Impact of fixed orthodontic appliances on blood count and high-sensitivity C-reactive protein levels: A prospective cohort study

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Introduction: The aim was to elucidate the magnitude of alterations in systemic blood counts in healthy patients during the first 14 days after fixed orthodontic appliance placement. **Methods:** This prospective cohort study consecutively included 35 White Caucasian patients starting orthodontic treatment with fixed appliances. The mean age was 24.48 ± 6.68 years. All patients were physically and periodontally healthy. Blood samples were collected at 3 time points: (1) baseline (exactly before the placement of appliances), (2) 5 days after bonding, and (3) 14 days after baseline. Whole blood and erythrocyte sedimentation rates were analyzed in automated hematology and erythrocyte sedimentation rate analyzer. Serum high-sensitivity C-reactive protein levels were measured by the nephelometric method. Standardized sample handling and patient preparation procedures were adopted to reduce preanalytical variability. **Results:** A total of 105 samples were analyzed. All clinical and orthodontic procedures were performed without complications or side effects during the study period. All laboratory procedures were performed per protocol. Significantly lower white blood cell counts were detected 5 days after bracket bonding, compared with baseline ($P < 0.05$). Hemoglobin levels were lower at 14 days than baseline ($P < 0.05$). No other significant shifts or alteration patterns were observed over time. **Conclusions:** Orthodontic fixed appliances led to a limited and transient change in white blood cell counts and hemoglobin levels during the first days after bracket placement. The fluctuation of high-sensitivity C-reactive protein levels was not significant, demonstrating a lack of association between systemic inflammation and orthodontic treatment. (Am J Orthod Dentofacial Orthop 2023;■:■-■)

Orthodontic treatment with fixed appliances remains the treatment for malocclusions that interfere with function and affect facial appearance.[1] Orthodontic tooth movement includes a variety of biological reactions after force application and involves bone resorption, periodontal ligament compression, and bone formation, mainly in the form of a local inflammatory process.[2] Release of locally produced inflammatory mediators in the circulation is, in turn, a mechanism that activates systemic inflammation. This occurs mainly in the liver, where the circulating inflammatory mediators trigger the secretion of acute-phase proteins, resulting in low-grade systemic inflammation, which then can affect the physiological function of tissues and organs. For example, a well-established marker of inflammation is C-reactive protein (CRP), which is released in response to various stimuli and can increase dramatically within 72 hours from tissue trauma or infection.[3] Another marker of systemic inflammation because of infection or injury is the white blood cell count (WBC).[4] In this context, acute changes in the systemic inflammatory status or hemostatic system because of dental treatment, although transient, may be a matter of concern in patients with high risk for cardiovascular events. Indeed, it has been previously

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reported that invasive dental treatment significantly increased the rate of important vascular events within 4 weeks after the intervention, irrespective of traditional risk factors.[5] Furthermore, it has been shown that patients suffering from common respiratory or urinary infections had a significantly increased transient risk for a vascular event (ie, a first or a subsequent myocardial infarction or stroke), the mechanism behind this being the acute increase in systemic inflammation.[6]

Limited data suggested a systemic inflammatory response to orthodontic tooth movement in humans. MacLaine et al[7] reported a trend of increased concentration of high-sensitivity C-reactive protein (hsCRP), interleukin-6, and tumor necrosis factor-α levels from blood samples before initiating orthodontic treatment and at 2, 4, and 6 months of treatment. More recently, a significant elevation was reported in hsCRP, WBC, and neutrophil counts on days 1 and 7 and 3 months after bracket placement, accompanied by a significant decrease in levels of lymphocyte count and sodium levels and a significant decrease in potassium levels on day 1.[8] Although the changes early after initiation of orthodontic therapy are likely because of the local tissue trauma, the late changes reported in these 2 studies may be likely due to a combination of factors (ie, local tissue trauma from the continued orthodontic movement and infection from the suboptimal plaque control), often observed in patients with fixed appliances; nevertheless, the relative contribution of these 2 factors is not known. Indeed, in controlled animal model studies of orthodontic force-induced systemic inflammatory responses, the maximum induction of interleukin-1β and interleukin-6 was observed on day 3 of tooth movement on the compression side, followed by a decrease until day 7, ultimately leading to undetectable levels on day 10[9]; systemic inflammatory monocyte percentages have also been shown to decrease until day 3 post-bracket placement and then recovered later at day 7.[10]

Thus, we hypothesized that in humans, orthodontic treatment with fixed appliances would also impact systemic circulatory markers during the very early time after fixed appliance placement, which then dissipates. To test this hypothesis, this study was designed to elucidate the magnitude of alterations in systemic blood counts in healthy patients, without extensive periodontal inflammation and with a good standard of oral hygiene, during the first 14 days after fixed orthodontic appliance placement.

