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Original Article

Serum IL-6 and CRP levels in patients with trauma involving low-extremity bone fractures

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Abstract. Cytokines and acute phase proteins have been implicated in the systemic response to trauma. The aim of this study was to measure the IL-6, CRP, ALP, calcium and phosphorus levels in patients with trauma involving low-extremity bone fractures at 6, 24 and 48 hours following trauma.

Serum samples were obtained from 21 trauma patients with femoral or tibial fractures at 6th, 24th and 48th hour following trauma. Serum IL-6, CRP, ALP, calcium and phosphorus levels were measured in these samples.

Serum levels of IL-6, CRP and ALP at 24th hour were found to be significantly elevated in comparison to their levels at 6th hour ($p=0.01$, $p<0.01$ and $p<0.05$, respectively). Moreover, the levels of CRP and ALP were found to be significantly elevated at 48th hour compared to their levels at 6th hour ($p<0.01$). However, serum IL-6 levels at 48th hour were found to be lower than the levels at 6th hour ($p<0.01$). There was not a significant correlation between CRP and IL-6 levels at different time points. Serum calcium and phosphorus levels did not change significantly.

We have demonstrated that IL-6 reached its peak level in 24 hours after trauma and its level in the 48th hour decreased below that of 6th hour. CRP, as an acute phase reactant, increased for the first 24 hours and stayed elevated for the next 24 hours. Further studies should be conducted to demonstrate the correlation between the extent of injury and IL-6 and CRP levels.

Key words: Trauma, IL-6 and CRP

1. Introduction

Major trauma is one of the top reasons of death. Trauma involving a broken bone or bones ranges from small fissures to complete breaks. Skin, muscles, tendons, ligaments, blood vessels, neurons, or organs near the breaks can be wounded or totally damaged. Trauma is not only a local pathologic event; it can be defined as an event that affects the whole body because of the systemic complications such as bleeding, shock, and embolism (1). Cytokines are proteins that play an important role in inter-organs and intercellular communication, resulting in the control of intracellular events. Cytokines are synthesized by a wide range of cell types, and are often involved in immune responses including inflammation and hematopoietic regulation. Cytokines have also been implicated in the systemic response to trauma (2).

The cytokine interleukin-6 (IL-6) is a polypeptide that weighs 26 kDa and is synthesized by a broad range of cell types. IL-6 acts as a mediator in lymphocyte functioning and cell differentiation. IL-6 is an important regulator in remodeling of the bone. In young adults, there is a balance between the bone resorption by osteoclasts and bone formation by osteoblasts. This remodeling of the bone is regulated by the local factors named as osteotropic cytokines, such as IL-1, TNF-α and IL-6. Local production of IL-6 by osteoblasts is induced by other cytokines,

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such as IL-1 and TNF or parathyroid hormones (3,4). IL-6 can also be produced by osteoclasts. IL-6 induces production of early osteoclast precursors in colony forming units of granulocytes-macrophages (CFU-GM) (4,5). Experimental studies demonstrated that, in fetal rat bones, IL-6 increases calcium release and osteoclastic production and stimulates bone breakdown together with IL-1 (5). 17-beta-estradiol suppresses IL-6 production by bone and bone marrow stromal cells (6). Increased osteoclastic activity can be suppressed in vivo by anti-IL-6 antibodies (7).

IL-6 also induces the synthesis and release of the acute phase proteins (8,9). Acute phase reactants are made up of a combination of functionally and structurally heterogenous protein groups. Acute phase reactants help tissue repair and eradication of the inflammation by providing coordinated changes in physiologic and biochemical homeostasis. C–reactive protein (CRP) is a peptide that can precipitate with the C-polysaccharides of *Streptococcus pneumoniae* bacteria. After the beginning of inflammation, CRP production in the liver increases; IL-6 is released from activated macrophages resulting in rising CRP levels (10).

Alkaline phosphatases (ALP) are enzymes which hydrolyze monophosphate esters. Tissue non-specific form of ALP (TNAP) is expressed on the cell membrane of osteoblasts (11). ALP is an important protein in bone mineralization. In fact, TNAP knockout mice show defects in bone mineralization. Moreover, osteoblasts from these mice cannot initiate mineralization in vitro and heterozygotes demonstrate delayed mineralization (12).

Our aim was to determine the changes in serum IL-6, CRP, ALP, calcium and phosphorus levels in blood samples taken from patients at 6, 24 and 48 hours following trauma involving low extremity bone fractures.

