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ORIGINAL ARTICLE

Inflammatory cytokines IL-6 and TNF-α in patients with hip fracture

D. Saribal[1] · F. S. Hocaoglu-Emre[2] · S. Erdogan[3] · N. Bahtiyar[1] · S. Caglar Okur[4] · M. Mert[5]

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Abstract

Summary Mortality and remaining bedridden following the hip fracture surgery are not rare. We tried to measure the levels of inflammatory markers tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) following the hip fracture surgery and compare their levels with controls. We aimed to show a relationship between the levels of these markers and post-operative mortality and walking capability.

Introduction Osteoporosis is a condition, causing the hip fractures in the elderly. Hip fractures have a high rate of overall mortality up to 30% following the incident. Cytokines such as IL-6 and TNF-α are suggested to play a role in bone resorption and, thus, in the etiology of osteoporosis.

Methods Plasma levels of IL-6 and TNF-α were measured pre-operatively and on the first and second days after the surgery in 40 Turkish hip fracture patients. The levels of these cytokines were compared with 40 Turkish age-matched healthy controls. The levels of these cytokines were compared between the deceased and surviving patients, as well as the existence of walking capability following the surgery.

Results Significantly higher IL-6 levels were shown on the first and second days after the surgery ($p = 0.005$; $p = 0.01$, respectively). The overall death rate of our study group within the 2-year follow-up time was found to be 35%. No statistical significance was found in the means of 2-year follow-up mortality between the patients. Presence of walking capability did not differ between the patients, as well.

Conclusion We demonstrated an association between IL-6 levels and hip fracture in our study group following the surgery. We also suggest that TNF-α and IL-6 levels are not related to the occurrence of death and walking capability after the surgery. However, these findings need further functional and clinical confirmation.

Keywords Hip fracture · TNF-α · IL-6 · Cytokines · Tumor necrosis factor-alpha · Interleukin-6

Introduction

Osteoporosis is a disease of elder adults, especially postmenopausal women, occurring as a result of poor bone quality and increases the probability of (susceptibility to) hip fractures (HFs) [1]. Individuals with HFs have a risk of overall mortality up to 30% within the first year, and even if they survive, the life quality decreases dramatically [2].

As the inflammatory pathways are shown to be involved in different conditions in the organism, the balance between osteoblastic and osteoclastic activity and bone remodeling are regulated by the elements of the immune system. Cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are secreted by different cells of immune system and play a role in bone resorption and, thus in the etiology of osteoporosis, with the recruitment of the immune processes [3, 4]. These pro-

✉ F. S. Hocaoglu-Emre
sinemhocaoglu@yahoo.com

1 Department of Biophysics, Istanbul University Cerrahpaşa Medical Faculty, Istanbul, Turkey

2 Department of Nutrition and Dietetics, Beykent University, Istanbul, Turkey

3 Department of Orthopedic Surgery, Istanbul Metin Sabanci Baltalimanı Training and Research Hospital for Bone Diseases, Istanbul, Turkey

4 Department of Physical Treatment and Rehabilitation, Bakirkoy Sadi Konuk Training and Research Hospital, Istanbul, Turkey

5 Department of Orthopedic Surgery, Yeniyuzyil University Medical School, Istanbul, Turkey

inflammatory cytokines are secreted as a result of acute injury or trauma and also increase the synthesis of acute phase reactants, especially C-reactive protein (CRP), triggering the different pathways for inflammatory response [5, 6].

High levels of these cytokines were shown to be related with an increased rate of bone resorption and incidence of hip fractures by promoting bone catabolism through their receptors on the osteoclasts [7]. The higher quartiles of these substances are associated with a poor outcome, following the hip implant surgery [8]. Receptors of IL-6 and TNF-α on the osteoclastic cells are currently used as targets for therapeutic drugs for the management of rheumatoid arthritis, autoimmune diseases, and ankylosing spondylitis [9].

Since the role of chronic inflammatory reactions in the pathogenesis of osteoporosis needs to be revealed, we aimed to evaluate the changes in the levels of IL-6 and TNF-α in the pre-operative period and following the first and second days of the surgery in HF cases. We also aimed to determine an association between the high levels of inflammatory markers and their impact on survival and outcome throughout the two-year follow-up period.

