Evaluation of inflammatory response in patients undergoing surgical treatment for early and delayed femoral fractures

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Abstract

Introduction: It has been shown that long bone fractures are correlated with the inflammatory response. In the initial injury, surgical reduction and fixation of fractures induce the immunoinflammatory response. This study aimed to evaluate serum variation of inflammatory markers in patients undergoing surgical treatment for early and delayed femoral fractures.

Material and methods: This study aimed to evaluate serum variation of inflammatory markers in patients undergoing surgical treatment for early and delayed femoral fractures. The patients were randomly divided into two groups using the method of block randomization including early surgery (within 24 h) and delayed surgery (after 48 h). Serum levels of inflammatory markers in both groups including interleukin (IL)-1, 5, 6, tumor necrosis factor α (TNF-α) and interferon γ (IFN-γ) were determined using specific kits. From each patient 10 ml blood was collected for cytokine assay in their serum.

Results: Our findings suggest that serum levels of IL-8 were markedly decreased from 12 h until 48 h postoperatively (p < 0.05). Moreover, the results indicated that serum levels of TNF-α were significantly increased in the early hours, but after 48 h a decreasing trend was detected (p < 0.05). Furthermore, serum levels of IL-10, IFN-γ, and IL-6 were significantly increased from 12 h until 48 h postoperatively (p < 0.05).

Conclusions: The inflammatory status of the patient may be a useful adjunct in clinical decisions. With an improved understanding of the molecular basis of the inflammatory response, and by identifying relevant clinical markers of inflammation, surgeons can better manage the timing of surgical stabilization.

Key words: inflammation, tumor necrosis factor, interleukin, patient, femoral.

Introduction

The inflammatory response has been recognized as a physiologic reaction to injury. Surgery was shown to be the cause of a systemic response, the extent of which is moderated by different parameters such as the health and nutritional status of the patient, the severity of recent trauma and the presence of any preexisting physiologic derangement, and
the magnitude, duration, and technique of surgery [1–4]. It has been shown that hip fracture and surgery in aged rats induced a systemic inflammatory response and lung injury correlated with increased susceptibility to infection during the acute phase after injury and surgery. It has been shown that long bone fractures are correlated with the development of the systemic inflammatory response syndrome and are strongly associated with multi-organ failure, sepsis, hospital length of stay, and mortality [5–7]. Different components of the immune system have been demonstrated to be involved in this process, such as inflammatory cytokines, leukocyte adhesion molecules, growth factors, nitric oxide, platelet-activating factors, and the activation of local and systemic polymorphonuclear neutrophils (PMNs), lymphocytes, and macrophages. This complex response arises from the interplay between various mediators produced at the site of injury, including cytokines [8]. These mediators can regulate gene transcription, and modify intracellular signaling pathways [9]. In the initial injury, surgical reduction and fixation of fractures induce the immunoinflammatory response [10]. Therefore, modulation of cytokine release has been considered a tempting strategy [11]. This study aimed to evaluate serum variation of inflammatory markers in patients undergoing surgical treatment for early and delayed femoral fractures.

Material and methods

Patients and serum parameters

This study is a randomized clinical trial and all samples were conducted among patients with femoral fractures, between 2014 and 2015 in Rasoul Hospital of Tehran. This study was approved by the Ethical Committee for Clinical Research of the Hospital, and informed consent was obtained from all the patients. It is worth noting that the criteria included ages of 20 to 50 years, and patients with femoral shaft fractures without injury in other parts of the body were recruited for our study. The patients were randomly divided into two groups using the method of block randomization including early surgery (within 24 h) and delayed surgery (after 48 h). Serum levels of inflammatory markers in both groups including interleukin (IL)-1, 5, 6, tumor necrosis factor (TNF)-α and interferon (IFN)-γ were determined by specific kits. From each patient 10 ml of blood was collected for cytokine assay in their serum.

Patients with the following criteria were excluded from the study: patients who had chronic inflammatory disease or a history of trauma in the last month, patients who had suffered multiple organ damage in their recent trauma, and patients with complex fractures.

ELISA analysis

Serum was also separated from blood using centrifugation (2000×g for 15 min at 4°C). All samples were frozen at −20°C in sterile tubes until used for cytokine measurements by the ELISA method using commercial kits (BIORBYT).

Statistical analysis

All variables were analyzed using the software SPSS version 16.0 (SPSS Inc, IL, USA). To compare levels of inflammatory markers including IL-1, IL-5, IL-6, TNF-α and IFN-γ the independent t-test was used. Differences were considered statistically significant when p was less than 0.05.