2. Materials and Methods

This study has been approved by the Institute of Health Sciences of Yuzuncu Yil University, Van, Turkey. It was performed on patients that were transferred to the emergency service of Yuzuncu Yil University Medical Faculty Hospital after trauma with fractured bones between December 2001 and April 2002. The age of the patients varied between 15 and 70 (the mean: 31.05±3.28) and consisted of 17 men and 4 women. Eleven patients had trauma related to femoral fracture and the other 10 had tibial fractures as was detected with X-ray. Patients did not have any major accompanying injuries, except for bruises and skin lacerations. Blood samples were taken at 6^{th}, 24^{th} and 48^{th} hours after the trauma while these patients were monitored in the emergency service. Then blood samples were centrifuged at 2000 rpm for 15 minutes to get serum samples, which were then stored at -70°C and analyzed within one month of collection.

Serum IL-6 concentration was measured with chemiluminescent method using commercially available IMMULITE® kit (DPC, USA) in IMMULITE hormone analyzer (Immulite-1000, USA). CRP concentration was measured with nephelometric method using commercially available mono Behring kit in Behring 100 nephelopmeter (Germany), whereas serum ALP, calcium (Ca^{+2}), and phosphorus (P) levels were detected with commercial kits (Biotrol, France) in Technicon RA-XT auto-analyzer (108-A OTO, Ireland). For the statistical comparison of the data, parameters were analyzed by Kolmogorov–Smirnov Goodness Fit test and the differences between time points were analyzed by paired Student's test. Pearson's correlation test was used to analyze the correlation in IL-6 and CRP levels at different time points. Values at $p<0.05$ were accepted as statistically significant.

3. Results

The average serum IL-6 levels at 6^{th}, 24^{th} and 48^{th} hours following trauma were 50.14±2.93, 86.84±6.78 and 32.45±3.13 pg/mL, respectively (Figure 1a); CRP levels were 8.14±1.85, 104.3±14.55 and 106.43±18.55 mg/L (Figure 1b); ALP levels were 88.52±13.89, 108.00±14.13 and 137.42±16.35 U/L; Ca levels were 8.65±0.37, 7.99±0.27 and 8.25±0.31 mg/dL; and P levels were 2.89±0.21, 2.62±0.16 and 2.38±0.18 mg/dL (Table 1). Serum levels of IL-6, CRP and ALP at 24^{th} hour were found to be significantly increased in comparison to the levels at 6^{th} hour ($p=0.01$, $p<0.01$ and $p<0.05$, respectively). Although 48^{th} hour serum CRP and ALP levels were higher than 6^{th} hour levels ($p<0.01$, for both), IL-6 levels were found to be lower ($p<0.01$). Calcium and phosphorus levels did not change significantly at any time point. Moreover, there was not a significant correlation between CRP and IL-6 levels at different time points.

4. Discussion

During tissue injury, physiologic and various metabolic changes occur to correct the altered homeostasis (13). There are many reports on the pathophysiology of metabolic changes after trauma (14,15).

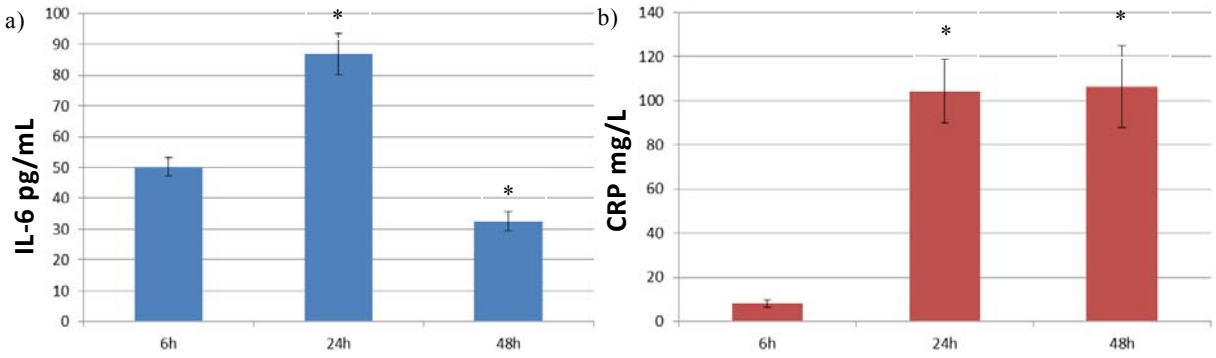

Fig. 1. IL-6 and CRP levels at 6th, 24th and 48th hours following trauma with bone injury. a) IL-6 levels at 24h significantly higher than 6h levels, however, 48h levels are significantly lower than 6h levels. b) CRP levels are significantly higher at 24h and 48h compared to the 6h levels. Asterisk indicates statistical significance ($p<0.01$). Error bars indicate SEM (Standard error of the mean).