Materials and methods

Patient population

Forty patients with hip fracture due to osteoporosis (12 M, 28 F; 74 ± 1 years) and 40 age-matched (67 ± 7 years) non-osteoporotic healthy controls (12 M, 28 F) were enrolled in this study.

Controls were selected using age- and gender-matched random sampling from the cases applied to physical treatment and rehabilitation clinic of the study center who reported no prevalent fractures.

All the patients in the study group received surgical treatment by the physicians from the department of orthopedics of one of the hospitals in Istanbul, Turkey.

Plasma levels of IL-6 and TNF-α were measured before and on the first and second days of the post-operative period. For the purpose of morbidity and mortality assessment, we used a phone questionnaire every 3 months for 2 years of the follow-up period. The length of time before starting walking and occurrence of death were recorded.

Written informed consent was obtained from all the participants. The study was approved by the local ethics committee and carried out in compliance with the Helsinki declaration.

Laboratory analysis

Non-fasting peripheral blood samples were collected from an antecubital vein into EDTA containing tubes (Becton Dickinson, Vacutainer Systems, NJ, USA) from all patients in the hip fracture group for three consecutive days following the surgery. In order to minimize the biological variation of these analytes, sampling time was fixed at the morning hours between 8:00–9:00 a.m. for both study and control group subjects. Blood samples from controls were also drawn non-fasting and at the similar time of the day. Processed serum samples were kept at − 80 °C until the analysis day.

IL-6 and TNF-α levels were measured using a quantitative sandwich enzyme immunoassay (Assaypro, St. Charles, MO, USA) according to the manufacturer's instructions. All samples were analyzed in duplicates, and control samples were analyzed in the first and last lines of each plate. If the difference between the analysis results were more than 10%, the samples were analyzed repeatedly. The mean values of duplicate analysis results were calculated and used.

The intra-assay coefficients of variation (CVs) of IL-6 and TNF-α were 3.6% and 4.9%, whereas inter-assay CVs were 9.5% and 10.2%, respectively. The assay ranges were 0.008–0.5 ng/ml for IL-6 and 0.016–1 ng/ml for TNF-α. The samples detected to be out of the range were diluted with a proportion of 1:1 and re-assayed.

Statistical analysis

All statistical analyses were performed with SPSS 16.0 statistical software.

The comparisons of pre- and post-operative values of IL-6 and TNF-α levels with control subjects were done using the Wilcoxon signed-rank test for paired data. In order to observe if there are differences in the levels of cytokines of interest following the hip fracture surgery, pre- and post-operative values were compared using two-tailed Mann-Whitney *U* test. Data for these analyses were expressed as median and interquartile range (IQR). Log transformation of the data was performed for comparison of the cytokine levels between individuals with different outcomes such as mortality and walking capability following the surgery. Log-transformed data was expressed as mean ± SD. Survival analysis was done using the Kaplan-Meier survival test.

The *p* values less than 0.05 were considered as statistically significant. Diagrams were drawn using MedCalc statistical software.

Results

We measured plasma IL-6 and TNF-α levels in 40 patients with HF and controls. The pre- and post-operative measurement results are shown in Table 1.

Patients with an HF had similar levels of these cytokines when compared with the control group in the pre-operative analyses ($p = 0.71$ for IL-6, $p = 0.96$ for TNF-α).

Table 1 The pre- and post-operative measurement and comparison results of IL-6 and TNF-α[a]

Parameter	Controls	HF Group	
Male gender, *n* (%)	12 (30%)	12 (30%)	
Age (years)	67 ± 7	74 ± 1	
IL-6 (pg/mL)			*p* value
IL-6 (pg/dL) day 0 (pre-op)	121 (46.99)	105 (110.40)	0.71
IL-6 (pg/dL) 24–30 h post-op		151 (86.93)	0.005*
IL-6 (pg/dL) 48–54 h post-op		151 (134.24)	0.01*
IL-6 (pg/dL) mean values post-op		157 (100.88)	0.004*
TNF-α (pg/mL)			
TNF-α (pg/dL) day 0 (pre-op)	121 (33.14)	122 (110.327)	0.96
TNF-α (pg/dL) 24–30 h post-op		133 (131.78)	0.18
TNF-α (pg/dL) 48–54 h post-op		138 (187.19)	0.09
TNF-α (pg/dL) mean values post-op		139 (131.40)	0.07