Results

Our findings suggest that serum levels of IL-8 were markedly decreased from 12 h until 48 h postoperatively (p < 0.05). Moreover, the results indicated that serum levels of TNF-α were significantly increased in the early hours, but after 48 h a decreasing trend was detected (p < 0.05). Furthermore, serum levels of IL-10, IFN-γ, and IL-6 were significantly increased from 12 h until 48 h postoperatively (p < 0.05) (Table I).

Discussion

The complex inflammatory response arises from the interplay between various mediators produced at the site of injury, including cytokines [8]. These mediators can regulate gene transcription, and modify intracellular signaling pathways [9]. In the initial injury, surgical reduction and fixation of fractures induce the immunoinflammatory response. Therefore, modulation of cytokine release has been considered a tempting strategy [10]. Zhang et al. reported that hip fracture and surgery in aged rats induced a systemic inflammatory response and lung injury correlated with increased susceptibility to infection during the acute phase after injury and surgery [11].

In the present study, our findings suggest that serum levels of IL-8 were markedly decreased from 12 h until 48 h postoperatively. Moreover, the results indicated that serum levels of TNF-α were significantly increased in the early hours, but after 48 h a decreasing trend was detected. Furthermore, serum levels of IL-10, IFN-γ, and IL-6 were significantly increased from 12 h until 48 h postoperatively.

Neumaier et al. [12] reported that the C-reactive protein (CRP) values were significantly lower in early surgery within 24 h after trauma than in delayed surgery. Moreover, they found that a lower postoperative inflammatory reaction after early surgery of hip fractures provides a better out-
Table I. Comparison of serum levels of inflammatory markers in patients with different times

| Number of patient | Gender | Sampling dates and times | IL-8 concentration [ng/µl] | TNF-α concentration [ng/µl] | IL-6 concentration [ng/µl] | IFN-γ [ng/µl] | IL-10 [ng/µl] |
|-------------------|--------|--------------------------|-----------------------------|------------------------------|----------------------------|---------------|---------------|
| 1                 | M      | 0                        | 0                           | 0                            | 0                          | 0             | 0             |
| 2                 | M      | 24 h                     | 0                           | 0                            | 0                          | 0             | 0             |
| 3                 | M      | 48 h                     | 0                           | 0                            | 0                          | 0             | 0             |
| 4                 | M      | 0                        | 0                           | 0                            | 0                          | 0             | 0             |
| 5                 | M      | 24 h                     | 0                           | 0                            | 0                          | 0             | 0             |
| 6                 | M      | 48 h                     | 0                           | 0                            | 0                          | 0             | 0             |
| 7                 | M      | 48 h                     | 0                           | 0                            | 88.27                      | 108.474       | 258.598       |
| 8                 | M      | 24 h                     | 0                           | 0                            | 28.871                     | 374.836       | 164.614       |
| 9                 | M      | 0                        | 0                           | 0                            | 1.679                      | 56.695        | 141.466       |
| 10                | F      | 48 h                     | 0                           | 0                            | 135.058                    | 393.762       | 104.554       |
| 11                | F      | 24 h                     | 0                           | 0                            | 176.806                    | 314.69        | 78.371        |
| 12                | F      | 0                        | 0                           | 0                            | 37.275                     | 79.316        | 6.946         |
| 13                | M      | 0                        | 0                           | 0                            | 33.393                     | 64.027        | 0             |
| 14                | M      | 48 h                     | 0                           | 0                            | 60.903                     | 16.411        | 3.572         |
| 15                | M      | 24 h                     | 0                           | 0                            | 60.903                     | 16.411        | 3.572         |
| 16                | M      | 0                        | 0                           | 0                            | 60.903                     | 16.411        | 3.572         |
| 17                | M      | 24 h                     | 0                           | 0                            | 46.439                     | 32.874        | 0             |
| 18                | M      | 24 h                     | 0                           | 0                            | 55.636                     | 11.52         | 86.575        |
| 19                | M      | 48 h                     | 0                           | 0                            | 76.802                     | 11.52         | 118.66        |
| 20                | M      | 24 h                     | 0                           | 0                            | 55.636                     | 11.52         | 86.575        |
| 21                | M      | 24 h                     | 0                           | 0                            | 67.127                     | 10.66         | 167.327       |
| 22                | M      | 24 h                     | 0                           | 0                            | 66.612                     | 4.822         | 23.186        |
| 23                | M      | 24 h                     | 0                           | 0                            | 318.514                    | 128.109       | 19.013        |
| 24                | M      | 24 h                     | 0                           | 0                            | 357.436                    | 107.241       | 41.772        |
| 25                | M      | 24 h                     | 0                           | 0                            | 33.933                     | 64.027        | 0             |
| 26                | F      | 24 h                     | 0                           | 0                            | 254.99                     | 21.558        | 96.848        |
| 27                | F      | 0                        | 0                           | 0                            | 20.93                      | 54.418        | 32.107        |
| 28                | M      | 24 h                     | 0                           | 0                            | 70.716                     | 4.822         | 11.433        |
| 29                | M      | 24 h                     | 0                           | 0                            | 58.805                     | 6.946         | 9.475         |
| 30                | M      | 24 h                     | 0                           | 0                            | 612.362                    | 11.52         | 24.638        |
| 31                | M      | 24 h                     | 0                           | 0                            | 228.051                    | 202.519       | 9.184         |
| 32                | M      | 24 h                     | 0                           | 0                            | 148.101                    | 29.939        | 35.193        |
| 33                | M      | 24 h                     | 0                           | 0                            | 214.574                    | 89.322        | 94.726        |
| 34                | M      | 0                        | 0                           | 0                            | 180.941                    | 74.811        | 74.866        |
| 35                | M      | 24 h                     | 0                           | 0                            | 28.871                     | 4.063         | 196.526       |
| 36                | M      | 24 h                     | 0                           | 0                            | 91.92                      | 185.003       | 4.822         |
| 37                | M      | 0                        | 0                           | 0                            | 28.277                     | 67.388        | 3.572         |
| 38                | M      | 24 h                     | 0                           | 0                            | 66.171                     | 115.362       | 64.406        |
| 39                | M      | 0                        | 0                           | 0                            | 230.739                    | 201.428       | 37.545        |
| 40                | M      | 24 h                     | 0                           | 0                            | 72.753                     | 0             | 15.458        |
| 41                | M      | 24 h                     | 0                           | 0                            | 155                        | 0             | 49.781        |
| 42                | M      | 0                        | 0                           | 0                            | 155                        | 0             | 49.781        |
| 43                | M      | 0                        | 0                           | 0                            | 350.672                    | 4.822         | 61.946        |
| 44                | M      | 24 h                     | 0                           | 0                            | 24.823                     | 253.136       | 74.811        |
| 45                | M      | 24 h                     | 0                           | 0                            | 117.261                    | 201.428       | 37.545        |
come when treated with arthroplasty. Findings of Harwood et al. [13] support the continued use of damage control procedures in severely injured patients and complement data already available, suggesting that a damage control orthopedics (DCO) approach reduces the subsequent inflammatory response. Moreover, they concluded that the inflammatory status of the patient may be important in clinical decision making regarding the timing of conversion to an intramedullary device.