MATERIAL AND METHODS

This prospective cohort study was approved by the 251 Greek Air Force Hospital's Education, Ethics and Research Committee (approval no. 076/7592/06.05.2015) and was conducted following the guidelines of the Declaration of Helsinki. Informed consent was obtained from all participants before the commencement of the study. This study included 35 (17 males, 18 females; mean age, 24.48 ± 6.68 years) White Caucasian patients, obtained from a pool of patients referred to the Department of Orthodontics of 251 Greek Air Force Hospital, Athens, Greece, for orthodontic treatment needs. The study was performed within the frames of an ongoing prospective controlled study assessing the occurrence of gingival recessions in patients undergoing orthodontic treatment; sample size calculation was also based on this primary outcome. Patients presenting with any acute systemic disease during the observation period were excluded.

Patients were further assessed for eligibility according to inclusion and exclusion criteria. The inclusion criteria were (1) healthy patients aged >18 years, (2) no previous orthodontic or extensive periodontal treatment, (3) need for orthodontic treatment with full fixed appliances, and (4) good oral hygiene. In contrast, the exclusion criteria were (1) pregnant or lactating females, (2) clinical signs of gingival conditions/diseases resulting in swelling of the gingiva (eg, gingivitis), (3) the presence of increased probing depths (eg, >3 mm), indicating possible periodontal inflammatory conditions, (4) intake of medication with any known effect on the gingiva or bone remodeling, and (5) the presence of congenital anomalies or dental structural disorders.

Orthodontic treatment included fixed appliances in the maxilla and mandible (In-Ovation R brackets 0.022-in slot and nickel-titanium wires (Dentsply GAC International, The Hague, Netherlands). All participants received oral health instructions and were informed of the recommended dietary habits immediately after placing the appliances. The initial archwire was 0.014-in with 80 g of force (Sentalloy; Dentsply GAC, Central Islip, NY).

All blood samples were collected in the Department of Orthodontics and Dentofacial Orthopedics at the 251 Greek Air Force Hospital and were analyzed in the Laboratory of Hematology and Biochemistry.

Blood samples were collected at 3 time points: (1) baseline (exactly before the placement of the fixed orthodontic appliances (T0), (2) 5 days after bonding (T1), and (3) 14 days after bonding (T2), as the physiological response to sustained pressure against a tooth takes place in the first 14 days after the initiation of orthodontic treatment.[11] Blood sampling was conducted during the morning hours (8 AM-10 AM) after an overnight fast, and the participants were also advised to avoid vigorous exercise the day before sampling.