[a] Data were presented as median. IQR was shown in parentheses. The comparison of pre- and post-op values with the controls was performed with the Wilcoxon signed-rank test for paired data. *p* values < 0.05 were accepted as significant

In the HF group, the post-operative mean levels of IL-6 and TNF-α on day 1 following the surgery were found to be 210 ± 156 pg/dL and 310 ± 412 pg/dL, respectively when compared with the controls (IL-6 = 136 ± 42 pg/dL; TNF-α = 167 ± 173 pg/dL). These findings were significantly higher for IL-6 ($p = 0.005$). Both IL-6 (215 ± 162 pg/dL) and TNF-α (325 ± 424 pg/dL) levels were higher on the second post-operative day compared with the controls. However, the result was statistically significant only for IL-6 ($p = 0.038$; $p = 0.09$). The mean values of post-operative levels of these analytes were also statistically significantly higher than the control values for IL-6 ($p = 0.01$). Distribution lots of these analytes were shown in Fig. 1.

There was no in-hospital death in the post-operative time. Eight patients died within the first year, whereas six patients were lost after the first year of the surgery. The overall death rate of our study group within the 2-year follow-up time was found to be 35%. One female patient who died due to cancer was not evaluated in this group.

The mean follow-up period was 21.03 ± 5.23 months (median, 24; range, 10–24 months), and the mean survival time was 21.02 ± 0.90 months (95% CI 19.25–22.80). Cumulative survival was 97.1 ± 2.8% in the 10th month, 77.1 ± 7.1% in the 12th month, 74.3 ± 7.4% in the 18th month, and 60 ± 8.3% in the 24th month while one patient who died due to cancer was excluded.

While the patients' cytokine levels were compared before and after the surgery, difference between the pre- and post-operative measures found to be statistically significant for IL-6 (Table 2).

When the patients were subdivided into two groups in the means of mortality within 2 years following the surgery, there was no statistically significant difference between the levels of these cytokines.

Twenty-two of 40 patients (55%) managed to walk following the surgery, whereas 18 (45%) remained bedridden. Categorization of patients as the ones who were able to or not able to walk after surgery did not reveal a difference, either. Detailed data were presented in Table 3.

Discussion

HF is a condition, resulting from osteoporosis in the elderly, and has a higher rate of morbidity and mortality within the same year following the injury [10]. There are several studies showing elevated levels of inflammatory cytokines in osteoporosis and HF patients [11, 12].

We demonstrated an association between IL-6 levels and HF in our study group following the surgery. Furthermore, the two-point measurement results of this cytokine were significantly higher than the controls on the first and second days following the surgery.

Stojanović et al. [13] reported that the initial levels of IL-6 and white blood cell (WBC) count but not CRP were associated with a significantly higher risk of HF in a large cohort group with a long follow-up period of 11 years comprised of individuals with different ethnic origins. Another case-cohort study with relatively smaller sample size and shorter follow-up duration in men showed that higher quartiles of TNF-α and its receptor levels are related with a higher incidence of hip and vertebral fractures. However, they could not find an association between IL-6 and its soluble receptor levels and the fracture risk [14].

