showed a strong positive relationship with BOP and CAL in Group 2 (P<0.001 for all). Strong positive correlations were also found between YKL-40 and Hs-CRP; CAL and BOP; and between Hs-CRP and CAL and BOP (P<0.001 for all) in all group correlation analysis.
DISCUSSION

This is the first study reporting serum YKL-40 levels in molar-incisor pattern of periodontitis and also investigating its association with Hs-CRP levels and clinical parameters. A specific form of periodontal diseases, molar-incisor pattern of periodontitis affects young individuals and is localized to molar-incisor teeth with rapid severe periodontal destruction that can lead to tooth loss in early ages of life. When this destruction pattern and its unique and different clinical and immunological features are considered, an effective biomarker is desirable in clinical periodontics to reflect the host response and to screen disease progression. It is well-known that the acute phase response is the primary response of the host defense mechanism and represents a systemic counterpart to the localized inflammatory response (16,17). Assessment of the circulation levels of acute phase proteins can provide information about the current state of disease activity and can predict the risk of systemic disorders, additionally. YKL-40 is a novel acute phase protein that can be a promising mediator in periodontal diagnosis (23-25,28). Hs-CRP is also an acute phase protein and has been reported to reflect host inflammatory response in periodontitis over a past few decades (12-14,18,22,35). Accordingly, we have focused on the concentrations of serum YKL-40 and also Hs-CRP in the patients with the specific and the distinct form of periodontitis.

The findings of the current study indicated that patients with molar-incisor pattern of periodontitis (stage 3, grade C) had elevated levels of YKL-40 in serum compared to periodontally healthy individuals. Hs-CRP levels were statistically significant from Group 1 (Mann–Whitney U test) Data are presented as box and whisker plots. The median value is indicated by the line within the box plot. The box extends from the 25th to the 75th percentiles. Whiskers extend to show the highest and lowest values.

Table 3. The Spearman rank correlation (rho) among groups with respect to YKL-40, Hs-CRP, CAL and BOP

<table>
<thead>
<tr>
<th></th>
<th>YKL-40 to Hs-CRP</th>
<th>YKL-40 to CAL</th>
<th>YKL-40 to BOP</th>
<th>Hs-CRP to CAL</th>
<th>Hs-CRP to BOP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.876*</td>
<td>0.090</td>
<td>0.468*</td>
<td>0.140</td>
<td>0.490*</td>
</tr>
<tr>
<td>P</td>
<td>0.000*</td>
<td>0.656</td>
<td>0.014*</td>
<td>0.487</td>
<td>0.009*</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.854*</td>
<td>0.828*</td>
<td>0.857*</td>
<td>0.834*</td>
<td>0.996*</td>
</tr>
<tr>
<td>P</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td>All groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.897*</td>
<td>0.861*</td>
<td>0.916*</td>
<td>0.758*</td>
<td>0.856*</td>
</tr>
<tr>
<td>P</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

CAL: Clinical Attachment Level; BOP: Bleeding On Probing; Hs-CRP: High Sensitive C-Reactive Protein

*Statistically significant (P<0.05)
†Statistically significant (P<0.001)
also higher in serum of these patients than healthy ones. Furthermore, YKL-40 and Hs-CRP were correlated with each other and with BOP scores for both groups of the study, separately. In all groups and in periodontitis group, both YKL-40 and Hs-CRP were positively related with CAL. Previous studies reported that YKL-40 is a new biomarker of inflammation in various systemic inflammatory diseases consisting of cardiovascular diseases, rheumatoid arthritis, diabetes mellitus, atherosclerosis and inflammatory pulmonary diseases (24-27,36). In patients with early rheumatoid arthritis, serum YKL-40 levels were increased and its levels were found to be correlated with clinical markers of disease activity (27). Although there are many clinical evidence showing that YKL-40 could be a novel indicator of such systemic inflammatory conditions, very few studies conducted on periodontal diseases (28-30). The researchers observed elevated levels of YKL-40 in both gingival crevicular fluid and serum of chronic periodontitis and gingivitis patients (28). A recent study in chronic periodontitis with/without diabetes mellitus indicated that gingival crevicular fluid YKL-40 levels were higher both in diabetic and non-diabetic periodontitis and significantly correlated with PD and GI scores (29). Similarly, in the other recent research investigating gingival crevicular fluid YKL-40 in chronic periodontitis patients with/without diabetes mellitus, there was a significant reduction in the glycoprotein levels after periodontal treatment (30). The current study findings are in accordance with these previous researches, although there are differences in the periodontal disease type and ages of included patients and also in sample sizes. It is also important to note that no further data of YKL-40 regarding periodontal conditions to compare and to our knowledge our data is the first demonstrating higher YKL-40 levels in the patients with molar-incisor pattern of periodontitis.