Table I. Descriptive statistics of blood count measurements

Measurements	*T0*	*T1*	*T2*
WBC	6.52 ± 1.68	6.26 ± 1.76	6.51 ± 1.66
NEU	3.94 ± 1.31	3.62 ± 1.18	3.77 ± 1.16
NEU%	59.68 ± 8.74	57.59 ± 9.06	57.71 ± 7.67
LYMPH	1.91 ± 0.59	1.96 ± 0.81	2.03 ± 0.72
LYMPH%	29.96 ± 7.99	31.32 ± 8.85	31.43 ± 8.20
MNC	0.48 ± 0.22	0.47 ± 0.17	0.48 ± 0.18
MNC%	7.28 ± 2.34	7.66 ± 1.73	7.48 ± 1.86
EO	0.16 ± 0.11	0.18 ± 0.12	0.18 ± 0.14
EO%	2.60 ± 1.96	2.93 ± 2.14	2.85 ± 2.39
BASO	0.01 ± 0.03	0.01 ± 0.03	0.01 ± 0.03
BASO%	0.48 ± 0.31	0.50 ± 0.19	0.55 ± 0.38
RBC	5.09 ± 0.68	5.01 ± 0.70	5.00 ± 0.62
HGB	13.79 ± 1.48	13.56 ± 1.31	13.54 ± 1.34
Ht	42.11 ± 4.14	41.38 ± 3.73	41.29 ± 3.54
MCV	83.42 ± 8.38	83.42 ± 8.47	83.29 ± 8.56
MCH	27.35 ± 3.26	27.38 ± 3.26	27.37 ± 3.23
MCHC	32.71 ± 0.85	32.77 ± 0.83	32.79 ± 0.79
RDW	13.74 ± 1.20	13.82 ± 1.21	13.88 ± 1.27
PLT	250.65 ± 56.99	256.56 ± 63.35	252.15 ± 64.69
MPV	8.65 ± 0.81	8.62 ± 0.89	8.64 ± 0.86
PDW	16.27 ± 0.43	16.31 ± 0.45	16.34 ± 0.44
PCT	0.21 ± 0.05	0.22 ± 0.05	0.22 ± 0.05
ESR	8.77 ± 9.44	8.97 ± 10.02	7.85 ± 7.14

NEU, neutrophils; *LYMPH*, lymphocytes; *MNC*, mononuclear cells; *EO*, eosinophil count; *BASO*, basophils; *RBC*, red blood cells; *HGB*, hemoglobin; *Ht*, hematocrit; *MCV*, mean corpuscular volume; *MCH*, mean corpuscular hemoglobin; *MCHC*, mean corpuscular hemoglobin concentration; *RDW*, red cell distribution width; *PLT*, platelets; *MPV*, mean platelet volume; *PDW*, platelet distribution width; *PCT*, procalcitonin test.

Standardized sample handling and patient preparation procedures were adopted to reduce preanalytical variability. Participants had peripheral blood collected from the median cubital vein at the antecubital fossa into an ethylenediaminetetraacetic acid-coated vacutainer. Blood samples were analyzed in an automated hematology analyzer (Unicel DxH 800; Beckman Coulter Diagnostics, Nyon, Switzerland) on the same day. Specifically, blood samples were centrifuged within 1 hour of collection to prevent hemolysis at 1500 g of force over 10 minutes to obtain supernatant, which was then divided into aliquots. Specimens were then directly transported and analyzed without refrigeration. The erythrocyte sedimentation rate (ESR) was measured with an automatic ESR analyzer (Monitor 100, Electa Lab, Italy). Serum hsCRP levels were measured by nephelometric method (CardioPhase hsCRP/BN II, Siemens Healthcare Diagnostics, Issaquah, Wash). Within-run and total percentage coefficient of variance values were 4.6 and 5.8 for serum samples, respectively. The analytical range was 0.16-10.00 mg/L.

Table II. Results of the Skillings-Mack test

Measurements	*Statistic*	P *value*	*Ties*
WBC	7.70	0.016	
NEU	3.167	0.1920	
NEU%	1.843	0.3979	No ties
LYMPH	1.921	0.3190	
LYMPH%	0.379	0.8274	No ties
MNC	0.018	0.9850	
MNC%	3.482	0.1753	No ties
EO	0.189	0.8200	
EO%	0.863	0.6500	
BASO	0.045	0.7140	
BASO%	2.206	0.3020	
RBC	4.561	0.1060	
HGB	5.864	0.0370	
Ht	2.470	0.3080	
MCV	0.318	0.8610	
MCH	0.614	0.7120	
MCHC	0.291	0.8560	
RDW	0.354	0.8020	
PLT	2.397	0.2800	
MPV	0.864	0.6180	
PDW	0.641	0.6880	
PCT	0.635	0.7330	
ESR	1.268	0.4740	
CRP	0.889	0.6411	No ties

NEU, neutrophils; *LYMPH*, lymphocytes; *MNC*, mononuclear cells; *EO*, eosinophil count; *BASO*, basophils; *RBC*, red blood cells; *HGB*, hemoglobin; *Ht*, hematocrit; *MCV*, mean corpuscular volume; *MCH*, mean corpuscular hemoglobin; *MCHC*, mean corpuscular hemoglobin concentration; *RDW*, red cell distribution width; *PLT*, platelets; *MPV*, mean platelet volume; *PDW*, platelet distribution width; *PCT*, procalcitonin test.

