ACTA ODONTOLOGICA SCANDINAVICA
2019, VOL. 77, NO. 2, 142–149
https://doi.org/10.1080/00016357.2018.1522447

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ORIGINAL ARTICLE

Association between C-reactive protein, neutrophil to lymphocyte ratio and the burden of apical periodontitis: a case-control study

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ABSTRACT

Objective: Endodontic originated chronic apical periodontitis (AP) is an inflammatory disease of periapical tissue. High-sensitivity C-reactive protein (hsCRP) as an inflammatory marker and hemogram indexes provide valuable information to clinicians for diagnosis, screening and follow-up of various diseases. The aim of this study was to investigate AP in terms of its association with hemogram indices and hsCRP levels.

Material and methods: Study includes 104 patients with AP and 40 participants as the control group. 160 teeth were diagnosed as AP through digital radiographic images and scored with respect to Periapical Index (PAI) scoring. Afterwards, patients were categorized into 3 grades in accordance with both the number and the severity of AP. AP grade 0 was considered for the control group with regard to a new scoring system. Patients with only one tooth involved with AP with a PAI score of 3 or 4 were categorized as an AP Grade 1, when a patient had more than one tooth with a PAI score of 3 or 4 he was classified as an AP Grade 2 and a patient with at least one tooth scored as a PAI 5 was rated as an AP Grade 3. Hemograms and hsCRP levels were measured for each individual to establish a correlation with inflammatory markers.

Results: The neutrophil/lymphocyte ratio (NLR) levels of patients with AP Grade 3 were significantly higher than all other AP grades ($p < .05$). hsCRP levels in patients with an AP Grade 2 and 3 were higher than both AP Grade 0 and 1 ($p < .05$).

Conclusions: hsCRP levels of patients were reliable predictive indicators for AP severity in correlation with the new proposed scoring system for AP.

ARTICLE HISTORY
Received 31 May 2018
Revised 5 September 2018
Accepted 6 September 2018

KEYWORDS
Apical periodontitis; endodontics; C-reactive protein; neutrophil-lymphocyte ratio; periapical index

Introduction

Apical periodontitis (AP) is an inflammatory process of the periapical tissues most commonly in response to an endodontic infection, but may also occur in the presence of severe periodontal disease [1,2]. Inflammation as an immune response to the infection within the endodontium underlies the pathogenesis [1–3]. Over time, dissolution of bone tissue due to replacement of periapical bone mineral with inflammatory cells is detected as findings of chronic AP on radiographs [3]. An understanding of the pathogenesis of these lesions at the molecular level is still questionable. Even though microbial factors play a major role in the aetiology of AP, the presence of lymphocytes, macrophages and neutrophils in periapical lesions indicates that the immune system is also involved in the process of pathogenesis [4].

Although periapical lesions are classified as an inflammatory disease, the use of inflammatory markers is rather limited in their assessment. C-reactive protein (CRP), one of the inflammatory markers, is a pentametric molecule in protein structures. It is produced in response to many systemic injuries in the body and is a sensitive but nonspecific response to inflammation. It is released during cell death and functions in the immunological system by binding to certain molecular structures found on pathogenic surfaces [5]. It has been reported that CRP is regulated by cytokines such as interleukin-6 (IL-6), interleukin-1β (IL-1β) and a tumour necrosis factor-α (TNF-α) [6,7]. Various studies have investigated the effects of marginal periodontitis on the release of these proinflammatory cytokines and inflammatory markers such as CRP and they have shown some systemic effects of marginal periodontitis, like its effects on cardiovascular diseases and pregnancy [8–11]. As marginal periodontitis and AP are similar in terms of being infectious diseases with anaerobic gram-negative weighted pathogens [1,4,12], AP has also been considered as a cause for inflammatory changes and the interaction of AP with systemic diseases has begun to be examined by evaluating biochemical parameters [13–17].

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Today, hemogram is used as a routine analysis in almost all patients. Platelet indices, including mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT) and platelet count (PLT), have been reported to provide useful information in many inflammatory, septicemic and fatal diseases. It has been reported that there is an increase in platelet consumption and MPV with acute infection in human studies [18–21]. It is also noted that MPV is prognostically important in the early stage of sepsis [22]. It has been reported that the lymphocyte count and neutrophil-to-lymphocyte ratio changes in chronic infections such as marginal periodontitis and glaucoma [23,24]. Unfortunately, platelet indices have not been studied adequately in chronic endodontic diseases. Although hemogram is not a regular test used in dentistry, its correlation with gingivitis and periodontitis scores is recommended [24]. Use of hemogram indices would provide important information particularly for immunocompromised patients such as those with AIDS or cancer.

