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Evaluation of inflammation during fixed orthodontic treatment

Fundagul Bilgic, DDS PhD, Assistant Professor[a,*], Ozlem Akinci Sozer, DDS PhD, Assistant Professor[a], Oguzhan Ozcan, PhD, Assistant Professor[b], Ahmet Burak Gurpinar, PhD, Specialist[c], Hakki Yilmaz, PhD, Research Assistant[a], Yazgi Ay, PhD, Assistant Professor[d]

[a] Department of Orthodontics, Faculty of Dentistry, Mustafa Kemal University, Hatay, Turkey
[b] Department of Biochemistry, Faculty of Medicine, Mustafa Kemal University, Hatay, Turkey
[c] Tokat Public Hospital, Tokat, Turkey
[d] Department of Orthodontics, Faculty of Dentistry, Adnan Menderes University, Aydin, Turkey

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ABSTRACT

Objective: The aim of this study was to assess effects of fixed orthodontic therapy on high-sensitivity C-reactive protein (hs-CRP) level, CBC parameters and levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), urea, creatinine, sodium (Na), potassium (K), calcium (Ca), total protein (TP), and albumin (Alb).
Design: Blood samples (7 ml) were drawn at baseline, on days 1 and 7, and three months after placement of braces in the study group, while only one blood sample was drawn at baseline in the control group. Serum hs-CRP levels were measured by nephelometric method. Friedman two-way variance analysis was used to assess values with skewed distribution obtained at baseline, on days 1 and 7, in the third month. Wilcoxon rank sign test was performed if median values were unequal.
Results: During measurement periods, there were significant increases in hs-CRP level, WBC count and neutrophil count while a significant decrease in Na level ($p < 0.05$). K level was significantly decreased on the day 1. No significant differences were detected in other biochemical parameters evaluated.
Conclusion: Elevation in serum hs-CRP levels and neutrophil: lymphocyte ratio within first 3 months indicates that a systemic immune response develops against therapy in patients undergoing fixed orthodontic therapy.

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1. Introduction

Currently, fixed orthodontic therapy is the most preferred therapeutic modality for treatment of malocclusions that affect mastication and facial appearance (Liu et al., 2014). Although successful outcomes are achieved by orthodontic therapy, orthodontic appliances can affect subgingival microbial composition and inflammatory reactions can develop in gingival tissues of patients particularly at early periods of therapy (Kim et al., 2012; Liu, Zhang, Wang, Guo, & Xiao, 2013; Martha, Mezei, & Janosi, 2013).

The most hazardous condition that causes systematic passage of chemical particles in orthodontics is braces left in the mouth for approximately two years (Kloukos, Pandis, & Eliades, 2013). Prolonged stay of these braces leads plaque accumulation and enhances enzymatic activation, particularly salivary esterase. The braces can hinder complete oral hygiene, promoting an inflammatory process (Boncuk, Cehreli, & Polat-Ozsoy, 2014; Dubey, Jalili, & Garg, 1993; Martha et al., 2013).

High-sensitivity C-reactive protein (hs-CRP) is a plasma protein and acute phase reactant that is synthesized by the liver. CRP is the first inflammation and tissue damage marker identified. CRP and other acute phase molecules are generally found in lower amounts in plasma; however, they may show dramatic increases within 72 h in the presence of tissue injury or infection. hs-CRP is the gold standard molecule that can be tested comprehensively in the assessment of inflammation (hs-CRP2) (Kalra, Pradeep, Priyanka, & Kumari, 2013; Pepys & Hirschfield, 2003).

Systemic elevation in C-reactive protein (CRP), is a mechanism that can explain response to oral stimulus. These stimuli can develop as a result of periodontal inflammation or systemic spread of oral bacteria. Periodontal inflammation can result from plaque accumulation due to orthodontic appliances as well as aseptic

* Corresponding author at: Mustafa Kemal Universitesi, Dis Hekimligi Fakultesi, Ortodonti A.D., 31000, Hatay, Turkey.
E-mail address: fundagulbilgic@hotmail.com (F. Bilgic).

