CLINICAL STUDY

Effects of Gender Difference in Early Cytokine Levels in Trauma Patients

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Abstract

Background: Experimental studies conducted on laboratory animals have demonstrated that gender differences affect the outcome following trauma-hemorrhage but, it is not clear yet whether the manipulation of sex steroids during clinical trauma affects the outcome. This study was designed to determine whether gender based changes occur in cytokine responses after trauma-hemorrhage.

Methods: Plasma cytokine, estradiol, and prolactin levels of 100 consecutive abdominal trauma patients admitted to an emergency unit were measured to determine if there is a gender based difference.

Results: There was no significant difference in trauma severity between male and female patients. Plasma interleukin-1β levels were found to be significantly higher in male patients compared to females following trauma hemorrhage (p=0.003). On the other hand, there was no significant difference in plasma interleukin-6, tumor necrosis factor-α, and prolactin levels between the male and female patients (p>0.05).

Conclusion: These results suggest that the role of sex steroids on immunomodulatory processes following trauma-hemorrhage should be further investigated before studies are undertaken to evaluate the effect of hormonal manipulation in patients with trauma (Tab. 1, Fig. 1, Ref. 26). Full Text (Free, PDF) www.bmj.sk.

Key words: trauma, gender difference, cytokines.

Experimental studies conducted on laboratory animals have demonstrated that gender differences affect the susceptibility to and morbidity from sepsis and trauma (1–4). There are also a few epidemiologic studies indicating gender differences in the susceptibility to sepsis or infection after trauma, with an increased death rate in male patients compared to females (5, 6). Meanwhile, studies including postmenopausal women with decreased estrogen levels have shown a higher death rate in female patients compared to males (7). Thus, it appears that female sex hormones may provide a survival advantage after traumatic injury. While the female sex hormones especially estrogen and prolactin promote the immune functions, male sex hormones have been demonstrated to suppress these immune functions (8). Suppression of androgens after trauma and hemorrhage has also been shown to prevent suppression of immune, hepatic, and cardiovascular response (9–11).

The effects of sex hormones on tissue and organ systems appear to be by means of cytokines. Cytokines are small polypeptides or glycoproteins produced by diverse cell types at the site of injury and by systemic immune cells. These are the effector molecules directing the inflammatory response to infection and injury (12, 13).

The aim of the present study therefore, was to determine if there is any effect of gender difference on proinflammatory cytokine release in patients admitted to an emergency unit with trauma-hemorrhage.

Patients and methods

This study was carried out in 100 consecutive abdominal trauma patients who were admitted to surgical emergency unit in Ankara Numune Training and Research Hospital between Janu-

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ary 2003 and May 2004. There were a total of 59 male patients (59%) with a median age of 38 years (range, 9–82 years), and 41 female patients (41%) with a median age of 30 years (range, 16–57 years). Patients with a history of traffic accident, any kind of penetrating injury, and deceleration injury who had abdominal trauma were included in the study. Patients dying in the very early period after arriving in emergency department and patients not needing hospitalization were excluded. Acute Physiologic Assessment and Chronic Health Evaluation (APACHE) II score, Revised Trauma Score (RTS), and Injury Severity Score (ISS) were used to assess the severity of trauma. Distribution of patients was similar in both sexes in terms of APACHE, RTS, and ISS.

Whole blood was obtained by venous puncture and placed in micro centrifuge tubes at the emergency department, 6 and 24 hours after the occurrence of trauma to prevent time dependent errors in assays of individual patients. The tubes were then centrifuged at 3500 cycles/min for 15 min at 4 °C. Plasma was separated, placed in pyrogen-free polystyrene tubes, immediately frozen, and stored at -20 °C until assayed for estradiol, prolactin, interleukin-1, interleukin-6, and tumor necrosis factor-α levels. Plasma cytokine levels at 6th hour and 24th hour were determined with a commercially available ELISA kit (Cytelisa-Cytimmune, Maryland) according to manufacturer’s guide. Plasma estradiol and prolactin concentrations were determined only in the 6th hour blood samples with a commercially available immunoassay kit by Modular Analytics E 170 (Elecys module) system.

A 30 day survival for all patients was recorded. Gender differences were compared for the above cytokine levels and mortality rates.

**Statistical analysis**

All data were collected and analyzed by using SPSS for Windows 10.0 computer software (SPSS Inc, Chicago, IL, USA). Values were expressed as means. Statistical analysis was performed by Mann Whitney-U test to compare continuous variables between groups and Pearson correlation test was used to test correlation between trauma severity and cytokine levels. A P value <0.05 was considered statistically significant for all tests.

**Results**

There were a total of 100 patients (59 males and 41 females). Mean age of the patients was 38.5±15.2 for males, and 33.0±10.8 for females. Demographic characteristics of patients were identical for both groups. Clinical and laboratory characteristics are summarized in Table 1. On admission, there was no significant difference in trauma severity between male and female patients in terms of APACHE II, RTS, and ISS.

Plasma interleukin-10 levels were found to be significantly higher in male patients compared to females following trauma hemorrhage. On the other hand, there was no significant difference in plasma interleukin-6, tumor necrosis factor-α, and prolactin levels between the male and female patients (p>0.05), (Fig. 1); Interleukin-1β level was 89.0±80.7 pg/ml for males and 68.9±92.2 pg/ml for females, (p=0.003). Interleukin-6 level was 156.6±134.2 U/ml for males and 174.3±144.4 U/ml for females, (p=0.19). Tumor necrosis factor-α level was 171.2±117.2 pg/ml for males and 187.9±111.7 pg/ml for females (p=0.28). Estradiol levels were significantly higher in females compared to males, 130.0±26.8 and 35.8±3.1 pg/ml, respectively (p=0.002). Prolactin levels were also statistically not significant between the groups, 23.8±24.8 ng/ml for males and 31.6±33.0 ng/ml for females (p=0.66). Although the observed mortality rate was higher in males, 10 patients (17 %) for males and 5 patients (12 %) for females, this did not reach statistical significance (p=0.51). Pearson correlation analysis revealed a positive correlation between APACHE II score and IL-6: r=0.448, p=0.00.