The effects of AP on systemic health are unfortunately still not well known. Therefore, the potential risks and the need for treatment of asymptomatic chronic AP are not clear enough for both patients and physicians today. For these purposes, this study aimed to investigate whether endodontic originated chronic AP, a low-intensity chronic inflammatory disease, is associated with hemogram indices (such as MPV, PDW, PCT, NLR) and high-sensitivity C-reactive protein (hsCRP) levels as an inflammatory biomarker in respect to the affected tooth and severity of the lesion.

Materials and methods

This study was conducted in the endodontics department of the institute and included assessment of patients who were diagnosed with chronic apical periodontitis from May 2016 to September 2017. Ethical approval was obtained from the Institutional Ethical Committee, and written consent was obtained from all the patients after explaining in detail the entire research protocol.

Clinical and radiographic evaluation

Medical and dental data were collected from medical records and from the patient's appointments with the dentists. The number of teeth, pocket probing depth (PPD), presence of carious lesions (crown cavities and/or root cavities), residual tooth roots and chronic AP were assessed clinically and with the aid of panoramic radiographs and periapical x-rays. Regarding chronic AP, only when the presence of a radiolucent image was accompanied by clinical evidence of pulp necrosis (darkened tooth, restorations or carious lesions close to the pulp, or altered response to pulp test) and/or radiographic evidence of previous endodontic treatment (intracanal posts and/or fillings), the tooth was considered positive for the presence of a periapical lesion [17]. Teeth were considered positive for the presence of AP when they had a radiolucent image that was larger than the space corresponding to the periodontal ligament space and associated with the apex of the root and the presence of occlusal trauma could be discarded. All teeth present in the oral cavity were radiographed and presence of radiolucent images associated with the periapical region was assessed. Panoramic radiographs in the study were obtained using a digital panoramic unit (Orthopantomograph OP200 D; Instrumentarium Dental, Tuusula, Finland), operating at 70 kVp and 4.9 mA with 14.1 s exposure time in standard mode. For periapical radiographs, the Radiovisiography (RVG; Suni Medical Imaging, San Jose, CA, USA) system was used with an x-ray unit setting of 70 kV, 8 mA (Evostyle EX; New Life Radiology, Italy) [25]. The bisecting angle technique was used to obtain the periapical images.

Table 1. Eligibility criteria.

Inclusion criteria
1. Healthy persons having a negative history of any systemic illness between the age group of 18 and 60 years
2. Teeth that were diagnosed with symptomatic chronic apical periodontitis and confirmed using periapical radiographs and pulp vitality tests
Exclusion criteria
1. Patients who had taken antibiotics and/or anti-inflammatory drugs within the last 6 months
3. Pregnancy or lactation
4. Patient with a history of smoking
5. Presence of marginal periodontitis

In order to be considered for inclusion in the study, patients should have an AP lesion, have a negative history of any systemic illness, not be currently undergoing dental treatment and not have used antibiotics and/or anti-inflammatory drugs in the last six months. The exclusion criteria were the following: pregnancy or lactating at the time of the study; history of smoking; presence of marginal periodontitis (Table 1). Finally, one hundred and forty-four patients were included in the study (104 patients for the study group and 40 patients for the control group, with similar BMIs). All patients included in the study were referred to the biochemistry polyclinic to give blood for hemogram and hsCRP tests.

Study design and laboratory parameters

From radiographic evaluation of the teeth, the presence and severity of AP were assessed with the Periapical Index (PAI) scoring system, which was published by Ørstavik et al. [26]. This scoring system numbers the tooth for AP from 1 to 5 according to radiolucency of the periapex on radiographs. This scoring is as follows: PAI 1: Normal periapical structures, PAI 2: Small changes in bone structures, PAI 3: Changes in bone structure with some mineral loss, PAI 4: Periodontitis with well-defined radiolucent area, and PAI 5: Severe periodontitis with exacerbating features. Teeth scored as PAI 1 and 2 were not included in the study because of the lack of apparent periapical pathology and the study group was composed of teeth scored as PAI 3, 4 and 5. When in doubt the higher score was given and for multirooted teeth, the highest score of each root was assigned as the PAI score of the tooth.