In their large cohort of subjects including white women and with a long follow-up duration, Barbour et al. [3] measured the levels of IL-6 and TNF-α along with their soluble receptors in the circulation of hip fracture patients. They found that the probabilities of HF for the highest quartile were increased

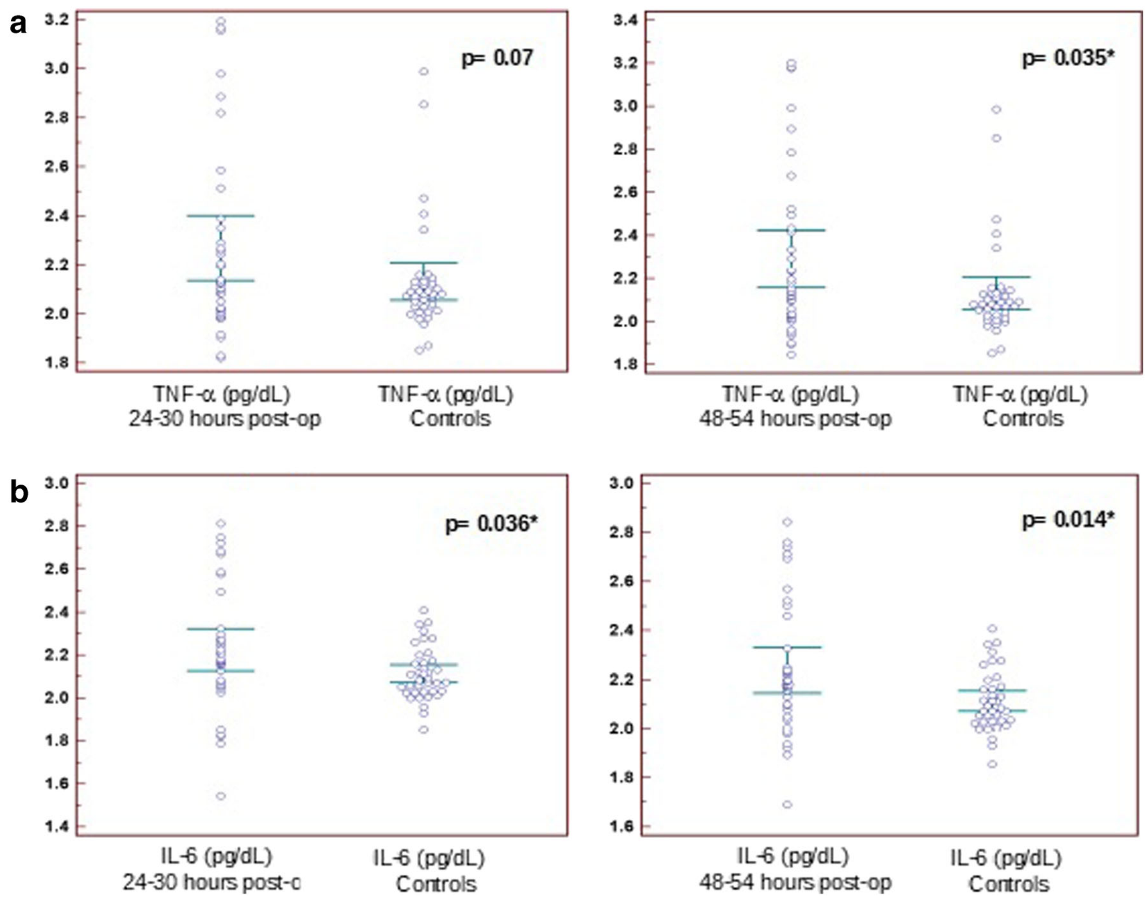

Fig. 1 **a** Comparison of TNF-α levels on the first and second days following the surgery. **b** Comparison of IL-6 levels on the first and second days following the surgery

1.64-fold for IL-6 and 2.05-fold for TNF-α soluble receptor levels. Additionally, women with two and more inflammatory markers in the highest quartile were found to be 1.42–1.51-fold more prone to HF. It has also been shown that using multiple regression models, post-operative levels of both IL-6 and TNF-α may be independent predictors of mortality at 1 year following the HF surgery due to adverse post-operative outcomes including pneumonia and organ dysfunctions [15].

In our study, we could not find a positive association between pre-operative plasma levels of IL-6 and TNF in HF cases when compared with the controls. Since IL-6 and TNF-α have a short life of less than 20 min, and the measurements in our study were performed at least a few hours following the trauma causing the fracture, and 24 h following the surgery, it could be possible that we might have skipped the peak concentrations of these analytes in the systemic circulation [16, 17]. However, post-operative values of IL-6 were still significantly higher than the control subjects in our study.