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necrosis due to orthodontic forces or combination. In case of the great orthodontic force a hyalinized zone which is an area of focal aseptic necrosis, occurs in the compressed periodontal ligament (PDL). Also an indirect resorption occurs in the tension side (Danesh et al., 2004; MacLaine, Rabie, & Wong, 2010; Rossi, Whitcomb, & Lindemann, 1996).

Previous studies suggested that release of cytokines (IL-1β, TNF-α, etc.) occurs in tissue response to mechanical stimuli at early phases of orthodontic tooth movement. IL-1β and TNF-α may be transported into the systemic circulation and stimulate hepatocytes in the liver to produce C −reactive protein (CRP) (Saadi & Ghaib, 2013). This mechanical stress can lead to development of acute inflammatory reactions in periodontal tissues, which promote tooth movement and biological processes, resulting in bone resorption (Sandy, Farndale, & Meikle, 1993).

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) are mainly found in the liver and assessed in the diagnosis of hepatic diseases. These enzymes are evaluated in the early diagnosis and prognosis of PDL inflammation (Ozmeric, 2004). Complete blood count (CBC) parameters are most commonly present at the initial inflammatory response (Martin & Leibovich, 2005).

The literature includes a limited number of studies about elevated levels of circulating inflammation markers by the response to periodontal inflammation due to orthodontic tooth movement. The present research aimed to study changes in hs-CRP level, CBC parameters, and levels of AST, ALT, GGT, ALP, urea, creatinine, sodium (Na), potassium (K), total protein (TB), calcium (Ca), and albumin (Alb) over time. This study may help to elucidate whether inflammation caused by orthodontic therapy and orthodontic appliances causes a toxic reaction in patients' livers and kidneys.

2. Materials & methods

This study was approved by an institutional ethics committee at 28.03.2014. The patients and controls were randomly chosen from among patients presented to the orthodontics clinic. The study group consisted of 37 patients scheduled for fixed orthodontic therapy, while the control group consisted of 40 patients without a need for orthodontic therapy. Overall, 77 patients were included in the study. There were 16 women (mean age 14,59 ± 2,16 years) and 21 men (mean age 14,86 ± 2,13 years) with mean age of 14,75 ± 1,89 years in the study group, and 21 women (mean age 13,75 ± 2,12 years) and 19 men (mean age 13.45 ± 2,31 years) with mean age of 13.68 ± 2,09 years in the control group. Eligibility criteria were lack of systemic disease, no drug use within the last four months, lack of gingivitis or periodontitis, and good oral care. The control group was comparable to the study group regarding sex, age, geographic location, and social characteristics.

Visible plaque index (VPI), gingival bleeding index were used for the measurement of the state of oral hygiene (Ainamo & Bay, 1975).

Plaque: The visible plaque index (VPI) was recorded as "1" when there was visible plaque and "0" when there was no plaque accumulation on the mesio-buccal tooth surface. Gingivitis: GBI for the gingival margin at the mesio-labial surfaces was assessed with 0.5 mm diameter periodontal probe which lightly inserted into the gingival crevice parallel to the long axis of the tooth with minimum axial force. Bleeding was recorded as 1, and no bleeding as 0.

The patients had "0" VPI and GBI scores were included in the study.

All cases in the study group had Class I malocclusion and were treated using straightwire fixed appliances. The slot size of Roth metal brackets (Dentaurum equilibrium 2, Phorzeim, Germany) was 0.018-in. tooth extraction. Tooth extraction process was delayed until the end of the study process in 23 patients treated with extraction.

Blood samples were drawn at baseline, on days 1 and 7, and three months after placement of braces in the study group, while only one blood sample was drawn at baseline in the control group. Permission was obtained from the patients and their parents for receiving blood.

2.1. Laboratory analyses

Early morning venous blood samples were drawn from an antecubital vein and collected into EDTA vacutainer by a pediatric nurse. Blood samples were analyzed in an automated hematology-analyzer (Sysmex XN-1000, Japan) within the same day. Fasting venous blood samples were centrifuged at 1500g over 10 min to obtain supernatant, which was then divided into aliquots and stored at −80 °C until assays. In the sera obtained, ALT, AST, GGT, and ALP activities were studied by an enzymatic-kinetic method, while Ca, TP, and Alb levels were measured by spectrophotometric method and Na and K levels were measured by indirect ISE method by using an auto-analyzer (Architect c8000; Clinical Chemistry Analyzer, Abbott, USA). Serum hs-CRP levels were measured by nephelometric method (Beckman Coulter Immage Nephelometer, USA). Within-run and total% CV values were 5.0 and 7.5 for serum samples, respectively. Analytical range was 0.02–6.0 mg/dl.