Since the purpose of the study was to examine the systemic effects of AP, while examining the patients with respect to AP, we thought taking into account both the

number of the tooth with AP and the severity of the AP present in the tooth would reflect the patients AP status better. In accordance with this purpose following the PAI scoring system, we categorized patients into 3 grades with respect to AP, considering both the severity and the number of teeth with AP they had. Namely; a patient with only a tooth involved with AP with a PAI score of 3 or 4 were categorized as AP Grade 1, when the patient had more than one tooth with a PAI score of 3 or 4 he was rated as AP Grade 2 and the patient with at least one tooth scored as a PAI 5 was classified as AP Grade 3. The control group was composed of people without any tooth involved with AP and classified as AP grade 0. The demographic characteristics of both the control group and all the patients were recorded, including age, sex, BMI, WC and teeth. Hemograms and hsCRP levels were also measured.

Panoramic radiographs and RVGies used in the study were evaluated by two observers including an experienced endodontist and a dentomaxillofacial radiologist. Radiographs were transferred to the hospital's picture archiving and communication system and examined with 20-inch wide LCD TFT monitors (HP, Houston, Texas, USA) via Extreme Pacs, version 4.3 (Ankara, Turkey) software in a dimly light room. Observers were allowed to adjust the contrast and the density and they used the image processing tools to zoom, for inversion and edge enhancement. A total of 36 panoramic radiographs and up to 40 RVGies, which corresponds to approximately 25% of the radiographs were evaluated each by two observers independently, two times with a three-week interval. The radiographic analysis of AP was evaluated independently two times in 45 days by an experienced endodontist and dentomaxillofacial radiologist. Agreement levels of both specialists yielded a Kappa of 0.932 for AP at the first evaluation and a Kappa of 0.945 for AP the second evaluation.

Hemogram tests were performed using the 'CELL-DYN Sapphire haematology system' hemogram instrument (Abbott Diagnostics, USA). Blood samples for hsCRP testing were centrifuged at 3500 rpm for 10 min. The sera were stored at −80 °C until analyzed. HsCRP levels of the sera brought to room temperature on the study day were also measured with ELISA (Biotec Inc., elx800, USA) using a hsCRP kit (DRG International, Germany), functional sensitivity = 0.1 mg/L, intra-assay precision (expressed as coefficients of variation) at 0.55 mg/L concentration = 7.5% and inter-assay precision at 0.49 mg/L = 4.1%.

Power analysis

In a priori power analysis based on a study comparing C-reactive protein with BMI and smoking behaviour in periodontitis conducted by Gupta et al. [27], we calculated that at least 15 controls and 15 patients were required for our study. In this context, we planned each group to have at least 30 patients to get a stronger result.

Statistical analysis

SPSS 15.0 statistics software and InStat3 GraphPad Software were used for all statistical analyzes. One-way ANOVA for comparison of parametric data and Kruskal–Wallis for nonparametric data comparison were used in independent groups with more than two groups. Pearson correlation analysis for parametric data and Spearman correlation analysis for nonparametric data were used. Fisher's Exact Test was used for relative risk analysis to determine the association between apical periodontitis and the inflammatory marker, hsCRP. The kappa test was used to assess intraobserver and interobserver agreement. The kappa value (k) obtained was interpreted according to the rank [28].

Results

Subject characteristics

A total of 40; 27.78% of the study participants, were women ($n = 28$; 70.00% in the study group and $n = 12$; 30.00% in the control group) and 104; 72.22% were males ($n = 76$; 73.08% in the study group and $n = 28$; 26.92% in the control group). While the average total age was 41 ± 18 years, the mean age of the study group was 40 ± 18 years (male: 41 ± 20 years, female: 39 ± 14 years, $p > .05$) and the mean age of the control group was 42 ± 15 years (male: 40 ± 14 years, female: 46 ± 18 years, $p > .05$). Body mass index (BMI) was 25.2 ± 3.9 kg/m^2 in the patient group and 26.9 ± 4.3 kg/m^2 in the control group, waist circumference (WC) was 91 ± 13 cm in the patient group and 93 ± 12 cm in the control group. For intraobserver and interobserver reliability of PAI scores almost perfect agreement was defined (k = 1.00 for control group and k = 0.96 for PAI scores). Table 2 shows the distribution of parameters in accordance with AP grades of the participants. Except for the number of teeth with AP, no difference was defined between AP grades with respect to other parameters ($p > .05$). Besides, there was a statistically significant variation between other AP grades in terms of the number of teeth with AP ($p < .05$) but between AP Grade 2 and 3 no difference was determined ($p > .05$).