Statistical analysis

All variables of interest were tested for normality with the Shapiro-Wilk test at any given time. The normality assumption was violated for the vast majority of the tested variables. Therefore, all the respective repeated measurements were analyzed with the nonparametric Skilling-Mack test. The Wilcoxon rank sum test of equality in distribution between genders was applied. The level of statistical significance was set to $\alpha = 0.05$. All statistical analyses were performed using Stata software (version 13; StataCorp LP, College Station, TX).

RESULTS

The fixed orthodontic appliances were placed uneventfully, and no side effect was recorded throughout the study. All participants reported good general health during the trial, and a complete set of blood samples was taken from all 35 patients (ie, 105 samples were analyzed). Significantly lower WBC counts were detected at T1 ($P < 0.05$) than T0. Hemoglobin levels were lower at T2 than T0 ($P < 0.05$). Although there were some

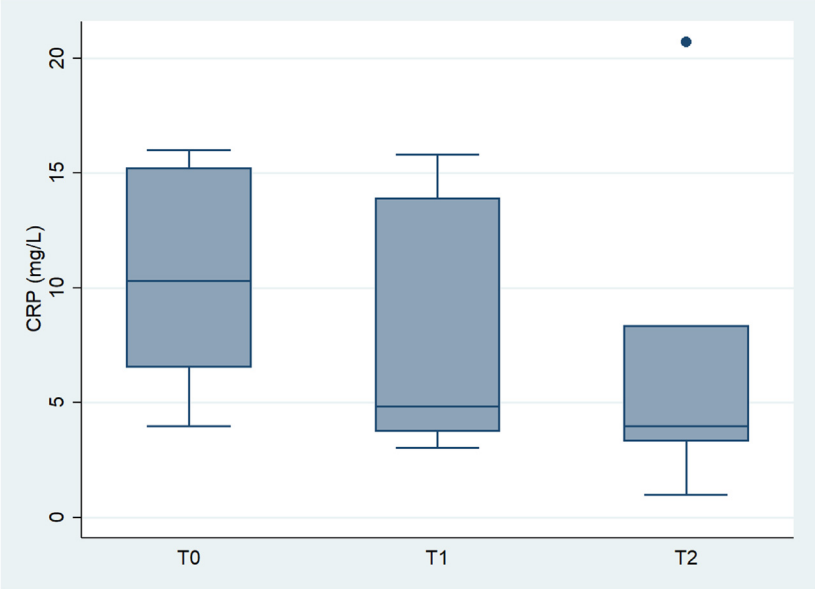

Fig. Box plots of hsCRP fluctuation during study follow-up.

variations, no other significant shifts were observed in the levels of the remaining parameters. Descriptive statistics for the measurements at each time point separately are presented in Table I. The Skillings-Mack test results are stated in Table II.

The results of the Wilcoxon rank sum test of equality in distribution of the differences (T2 − T0) between females and males reached marginal statistical significance for WBC ($P = 0.049$) and procalcitonin test ($P = 0.039$), but clinical significance remained negligible.

The fluctuation of hsCRP is presented in the box plot shown in the Figure, demonstrating a lack of association between systemic inflammation and orthodontic treatment (normal values <20 mg/L).

DISCUSSION

After a sustained force is applied against a tooth, a cascade of biochemical reactions is expressed in periodontal tissues.[11-13] The response of local multipotent mesenchymal cells to mechanical forces applied to teeth leads to alterations in the position, structure, and form of the periodontal tissues. These alterations promote bone resorption and deposition processes, and eventually, tooth movement can occur. The basis of these phenomena is inflammation, which, although largely confined within the local (oral) tissues, also has a systemic impact. As mentioned earlier, acute changes in the systemic inflammatory status or the hemostatic system because of dental treatment, although transient, could be a matter of concern in patients with a high risk for cardiovascular events.[5,6]

Concerns on the potential risk of dental treatment on high-risk patients have also been raised previously, specifically regarding periodontal treatment, as it is well established that scaling and root planing during the second step of periodontal treatment, especially the full-mouth protocols, results in acute and transient systemic perturbations, including the increase of systemic inflammation and endothelial cell activation and dysfunction.[14,15]

Thus, this study aimed to investigate the magnitude of the alterations in systemic blood counts during the first days after bracket placement in patients undergoing orthodontic treatment with fixed appliances and assess whether the possible changes are transient. Periodontally healthy patients with good oral hygiene receiving fixed appliances in both jaws to reduce confounding and assess the maximum effect of orthodontic therapy initiation, respectively, were included. The results showed that the assessed hematological parameters did not show substantial fluctuation or significant trends during the study follow-up.