In agreement with our study, they found that the pattern of serum IL-6, keratinocyte, IL-10, and IL-1 release was dynamic, but no significant elevation in TNF-α was detected. The early hepatic and pulmonary infiltration of polymorphonuclear cells occurred in the absence of significantly elevated serum cytokine levels, indicating that either early minor changes with an imbalance in inflammatory mediators or locally produced cytokines may initiate this process. Nakamura et al. in Japan reported that IL-1 and IL-6 and TNF-α were increased after femoral fractures and that they originated from synovial cells [14]. It has been reported that intramedullary nailing fixation resulted in an increase in the level of inflammatory cytokines in animal models. As a matter of fact, it has more adverse effects on the inflammatory response, system stress, and multiple organs [14].

A previous study found that the serum levels of IL-6 and IL-8 in the cerebrospinal fluid were increased, and it raises the possibility that IL-8, acting in the central nervous system (CNS), plays a role in the postinjury syndrome. The mechanism by which CNS IL-8 is produced in trauma is unclear, but a physiological role is supported by the known ability of the CNS to produce IL-8 and the presence of receptors for its action in the CNS [15, 16].

In an animal model, it has been reported that immune cell performance can be increased, and this phenomenon results in an increase in cytokine secretion levels [17]. A previous study evaluated the change in IL-6 levels perioperatively in patients treated for femoral shaft fracture. It was reported that damage control procedures provoked a significantly smaller increase in IL-6 levels when compared with those observed after primary intramedullary nail (IMN). Furthermore, similar studies on bone fracture were conducted previously by other authors [18–20]. In conclusion, the inflammatory status of the patient may be a useful adjunct in clinical decision making. With an improved understanding of the molecular basis of the inflammatory response, and by identifying relevant clinical markers of inflammation, surgeons can better manage the timing of surgical stabilization.

**Conflict of interest**

The authors declare no conflict of interest.
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