Comparison of groups

There was no difference between the AP grades with regard to haemoglobin, hematocrit, white blood cell (WBC), Neutrophil %, Lymphocyte %, Monocyte %, Eosinophil %, Basophil %, PLT, MPV, PCT and PDW were determined through hemogram analysis ($p > .05$) (Table 2).

When platelet indices were compared in respect of AP grades, no difference was observed in MPV/PLT, PDW/PLT, MPV/PCT, PDW/PCT, MPVxPDW/PLTxPCT and platelet to lymphocyte ratio. However, the neutrophil-to-lymphocyte ratio (NLR) value in AP Grade 3 was significantly higher than the other groups ($p < .05$) (Table 3 and Figure 1).

As the sample size of the control group was lower than one hundred twenty, Robust Method was used according to the recommendations of the Clinical and Laboratory Standard Institute (CLSI C28-A3) guideline [29]. Lower and upper limit of NLR reference interval was found 0.934 (90% CI = 0.865 to 1.067) and 4.361 (90% CI = 3.304 to 5.990), respectively, at 95% confidence intervals.

Table 2. Comparison of the distribution of patients data according to grades of the apical periodontitis (AP).

	AP Grade 0	AP Grade 1	AP Grade 2	AP Grade 3	*p*
n	40 (28%)	43 (30%)	31 (22%)	30 (21%)	–
Age, yr	42 ± 15.4	41 ± 18.3	41 ± 17.1	38 ± 20.5	[a].8016
Gender, male	28 (70%)	30(70%)	21 (68%)	25 (83%)	[b].4997
BMI, kg/m^2	26.9 ± 4.3	25.0 ± 3.8	25.7 ± 4.4	25.1 ± 3.7	[a].1476
WC, cm	93 ± 12.5	90 ± 13,4	92 ± 14.3	92 ± 12.7	[a].6390
RCT, *n*	1.3 ± 1.8	1.3 ± 1.6	2.1 ± 1.8	1.3 ± 1.1	[b].0511
Removable prosthesis, *n*	0.4 ± 0.7	0.1 ± 0.3	0.3 ± 0.6	0.2 ± 0.6	[b].1977
Crown, *n*	2.2 ± 3.4	3.0 ± 5.9	4.7 ± 7.6	2.6 ± 5.0	[b].6982
Composite filling, *n*	0.8 ± 1.0	1.6 ± 1.3	1.6 ± 1.1	1.0 ± 1.1	[b].5374
Amalgam filling, *n*	0.7 ± 0.9	1.7 ± 2.3	1.6 ± 2.1	1.8 ± 2.1	[b].1409
Total teeth in mouth, *n*	26 ± 7.5	27 ± 5.5	25 ± 7.3	27 ± 7.0	[b].5062
Apical Periodontitis, *n*	0.0 ± 0.0	1.0 ± 0.0	2.2 ± 0.5	1.6 ± 0.6	[b]<.0001[c]
WBC, 10^3/mm^3	7.4 ± 1,6	7.4 ± 2.2	7.0 ± 1.6	8.0 ± 2.3	[a].2952
Neutrophil, %	56 ± 7.5	58 ± 6.7	59 ± 6.0	61 ± 9.9	[a].1483
Lymphocyte, %	33 ± 7.3	32 ± 6.0	32 ± 5.1	29 ± 8.6	[a].0959
Monocyte, %	7.1 ± 1.85	7.1 ± 1.8	6.4 ± 1.5	6.4 ± 2.1	[a].1525
Eosinophil, %	2.4 ± 1.1	2.4 ± 1.7	2.1 ± 1.3	2.5 ± 1.6	[b].5365
Basophil, %	0.73 ± 0.38	0.69 ± 0.39	0.73 ± 0.47	0.52 ± 0.29	[b].1137
Hemoglobin, mg/dl	14.7 ± 1,3	14.3 ± 1.4	14.3 ± 1.7	14.5 ± 1.5	[a].6392
Hematocrit, %	43.4 ± 3.7	41 ± 3.9	41 ± 4.9	42 ± 4.4	[a].0741
PLT, 10^3/mm^3	244 ± 58	249 ± 51	229 ± 60	250 ± 72	[a].5825
MPV, fL	7.6 ± 0.8	7.7 ± 1.3	7.8 ± 1.0	7.3 ± 0.8	[a].1320
PCT, %	0.18 ± 0.04	0.18 ± 0.04	0.18 ± 0.04	0.17 ± 0.04	[a].3593
PDW,	15.8 ± 0.6	15.9 ± 1.1	15.8 ± 0.7	15.6 ± 0.4	[a].4623