2.2. Statistical analysis

Data were analyzed by MedCalc 12.7.4 (MedCalc Software bvba, Ostend, Belgium) statistic software. The Kolmogorv-Smirnove test was used to test normal distribution. All values except hs-CRP had normal distribution. Friedman test was used in hs-CRP value, while other biochemical parameters were analyzed with One-Way ANOVA. Wilcoxon rank signed test as post hoc test was used for non-parametric analysis and Duncan test was applied for parametric test. $p < 0.05$ was considered statistically significant.

3. Results

No significant difference was detected in biochemical parameters obtained at baseline between study and control groups. Table 1 presents hs-CRP and biochemical values obtained at the time points studied (baseline, day 1, week 1, and month 3). Significant differences were detected in hs-CRP ($p \leq 0.0001$) (Fig. 1, Table 1), Na ($p \leq 0.0001$), and K values ($p = 0.0002$), while no significant differences were detected in albumin, ALP, ALT, AST, Ca, creatinine, GGT, TP, and urea values among the time points studied ($p > 0.05$) (Table 1).

The Fig. 2 and Table 2 present changes in CBC parameters between baseline and month 3. Significant increases were detected in WBC ($p = 0.0040$) and neutrophil counts ($p \leq 0.0001$), while the lymphocyte count ($p = 0.0089$) decreased.

Base-line plasma levels of the hs-CRP were significantly lower than day 1 and month 3 levels of the hs-CRP. Base-line plasma levels of the Na were significantly higher than day 1 and month 3 levels of the Na, whereas levels of K were significantly higher than only day 1 plasma levels of K. Base-line plasma levels of the WBC and neutrophil counts were significantly lower than month 3 levels the WBC and neutrophil counts, whereas levels of lymphocyte count were significantly higher than month 3 plasma levels.

4. Discussion

Areas with plaque accumulation are at risk for gingival inflammation in orthodontic therapy. Bacterial plaque

Table 1
Comparison of hs-CRP and biochemical parameters obtained at the time points studied (baseline, day 1, week 1, and month 3).

Variables		Time of Assessment Mean ± SD			
		Baseline	Day 1	Day 7	Month 3
Albumin (g/dl)		4,70 ± 0,26[a]	4,71 ± 0,26[a]	4,68 ± 0,24[a]	4,67 ± 0,22[a]
ALP (U/L)		150,3 ± 1,47[a]	114,12 ± 1,08[a]	122,48 ± 0,62[a]	126,5 ± 1,29[a]
ALT (U/L)		6,7 ± 1,47[a]	6,41 ± 1,08[a]	6,28 ± 0,62[a]	6,82 ± 1,29[a]
AST (U/L)		14,2 ± 2,78[a]	14,8 ± 4,1[a]	13,6 ± 2,9[a]	14,1 ± 4,3[a]
CA (mg/dl)		9,7 ± 0,33[a]	9,5 ± 0,36[a]	9,7 ± 0,36[a]	9,65 ± 0,34[a]
Creatinine (mg/dl)		0,6 ± 0,07[a]	0,6 ± 0,12[a]	0,7 ± 0,09[a]	0,7 ± 0,28[a]
GGT (U/L)		12,2 ± 3,8[a]	11,7 ± 4,2[a]	11,2 ± 4,2[a]	12,4 ± 5,7[a]
K (mmol/L)		4,3 ± 0,32[a]	4,006 ± 0,28[b]	4,34 ± 0,35[a]	4,3 ± 0,44[a]
Na (mmol/L)		140,2 ± 3,46[a]	128,1 ± 8,15[b]	141,3 ± 1,34[a]	133,6 ± 9,61[c]
TP (g/dl)		7,3 ± 0,41[a]	7 ± 0,46[a]	7,3 ± 0,47[a]	7,3 ± 0,42[a]
Urea (mg/dl)		10,6 ± 2,6 [a]	9,9 ± 2,8[a]	9,8 ± 2,8[a]	10,4 ± 2,9[a]
[a]Hs-CRP (mg/dl)	Mean ± SD	0,184 ± 0,06[a]	0,35 ± 0,09[b]	0,20 ± 0,08[a]	0,32 ± 0,19[c]
	Median	0,174[a]	0,344[b]	0,184[a]	0,279[c]