Table 1. Serum Alkaline phosphatase (ALP), Calcium (Ca) and Phosphorus (P) levels after 6, 24 and 48 hours following bone related injury.

Parameter	6th h	24th h	48th h
ALP (U/L)	88.52 ± 13.89	108.00 ± 14.13[b]	137.42 ±16.35[a]
Ca (mg/dL)	8.65 ± 0.37	7.99 ± 0.27	8.25 ± 0.31
P (mg/dL)	2.89 ± 0.21	2.62 ± 0.16	2.38 ± 0.18

[a]$p<0.01$, [b]$p<0.05$. Results are shown as mean ± SEM (Standard error of the mean).

The majority of these reports focused on the diagnostic value of cytokines in traumatic injury (16-19). IL-6 levels elevate in correlation with the severity of the trauma in patients with bone and soft tissue injury (16). Plasma IL-6 levels are significantly related to the severity of the head trauma (17). Moreover, peak plasma levels of IL-6 are found after 4 and 6 hours following injury in patients with multiple trauma, while acute phase reactant protein levels are yet to be elevated (18). Furthermore, serum IL-6 is significantly elevated in the first 2 hours of the trauma, continues to elevate for the next 24 hours, but significantly decreases in the next 24 hours in patient group with severe injury compared to the groups of mild or moderate injury (19). We found that plasma IL-6 levels reached their peak in the 24th hour of the trauma and then declined for the next 24 hours below their level in the 6th hour of the trauma. These data indicate that IL-6 production as an organism's response to the trauma increases in the first 24 hour period following trauma.

In our study, CRP levels increased in the first 24 hour following injury and stayed elevated at 48th hour after the trauma. It was demonstrated by immunohistochemistry that CRP production is stimulated in hepatic lobules during inflammation (20). It was also shown that CRP levels increase in the first 6 hours after surgery and reach its maximum at 48th hour (21). Moreover, the amount of CRP and acute phase protein release is directly related to the extent of the inflammation (22) and the maximum level of CRP is reached in the third day after the trauma (23). Our result is consistent with the aforementioned studies confirming that CRP levels increase for at least the next 48 hours following trauma. ALP is an important protein in bone mineralization and is a marker of anabolic processes in the skeleton (24). This protein reflects the biosynthetic activity of osteoblasts (25). Our results show that ALP levels become elevated for the first 48 hours after trauma, indicating increased activity of osteoblasts during this period which is needed for increased bone formation.

In our study a correlation between IL-6 and CRP levels at 6th, 24th and 48th hours after trauma could not be established. It is now considered that IL-6 is the main stimulant of CRP release. Since we could not establish a direct relationship between the serum IL-6 levels and CRP release, we favor a model where IL-6 is not the single stimulant of acute phase reactant production in the liver. Indeed, it was demonstrated that recombinant IL-6 is only partially responsible for the stimulation of CRP synthesis (26). In addition, optimum acute phase protein synthesis in HepG2 cells requires stimulation by a

combination of IL-6, IL-1 and dexamethasone (27). All these data lead us to suggest that IL-6 is just one of the factors that induce CRP release in response to trauma. Further studies are needed to elucidate this subject.

Ca and P levels did not change at any time point during this study. This indicates that trauma does not have major impact on the levels of these electrolytes in the blood. However, serum ALP levels were increased after 24 and 48 hours following trauma in comparison to its levels at 6th hour. Since ALP is important in bone mineralization (11,12), increased levels of this enzyme may indicate increased activity of osteoblasts and bone formation at the site of injury.

In conclusion, we showed that IL-6 reached its peak level in 24 hours after trauma and its level in the 48th hour decreased below that of 6th hour. CRP, as an acute phase reactant, increased for the first 24 hours and stayed elevated for the next 24 hours. These results may lead us to the assumption that measurement of IL-6 levels in the first 24 hours of the trauma with bone fractures may be informative about the extent of the injury. Further studies should be conducted to demonstrate the correlation between the extent of injury and IL-6 levels. In that case, IL-6 levels may have a potential prognostic value for the patients.

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