[a]One-way ANOVA (parametric), [b]Nonparametric ANOVA (Kruskal-Wallis test). If *p*-value obtained by ANOVA is <.05, *p*-values between the subgroups (AP grade 0–1, 0–2, 0–3, 1–2, 1–3 and 2–3) are compared with post-test: [c]Respectively $p < .001$, $p < .001$, $p < .001$, $p < .001$, $p < .05$ and $p > .05$. *n*: number; yr: year; BMI: body mass index; WC: waist circumference; RCT: root canal treatment; WBC: white blood cell; PLT: platelet count; MPV: mean platelet volume; PCT: plateletcrit, PDW: platelet distribution width.

Table 3. Comparison of the hemogram indexes of patients according to grades of the apical periodontitis (AP).

	AP Grade 0	AP Grade 1	AP Grade 2	AP Grade 3	*p*
MPV/PLT[b]10^5	3.30 ± 0.87	3.40 ± 1.05	3.69 ± 1.27	3.19 ± 1.17	[a].3142
PDW/PLT[b]10^5	6.77 ± 1.41	6.91 ± 1.64	7.34 ± 1.87	6.78 ± 2.01	[a].5039
MPV/PCT	42.9 ± 8.8	43.6 ± 9.7	46.1 ± 1.87	44.9 ± 11.8	[a].5701
PDW/PCT	88.7 ± 16.9	89.9 ± 18.5	93.1 ± 17.1	96.5 ± 21.5	[a].3056
MPVxPDW/PLTxPCT	3.02 ± 1.17	3.16 ± 1.56	3.58 ± 1.71	3.25 ± 1.86	[a].5042
PLR	108±41	113 ± 33	106 ± 29	117 ± 37	[a].5824
NLR[b]	1.85 ± 0.80	1.92 ± 0.61	1.91 ± 0.51	2.46 ± 1.34	[a].0165
hsCRP[c], g/dl	1.75 ± 1.23	1.65 ± 0.77	3.31 ± 3.48	6.30 ± 7.59	[a]<.0001

[a]One-way ANOVA (parametric), *p*-values between the groups (AP grade 0–1, 0–2, 0–3, 1–2, 1–3 and 2–3) are compared with post-test. [b, c]Comparison between sub groups is shown in Figure 1. MPV: mean platelet volume, PLT: platelet count, PDW: platelet distribution width, PCT: plateletcrit, PLR: platelet to lymphocyte ratio, NLR: neutrophil-to-lymphocyte ratio, hsCRP: high-sensitivity C-reactive protein.