Different letters indicate statistically significant differences between periods (table columns) ($p < 0.05$.).
[a] For CBC parameters, median values were assessed ($p < 0.05$).

accumulation makes it difficult to ensure oral hygiene after implementation of braces, bands, and arc wires, resulting in gingival inflammation and hyperplasia (Boncuk et al., 2014; Dubey et al., 1993; Kim et al., 2012; Kloukos et al., 2013; Liu et al., 2013; Martha et al., 2013). Our study aimed to determine whether inflammation develops in patients undergoing fixed orthodontic therapy and whether substances released from material used during therapy cause hepatic and renal toxicity, thereby determining whether relevant changes occur in the health status of orthodontic patients due local inflammation, tissue damage, or systemic release of toxins due to orthodontic therapy. Furthermore, information about the effects of materials used in orthodontic therapy on the health of patients would be gained.

Naranjo, Trivino, Jaramillo, Betancourth, and Botero (2006)) investigated clinical and microbiological changes before and 3 months after placement of braces in 30 patients and compared this group of patients with 30 controls without braces application. The authors found increases in plaque amounts and clinical symptoms of gingival inflammation as well as a number of super-infected microorganisms and periodontal agent pathogens at the end of month 3. Many studies have reported that bacteremia develops during orthodontic procedures such as banding, debanding, or separator placement (Erverdi, Biren, Kadir, & Acar, 2000; Lucas, Omar, Vieira, & Roberts, 2002). In our study group, plaque index of the patients slightly increased three months after placement of braces. Any local soft tissue injuries were not observed in our study group.

The greatest tissue damage is seen in periodontal disorders caused by indirect injury due to immunoinflammatory response. The primary cause is inflammation in periodontal tissues (Kayal, 2013). Previous studies have proposed that hs-CRP level, a marker of inflammation and a risk predictor for coronary heart disease, is increased in patients with periodontitis and that periodontal treatment should be delayed in such patients (Loos, 2005; Persson & Persson, 2008). Although local infection influences systemic infection, other risk factors such as genetics can also affect CRP synthesis (hs-CRP5) (Miller et al., 2005).

In our study, blood samples were drawn at baseline, on days 1 and 7, and three months after placement of braces in the study group. The time points are needed to identify whether a potential immune response develops. Blood samples were drawn only at baseline in the control group, because no treatment protocol was applied to these patients and we just wanted to specify that those individuals were selected from healthy population. At the same