CRP is a widely used marker of inflammation. Increasing evidence suggests that CRP not only reflects the level of inflammation in the body but may also have a regulating effect in this process. Moreover, this protein can be deposited at naturally occurring or experimental traumatic injury or inflammation sites.[16] One could hypothesize that the elevation of the CRP levels would follow bracket placement and force application.

In a previous study, increased levels of high-sensitivity CRP were detected on day 1 of orthodontic treatment, possibly matching an acute response to mechanical stimulus; they were then found to be lower on day 7, ultimately reaching their highest level at month 3 of orthodontic treatment. The latter measurements could be attributed to a somewhat compensated oral hygiene status because of the appliances.[8] In contrast, MacLaine et al[7] found no differences in long-term CRP concentrations at 2, 4, and 6 months after treatment. The findings were in accordance with this study, which showed that CRP levels did not fluctuate significantly during the early observation period.

WBC counts exhibited a transient decline on day 5, followed by a recovery on day 14 after treatment initiation. This finding parallels the outcomes of another study on the systemic inflammatory responses induced by orthodontic force, in which the percentages of inflammatory monocytes were decreased from day 1 to 3 of treatment and then recovered on day 7 in the blood and spleen. Inflammatory monocyte recruitment can begin 2 hours after a local inflammatory environment becomes present,[17] and it takes 4 days for the bone marrow to compensate for the immune cell reduction in the bloodstream.[18] In contrast, in another study, increased neutrophil counts were detected up to 6 months after orthodontic treatment initiation, which could support persistent systemic inflammation.[8] In a rat study, the findings revealed reduced neutrophil counts after bracket removal.[19] In the frame of this study, WBC fluctuation could not be linked to a similar variation of other markers such as ESR and CRP, which probably precludes an indication of systemic inflammation as a response to orthodontic treatment.

Hemoglobin count directly measures the oxygen-carrying capacity of the blood, and it can even imply anemia when hemoglobin levels are low. The reference ranges for Hb concentrations are expressed in grams per deciliter, and the normal Hb level for adults is 14-18 g/dl for males and 12-16 g/dl for females.[20] In this study, Hb levels remained within the normal range, even if the observed decline from T0 to T2 was statistically significant. Because no other parameter of the red line was reported as significant, clinical importance is negligible. Nonetheless, an association between the decreased Hb levels and local inflammation induced by fixed appliances is not excluded. An interesting case report has shown a remarkable amelioration of severe anemia 4 months after treatment of severe periodontitis and full-mouth rehabilitation. All blood parameters returned to their normal value without medication.[21] Chronic periodontitis can constitute a significant cause of anemia through the induction of chronic inflammation and immune response and then by impairing the process of erythropoiesis, ultimately resulting in anemia.[22] This was not the case in this study, as only systemically and periodontally healthy patients were recruited. None of the other blood test parameters seemed to be affected during the first 2 weeks after initiating orthodontic treatment.

CONCLUSIONS

Orthodontic tooth movement with fixed appliances led to a limited and transient change in WBC and Hb levels during the first days after bracket placement in patients undergoing orthodontic treatment with fixed appliances. The fluctuation of hsCRP levels was not significant, demonstrating a lack of association between systemic inflammation and orthodontic treatment.

AUTHOR CREDIT STATEMENT

Dimitrios Kloukos contributed to conceptualization, methodology, and original draft preparation; Eleni Kalimeri contributed to data curation; Sofia Gkourtsogianni contributed to original draft preparation; Alpdogan Kantarci contributed to visualization and manuscript review and editing; Christos Katsaros contributed to supervision and manuscript review and editing; and Andreas Stavropoulos contributed to supervision, validation, and manuscript review and editing.

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