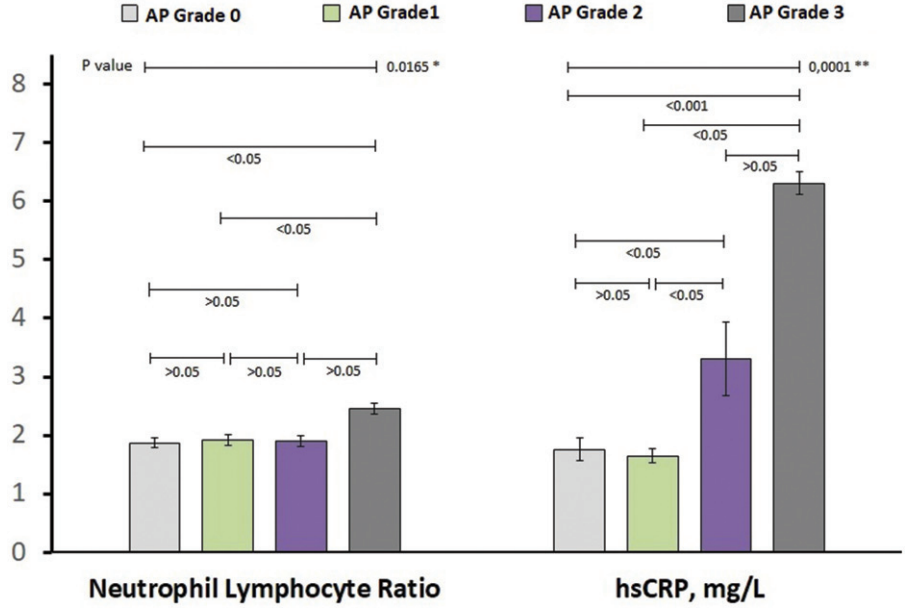

Figure 1. Comparative graph of mean and standard error values of Neutrophil-to-lymphocyte ratio and hsCRP according to grades of apical periodontitis (AP). * One-way ANOVA (parametric), ** Nonparametric ANOVA (Kruskal–Wallis test. If *p*-value obtained by ANOVA is <.05, *p*-values between the groups (AP Grade 0, 1, 2 and 3) are compared with post-test.

While there was no statistical difference between AP Grade 0 and 1 and between AP Grade 2 and 3 in terms of hsCRP levels ($p > .05$); hsCRP levels of AP Grade 2 and 3 were significantly higher than both AP Grade 0 and 1 ($p < .05$) (Table 3 and Figure 1).

Correlational studies

The relationship of AP grades with NLR values and hsCRP levels were investigated, while no significant correlation was determined with NLR values (Spearman $r = 0.177$ $p < .05$), a moderate correlation was observed with hsCRP levels (Spearman $r = 0.443$ $p < .001$). This finding suggests that as the severity of AP increases from AP grade 0 to 3, there is also an increase in hsCRP levels (Figure 2).

The relative risk results

For relative risk analysis, the cut-off value of 2.5 mg/L was adjusted for hsCRP based on hsCRP levels of the control group and research conducted by Osman et al. [30]. According to this, the relative risk for elevated hsCRP was found as 2.067 (95% Confidence Interval: 1.068 to 4.003, *p*: .0195). This means that the risk for hsCRP elevation in patients with AP was two times higher than the control group.

Discussion

AP is an inflammation of the periodontium which mostly originates from infection of tissue in the root canal system and the surrounding dentin [31]. Although it may be acute and painful, sometimes it is chronic and asymptomatic and it is mostly preventable or treatable via root canal therapy. However, treatment may not always be successful in healing or preventing the recurrence of AP [4]. For the evaluation of the presence of chronic AP, progress or healing is possible by estimating the density changes of the periapical bone tissue of the related teeth from radiographs [32]. AP and periodontal infections with different aetiology and pathogenesis have been reported to contain similar pathogens of anaerobic gram-negative bacteria. Elevation of the cytokine level in both is also similar. Some evidence has been established concerning production of these cytokines by inflamed pulp and periapical granulomatous tissue [4,33,34].

In the diagnosis and treatment of chronic AP radiography has an essential role. Both developmental and healing stages are possible to follow through the radiographic appearance of the periapical area. The radiographic diagnosis of AP is confirmed by deviation from the healthy periapical structure appearance. Resorption and remodelling activities of the bone as a response to the inflammation cause changes that are visible on radiographs [31]. In this study, the rare osteitis caused by AP was initially determined on panoramic