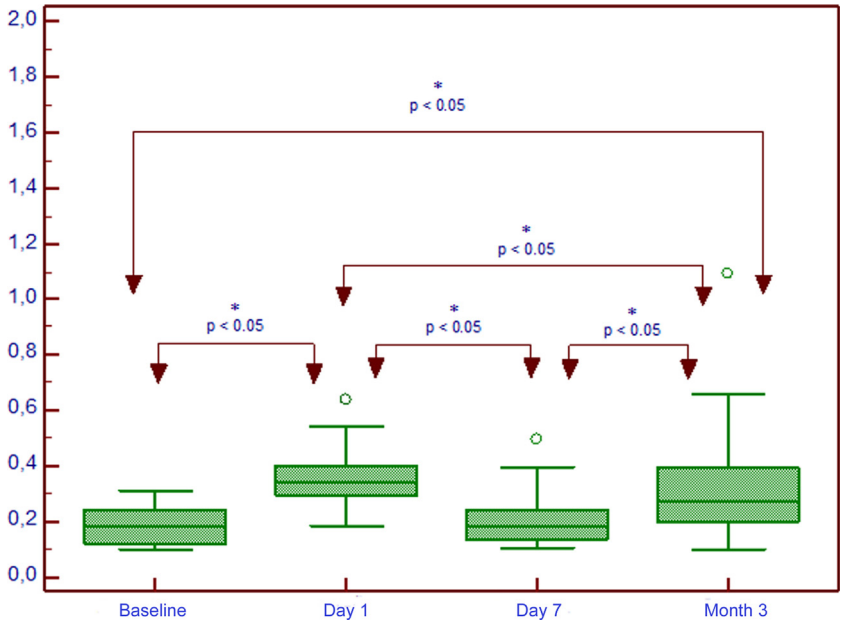


Fig. 1. Levels of high-sensitivity C reactive protein (hs-CRP) at baseline, day 1, week 1, and month 3.

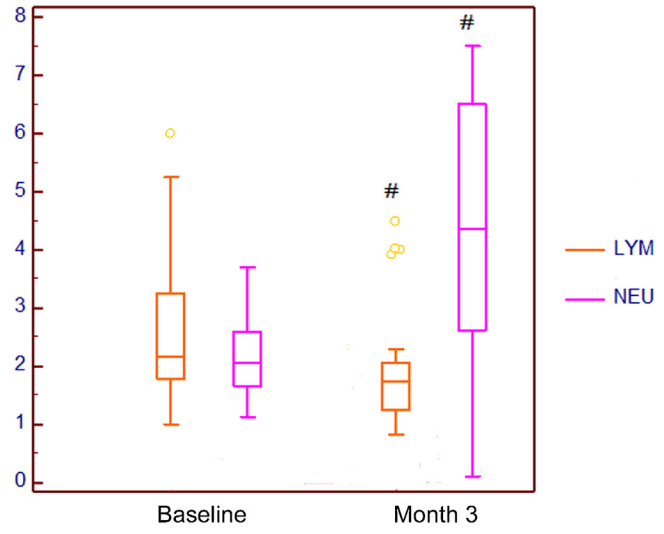


Fig. 2. Levels of complete blood count (CBC) parameters (WBC, neutrophil and lymphocyte) between baseline and month 3.

time, we selected healthy individuals as the control group with no need of orthodontic treatment to show that the patients in the study group had no medical disease.

Previous studies found that hs-CRP level increases in some patients as a result of systemic effects of periodontal infection or severe local injury (Carlson et al., 2005; Miller et al., 2005). Ide et al. (2004) detected a significant increase in CRP levels within hours after subgingival treatment. However, D'Aiuto, Parkar, and Tonetti (2005) found a moderate response in inflammatory markers at the end of week 1, which returned to normal at the end of day 30. Previous studies have suggested that salivary CRP levels reflect local inflammation in the mouth. Saadi and Ghaib, (2013) found that there was marked increase in salivary CRP one hour after replacement of braces, which returned to normal in salivary samples obtained in weeks 1 and 2. Authors linked this decrease to decreased stimuli on bone resorption.

In our study, hs-CRP levels were significantly increased on day 1 but decreased by the end of week 1. hs-CRP level reached peak level at month 3. We think that the elevated hs-CRP level on day 1 can be explained by local inflammation caused by force applied on PDL after placement of braces. Normal hs-CRP level at the end of week 1 and subsequent elevation to peak level at month 3 indicate enhancement in inflammation during treatment. We argue that the elevated hs-CRP level is caused by gingivitis due to inadequate plaque control by orthodontic patients and by local tissue inflammation, as alveolar bone resorption is caused by orthodontic force on periodontal ligament. Kumar et al. detected that the level of CRP was increased in gingival crevicular fluid correlated with the periodontal disease (Kumar et al., 2013).

Previous studies reported that a periodontal condition affects neutrophil count with increases in cases of periodontal disorders (Petropoulos, McKay, & Hughes, 2004; Ujiie, Oida, Gomi, Arai, & Fukae, 2007). In a rat study, Pereira, Kaminagakura, Bonan, Bastos, and Pereira (2008) found that there was a decrease in neutrophil count after removal of braces previously inserted. Miyamoto et al. (2013) found that neutrophil count was significantly higher in patients who underwent orthodontic therapy when compared to controls. In our study, significant increases were detected in WBC and neutrophil counts. The elevation in the neutrophil: lymphocyte ratio supports increased inflammation three months after orthodontic therapy.