It has been shown that HF mimics an acute stress response in the organism, and the proper functioning of polymorphonuclear cells (PMN) was affected after the HF before the operation suggesting the impaired signaling of PI3K and NF-κB pathways [10]. Therefore, similar results of IL-6 with the control group after the HF in the pre-operative period in our study might be a result of decreased immune response since one of the sources of these cytokines is PMN cells [18].

Expected values in normal, healthy individuals were shown to be < 445 pg/dL for IL-6 and < 253 pg/dL for TNF-α (for the 95th percentile) [19]. However, the mean post-operative levels of these cytokines were found to be 214 ± 153 pg/dL for IL-6 and 324 ± 420 pg/dL for TNF-α in the HF group. These levels for IL-6 were significantly higher than the control subjects. The 95th percentile results for mean post-operative values of IL-6 and TNF-α were found to be 522 pg/dL and 1147 pg/dL, respectively.

Our data indicates that eight (20%; 6 F, 2 M) patients out of our study group were lost due to complications and as a result of advanced age within the first year following the surgery. Six (15%; 4 F, 2 M) patients died following the first year and within the 2-year follow-up period. One female patient who died due to cancer is not among these six patients. Thus, the percentage of patients who died within 2 years following the HF and surgery was found to be 35% in our study group. This ratio is consistent with the data [1, 20]. Furthermore, a large cohort study showed increased rates of mortality in women who are in the highest quartiles of inflammatory markers.

Table 2 Comparison of IL-6 and TNF-α levels before and after the HF surgery[a]

TNF-α (pg/dL)		*p* value
Day 0 (pre-op)	24–30 h post-op	
122 (110.327)	133 (131.78)	0.38
Day 0 (pre-op)	48–54 h post-op	
122 (110.327)	138 (187.19)	0.22
Day 0 (pre-op)	Mean values post-op	
122 (110.327)	139 (131.40)	0.17
24–30 h post-op	48–54 h post-op	
133 (131.78)	138 (187.19)	0.72
IL-6 (pg/dL)		*p* value
Day 0 (pre-op)	24–30 h post-op	
105 (110.40)	151 (86.93)	0.018*
Day 0 (pre-op)	48–54 h post-op	
105 (110.40)	151 (134.24)	0.017*
Day 0 (pre-op)	Mean values post-op	
105 (110.40)	157 (100.88)	0.016*
24–30 h post-op	48–54 h post-op	
151 (86.93)	151 (134.24)	0.96

[a] Data were presented as median. IQR was shown in parentheses. The comparison of data was performed with Mann-Whitney *U* test. *p* values < 0.05 were accepted as significant

Additionally, while the number of cytokines in the highest quartile increases, mortality rate was boosted up to 17.5% per year [3]. This data is also consistent with our 2-year mortality rate. However, there is a great variety of reasons underlying the mortality following the hip fracture surgery.

TNF-α and IL-6 are cytokines, produced by most of the cells playing a role in the inflammatory response, and their levels have been shown to be associated with undesirable outcomes and mortality in patients with different conditions such as sepsis, meningitis, and trauma [19]. However, there are conflicting data in the literature on the use of these analytes as a marker of mortality and morbidity. Giannoudis et al. [16] suggest that clinical or subclinical inflammation might be leading to the death of HF patients and inflammatory markers may predict short- or long-term outcomes.

In our study group, both TNF-α and IL-6 levels were not found to be related with the occurrence of death when the levels of these analytes were compared between dead and living HF patients. Although there are different comorbidities underlying the increased levels of inflammatory markers in the elderly, it is possible to suggest that either HF itself or the surgical trauma alone significantly increases the levels of these cytokines when compared with the age-matched control group. However, whether the increased levels of these cytokines decline back to their initial levels several days or weeks after the HF and/or surgery needs to be studied.

Miller et al. [8, 21, 22] showed that the IL-6 levels of HF patients never reached the initial levels 1 year after the surgery and higher levels of inflammatory markers are related with poor physical activity capacity. Beloosesky et al. [23] reported increased levels of IL-6 and TNF-α after the surgery, similar to our findings. They also showed that not the TNF-α, but IL-6 levels decreased 1 month after the operation.