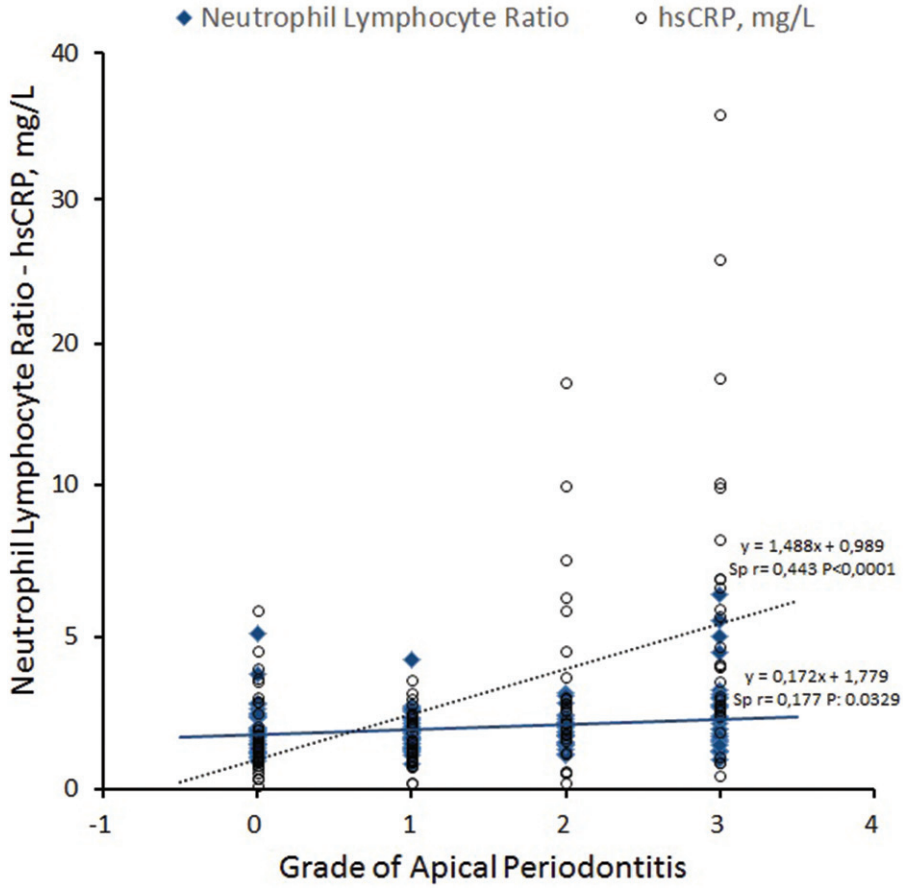

Figure 2. Spearman correlation graph between the grades of apical periodontitis and Neutrophil-to-lymphocyte ratio and hsCRP. HsCRP showed a higher correlation with the AP grades than Neutrophil-to-lymphocyte ratio.

radiograph and then evaluated in detail by the use of RVG technology using the bisecting angle technique. For the purpose of estimating periapical lesion size via periapical radiography, both the bisecting angle and the paralleling technique are appropriate [35]. The bisecting angle technique was preferred in this study because of the convenience in clinic application. RVG allows detailed viewing of the periapical image with reduced radiation dose according to panoramic and conventional radiography with the advantages of offering image processing tools such as; zoom on an image, inversion of the grey scale, edge enhancement and the ability to adjust the contrast and density of the image [36]. In addition, it provides instant image acquisition without the need for developing a bath and offers convenience in image archiving [25].

The PAI system was developed to standardize the scoring of periapical pathologies. PAI assesses the presence and severity of AP according to radiographic appearance [26]. This scoring system was used in varied epidemiological studies and later was well accepted for the evaluation of AP [32,37,38]. In studies investigating the association of AP with inflammatory markers and systemic diseases, it was mostly taken into account whether AP was present or not [14,17,39,40]. Both the number of teeth involved with AP and the effects of the severity of the present AP in teeth are ignored in those studies [14,17,39,40]. When researchers aimed to examine AP in more detail considering both the number and the severity of it, use of more than one system for instant diagnoses using the Modified Total Dental Index and PAI independently were necessary [38]. Because while PAI is being used for defining the severity of the AP, the Total Dental Index [41] takes the number of the lesions into consideration but not the severity and according to our knowledge no other index is available to evaluate both the number and the severity of AP. We have created a new scoring system in this study, which allows assessment of AP together with both the number of affected teeth and the severity of the disease. With this new system, the described AP grades served as data to be used in analysis but contain versatile information. If we had used two independent indexes, we would have had a large number of data which would have been difficult to interpret along with other variables. In this respect, we think this scoring system will be beneficial for studies for the evaluation of AP.

Oral infections produce significant increases in systemic inflammatory responses, manifested by cytokines and acute-phase reactants [6,7]. Acute-phase proteins like CRP may provide important mechanisms to modulate macrophage function since macrophages possess CRP receptors and CRP can potently upregulate proinflammatory cytokine production [42]. Thus, CRP has been shown to induce the synthesis of IL-1α, IL-1β, tumour necrosis factor α and IL-6 in human peripheral blood mononuclear cells and alveolar macrophages, suggesting that one of its physiological roles may be the amplification of inflammatory responses; although CRP probably plays more of an anti-inflammatory role [43].