Table 2
Comparison of complete blood count (CBC) parameters (WBC, neutrophil and lymphocyte) between baseline and month 3.

Variables	Time of Assessment Mean ± SD	
	Baseline	Month 3
WBC	$5{,}91 \pm 1{,}52^{a}$	$7{,}58 \pm 2{,}54^{b}$
Neu	$2{,}18 \pm 0{,}69^{a}$	$4{,}35 \pm 2{,}19^{b}$
Lym	$2{,}62 \pm 1{,}24^{a}$	$1{,}91 \pm 1{,}24^{b}$

Different letters indicate statistically significant differences between periods (table columns) ($p < 0.05$).

Many studies have reported that dental composites and their components cause cytotoxicity. Resolution of residual unpolymerized monomer is implied to be the primary cause of cytotoxic effect (Caughman, Caughman, Shiflett, Rueggeberg, & Schuster, 1991; Schedle et al., 1998). In addition, free radicals released from braces can cause changes in some tissues and organs in lower toxic concentrations (San Miguel, Opperman, Allen, Zielinski, & Svoboda, 2013). Daltaban, Saygun, Bal, Balos, and Serdar (2006) found no significant difference in ALP concentrations between periodontitis and control groups. Shirazi, Khosrowshahi, and Dehpour (2001) detected no significant differences in urea and creatinine levels of rats in which orthodontic tooth movements were achieved. Yamamoto et al. (2014) found no differences in creatinine and AST levels in saliva during orthodontic therapy with two distinct arc wires. Kolesarova et al. (2008) suggest that no significant differences of AST, ALT, ALP, GGT concentration in serum after nickel treatment which is widely used in orthodontic appliances.

Mikulewicz, Wotowiec, Janeczek, Gedrange, and Chojnacka (2014) were evaluated the accumulation of metal ions released from orthodontic appliances in the hair, kidney, liver, lung, aorta, and oral mucosa cells. After 3 months, the greatest differences were found in the aorta, cheek and hair. But toxic effect did not occur because of the low doses metal ions were released from the appliances.

In our study, no significant differences were detected in ALT, AST, GGT, urea, and creatinine values in blood samples drawn. These results suggest that materials used during orthodontic therapy have no toxic effect on hepatic and renal functions.

Some studies suggest that orthodontic therapy has no association with systemic immune response. It has been reported that elevation in inflammatory markers could be due to chronic health problems and that stress resulting from orthodontic therapy could cause acceleration or promotion of atheromatous plaque development; however, orthodontic therapy does not indicate risk for cardiovascular disorders in young healthy adults. Orthodontic treatment is a risk factor for cardiovascular disorders in adult patients if inflammatory markers are elevated due to orthodontic therapy. In young patients, elevated inflammatory markers can be associated with depression and obesity, which affects 5–16% of the adolescent population (Essau & Dobson, 1999; Ogden, Carroll, & Flegal, 2008).

From the perspective of health, it is important to ensure immunological safety of orthodontic therapy. In a controlled clinical trial, it was reported that there was no significant periodontal difference between patients who underwent orthodontic therapy at least 10 years ago and those with an orthodontic anomaly in an adolescence period but who did not undergo orthodontic therapy (Polson et al., 1988). Other studies report that orthodontic therapy does not cause pathological conditions, that there is no activation in the immune system during the active therapy phase, and that orthodontic therapy is safe from an immunological perspective (MacLaine et al., 2010).

Oral health has a major role in nutritional status. Orthodontic therapy can affect the pattern and quality of feeding and the amount of nutrients ingested. A previous study reported that poor dietary habits are present in age groups undergoing orthodontic therapy despite higher nutritional requirements (Riordan, 1997). In

our study, we think that significant changes in Na and K levels can be explained by altered feeding patterns and resultant decreases in fluid intake.

5. Conclusion

Based on our results, elevation in serum hs-CRP levels within the first three months in patients undergoing fixed orthodontic therapy indicates that a systemic immune response develops against therapy in these patients. During the first three months of treatment inflammation increased the possibility of hs-CRP, but materials used during orthodontic therapy caused no toxic effect on hepatic and renal functions.

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