While we compared the intra-individual levels of cytokines, we observed a significant difference in IL-6 levels

Table 3 Comparison between patient groups in terms of occurrence of death and walking capability following the HF surgery[a]

Death within 2 years			
Parameter	Yes $n = 14$ (35%)	No $n = 25$ (65%)	*p* value
Log10 IL-6 (pg/dL) day 0 (pre-op)	2.08 ± 0.40	2.02 ± 0.41	0.68
Log10 IL-6 (pg/dL) 24–30 h post-op	2.28 ± 0.27	2.18 ± 0.30	0.32
Log10 IL-6 (pg/dL) 48–54 h post-op	2.29 ± 0.27	2.20 ± 0.27	0.33
Log10 IL-6 (pg/dL) mean values post-op	2.29 ± 0.26	2.19 ± 0.28	0.30
Log10 TNF-α (pg/dL) day 0 (pre-op)	2.23 ± 0.43	2.19 ± 0.45	0.78
Log10 TNF-α (pg/dL) 24–30 h post-op	2.29 ± 0.39	2.25 ± 0.40	0.77
Log10 TNF-α (pg/dL) 48–54 h post-op	2.34 ± 0.38	2.23 ± 0.39	0.46
Log10 TNF-α (pg/dL) mean values post-op	2.34 ± 0.37	2.25 ± 0.39	0.50
Walking following the surgery			
	Yes $n = 22$ (55%)	No $n = 18$ (45%)	*p* value
Log10 IL-6 (pg/dL) Mean values post-op	2.28 ± 0.34	2.19 ± 0.21	0.43
Log10 TNF-α (pg/dL) Mean values post-op	2.35 ± 0.44	2.16 ± 0.16	0.19

[a] Data were presented as mean ± standard deviation. The comparison of outcomes following the surgery was done using the log-transformed data in order to supply the normal distribution. Comparisons of the data were performed with two-tailed probability *t* test. *p* values < 0.05 were accepted as significant. Kaplan-Meier test was performed for survival analysis

following the surgery. This data is consistent with the earlier findings suggesting that IL-6 has a more evident role in inflammatory response following the HF surgery [24, 25].

Our study has a number of strengths. We evaluated novel and less-analyzed inflammatory cytokines in our patient group. Additionally, we made a 2-year follow-up and assessed the occurrence of death and presence of ability to walk after the surgery. We also compared the levels of these inflammatory cytokines between the poor and deserved outcome groups. Also, our study has several limitations, the most important of which is the sample size from a single center. Due to the relatively smaller sample size, we did not constitute subgroups according to fracture types. Also, the analyses of the levels of these cytokines were made from the peripheral blood samples, thus, local measurements might give different results. In addition, the levels of the receptors for these analytes and a follow-up period of more than 54 h would broaden the understanding of the systemic inflammatory response to HF and surgery.

The limitations of our study include low sample size that makes further statistical analyses inapplicable. Besides, we did not include potential confounders including BMI, self-reported health, medical history, NSAID, steroids and/or hormone replacement use, bone mineral density, presence of any chronic disease, and physical activity levels of the study subjects.

In conclusion, in our study of HF patients, we demonstrate higher post-operative plasma levels of IL-6 and TNF-α compared with healthy controls. We did not find an association between the levels of these analytes and life expectation or life quality of the cases. We believe that our data are consistent with the findings of study groups including the ones with small sample size, one-center based prospective studies, and large cohorts suggesting that the inflammatory markers and/or their receptors might be targets to decrease the inflammatory response following the HF. Additionally, evaluation of the levels of different types of cytokines, as well as the levels of their receptors in circulation and on osteocytes' surface, might help in the better understanding of HF etiology and underlying pathways on the pathogenesis of bone fractures and high rates of mortality following the HFs in the elderly.

Compliance with ethical standards

Written informed consent was obtained from all the participants. The study was approved by the local ethics committee and carried out in compliance with the Helsinki declaration.

Conflict of interest None.

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