In the literature, many studies have been published associating marginal periodontitis cases with high serum CRP levels [8–10]. In recent years, studies have focused on whether there is such a relationship between CRP and AP too. The relationship of AP with inflammatory markers and proinflammatory cytokines was examined, Vidal et al. [17], reported high serum levels of CRP in the presence of AP for severely hypertensive patients. In a study by Garrido et al. [15], including the histologic investigation of extracted teeth they found significantly higher CRP levels in the periodontal ligaments of teeth with an apical lesion of endodontic origin when compared with healthy teeth. A few studies focused on the effects of AP on inflammatory markers and showed a high production of inflammatory markers with AP [14,16,44]. On the other hand, in a study examining the relationship between serum CRP levels and the number of teeth with root canal treatment, the number of teeth with root canal treatment was found insignificant for CRP levels [13]. In our study, hsCRP levels were significantly higher in patients with AP Grade 2 and 3 when compared with AP Grade 0 and 1; furthermore, there was a correlation between AP grades and hsCRP levels. In an analysis to display the relationship between AP grades and hsCRP levels, determination of a moderate correlation between these two demonstrates that hsCRP can provide meaningful information with periapical radiographs for an estimation of the severity of AP. The risk for high hsCRP was two times higher in patients with AP with a relative risk analysis result and also supports this finding (hsCRP cutoff: 2.5 mg/L).

The possible association between endodontic variables and smoking and between endodontic variables and periodontal disease are presented [8,9,45,46]. It has been shown that smoking induces a stronger systemic inflammatory reaction, increasing CRP levels in serum and the release of potentially tissue destructive substances such as reactive oxygen species, collagenase, serine proteases and the pro-inflammatory cytokines IL-1 β and TNFα [45,46]. Similarly, the effects of marginal periodontitis on the release of these proinflammatory cytokines and CRP has also been shown [8,9]. Therefore, the impact of periodontal disease and smoking on periapical health also needs to be further investigated.

A recent study reported a positive association between BMI and periodontitis and CRP [27]. For this reason, it is important that the working groups are not different in terms of obesity in order to eliminate the high level of hsCRP, which may be due to obesity. Thus, the difference in hsCRP detected between the groups is due to AP. In this study, there was no difference between groups in terms of BMI and WC, suggesting that the main reason for the increase in hsCRP levels was AP.

Today, hemogram analysis is used for the diagnosis, screening and follow-up of various hematologic, infectious, inflammatory and malign diseases. The results are obtained in a few minutes and are very low cost. In addition, hemogram indices (platelet indices, PLR and NLR) can be easily calculated with this test in a meta-analysis by Atalay et al. [23], the use of hemogram indices with other biological markers of systemic inflammatory response has been recommended for epidemiological studies [24]. Similarly, a recent study showed that an increase in NLR was associated with

marginal periodontitis [47]. The cutoff value of the NLR was reported as 0.52–3.5 ng/ml [29]. However, the reference interval of NLR was 0.934–4.361 ng/ml in the present study. This difference might be explained by our lower sample size and therefore use of Robust test based on the recommendations of CLSI C18-A3 guidelines. In our opinion, further studies with higher sample size might find the similar limits of the values of the NLR [29].

An alteration was detected only in NLR among the hemogram indices in the present study. The NLR value of AP Grade 3 was significantly higher than the others. This was attributed to the presence of PAI 5 scored teeth in AP Grade 3, which is the most severe phase of AP. It is likely that the neutrophil ratio (relative to lymphocytes) has increased in the WBC due to excessive periapical tissue damage and a strong inflammatory response to microbial and nonbacterial irritants diffusing from the necrotic pulp [48]. Therefore, the strong inflammatory response increases NLR. Whereas, there is no significant change in NLR in AP Grade 1 and 2 without severe periapical tissue destruction. The lack of significant correlation between AP grades and NLR supports this as well. As PAI score 5 shows severe apical periodontitis with exacerbating features, it would be good to perform a follow-up study on patients who have PAI 5 scored teeth to assess the association between the PAI score (thus severity of lesion) and biological mechanisms.

Conclusion

In conclusion, hsCRP levels can be used as a useful additional test when evaluating systemic inflammation in patients with AP. In addition, the new proposed scoring system considers both the number of teeth involved with AP, the severity of the involvement, and can be used for epidemiological analyses. Further prospective studies are required to investigate the impact of periodontal disease and smoking on periapical health.

Disclosure statement

No potential conflict of interest was reported by the authors.

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