**Discussion**

Numerous animal studies have been carried out in recent years to investigate the effects of hemorrhagic shock, alone or in conjunction with soft tissue trauma, on cell-mediated and humoral immunity. But the number of clinical studies on humoral immunity in trauma patients is very limited. Studies on animal models have shown depressed immune functions after experimental hemorrhagic shock or sepsis, especially in male sex (2). Clinical observations and experimental studies suggest an important suppressive effect of male sex steroids on immune functions. Castration studies in animal models of autoimmune diseases have shown a potent protective role of androgens in suppressing the autoimmune disease process, which indicates a significant depression and/or control of immune system by androgens (8, 14). Moreover, it has also been shown that administration of a testosterone receptor antagonist flutamide improved the immune, hepatic, and cardiac functions and increased survival after trauma-hemorrhage (10, 11).

Sex steroids exert most of their effects on immune functions by mediators called cytokines. Cytokines are the key mediators of organisms in response to trauma. TNF-α and IL-1β are the
proximal proinflammatory mediators. These cytokines initiate elaboration and release of other cytokines. They also stimulate the hepatic acute-phase response. In animal models, administration of TNF-α and IL-1β causes septic signs like hypotension, tachycardia, and tachypnea. Although commonly grouped with TNF-α and IL-1β as a proinflammatory cytokine, IL-6 does not produce signs of septic shock when administered to humans. The exact role of IL-6 in the inflammatory response remains unclear (13).

Estrogen is the predominant sex hormone in circulation of females. Previous studies have shown that estradiol improves immune functions following trauma-hemorrhage. Knöferl demonstrated that administration of 17[beta]-estradiol at the beginning of resuscitation normalized the depressed splenocyte as well as splenic and peritoneal macrophage function after trauma-hemorrhage in male mice (4). On the other hand, IL-6 levels were significantly attenuated in animals receiving 17[beta]-estradiol. These findings are in line with the results of studies by Zuckerman et al. who demonstrated significantly decreased levels of plasma IL-6 in mice pretreated with 17[alpha]-ethynyl estradiol and subjected to endotoxemia (15). Chao also demonstrated that 17[beta]-estradiol at concentrations within the physiological range increases male rat peritoneal macrophage tumor necrosis factor-[alpha] release capacity in vitro (16).

Gregory showed that cellular immune response secondary to burn injury was changing first 10 days in relation to changing estradiol level (17). This study revealed that delayed hypersensitivity reactions and splenocyte proliferation was suppressed in
male rats especially between the first and fourth days but, no such depression was observed in females. After the tenth day immune functions were suppressed in females secondary to increased plasma IL-6 levels.

In the study of Minutia et al, there was no suppression in cardiac output and hepatocellular functions in female rats in the first 24 hours following the experimental hemorrhagic shock (18, 19). On the other hand, male rats and ovariec-tomized female rats showed deterioration in both cardiac and hepatocellular functions. This deterioration in these two groups was associated with increased plasma IL-6 levels.

Another hormone effective in immune functions is prolactin. It is known that this hormone has immune improving effect and eliminates the immune-suppressive effects of glucocorticoids and cyclosporine. In the Zellweger’s study, administration of prolactin in males after trauma-hemorrhage has been shown to restore the depressed splenocyte and macrophage immune responses and to decrease mortality rates due to subsequent sepsis (20, 21).

We found that there is a positive correlation between the APACHE II score and plasma IL-6 levels. This was in concordance with the literature that increased IL-6 levels are associated with suppressed immune response and decreased survival (22, 23).

Unfortunately, our results related to plasma cytokine levels in this study were not consistent with the results obtained in previous experimental studies (24, 25). We found higher plasma IL-1β level in males compared to females which was inconsistent with the literature that Kahlike et al found the plasma IL-1β level to be higher in young female rats compared to males following trauma-hemorrhage (25). There was also no significant gender-specific differences in IL-6, TNF-α, and prolactin levels in our study; this may be related to the early time point (6 hours) used in this study. Similarly, Eachempati analyzed the data of 443 consecutive patients with SIRS (Systemic Inflammatory Response Syndrome) and a diagnosis of infection, retrospectively (26). He found that women had worse outcome than males in contrast to the hypothesis that females are superior to males in responding to infection. In a prospective study of 52 patients with surgical sepsis, Schröder et al examined gender-related differences in outcomes and certain mediator levels (6). Hospital mortality in that study was 70 % in males but only 26 % in females. Males had higher tumor necrosis factor [alpha] levels and lower IL-10 levels than women. In our study, although the female patients experienced less mortality (12 %) compared to male patients (17 %), this did not reach statistical significance (p=0.51). The discrepancy in our results may be due to small cohort size and mild to moderate trauma severity in most of the patients (mean APACHE II score was 8-10 and RTS was 10 for both sexes).

In summary, the results of clinical studies are conflicting and it remains unclear whether estrogen therapy or testosterone antagonists will be effective in the management of trauma patients. Before studies are undertaken to evaluate the effect of hormonal manipulation in patients with trauma, more clear demonstration of the role of sex steroids on immunomodulatory processes in trauma patients is needed.

References


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