Hs-CRP concentrations were also determined in our study and found to be elevated in serum of these patients than those of healthy controls. CRP is synthesized by the liver primarily and its circulating levels have been documented to be related with periodontal diseases in a variety of studies (12-14,18,19-22). According to the 1999 periodontal diseases and conditions classification, limited studies evaluating aggressive periodontitis patients reported elevated levels of CRP in both localized and generalized form of the disease compared to healthy individuals (13,14). In addition, Mysak et al. stated that serum CRP levels showed significant decreases after nonsurgical treatment in localized aggressive periodontitis patients (14). Our results confirm these previous findings revealing that the aggressive form of periodontitis is significantly associated with serum CRP concentrations (13,14,18,21).

Considering the data of YKL-40 and Hs-CRP in this study, the aggressive nature of this pattern of periodontitis seems to be adequate to affect the acute phase response despite the localized nature of its occurrence. It is also important to note that Hs-CRP and YKL-40 have different origin. Hs-CRP is secreted by mainly hepatocytes in the liver, while YKL-40 has been shown to be secreted by locally activated macrophages and neutrophils at the site of inflammation (15,25). In inflammatory conditions, YKL-40 concentrations exhibit a more rapid peak or a more rapid decrease because of its local production. Unlike YKL-40, CRP is a marker of systemic inflammation and its levels increases or decreases slowly (25,36). Hence, YKL-40 might better reflect the site of inflammation. Besides that, elevated circulation levels of Hs-CRP in addition to YKL-40 and also the observed correlation between YKL-40 and Hs-CRP, clearly showed that the inflammation localized to the molars and incisors in the mouth was such destructive and rapidly progressive to produce a systemic inflammatory response. Significant correlations of both biomarkers with clinical periodontal parameters reinforced this view. It should also be taken into consideration that increased levels of YKL-40 and also Hs-CRP due to the periodontitis may be of particular concern in younger individuals, as represented by the molar-incisor pattern of periodontitis patients, where they may contribute to early and more rapid systemic disorders in susceptible individuals.

One likely limitation of this study may be the lack of follow-up data of the patients; this could give more information about the role of YKL-40 in healing or progression process.

**CONCLUSION**

In conclusion, our study presented here is the first to provide evidence that YKL-40 could have a role in the pathogenesis of molar-incisor pattern of periodontitis. Based on the data of the present study, serum YKL-40 has been suggested to be a promising indicator of both localized inflammation of periodontal tissues and the risk of systemic conditions in this distinct form of periodontitis patients. Additionally, elevation of the circulating Hs-CRP such as that seen in these patients may supplement the systemic inflammation and this finding adds to the evidence on the involvement of Hs-CRP in the pathogenesis of periodontal diseases. Longitudinal studies on YKL-40 levels supported by microbiological and genetic analyzes are needed to better define its role in molar-incisor pattern of periodontitis.

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**Competing interests:** The authors declare that they have no competing interest.

**Financial Disclosure:** There are no financial supports.

**Ethical approval:** The study was approved by the Ethics Committee of Giresun University, Giresun, Turkey with Protocol number: KAEK-75.
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