Original Article

Serum Antithrombin III level in multi-trauma Patients who Develop Sepsis

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Abstract

Background: Sepsis is one of the most important causes of mortality in trauma patients. Sometimes its diagnosis could be difficult, especially at early stages. It is clear that early diagnosis of sepsis, especially with serum markers, may decrease the rate of death in trauma patients. Investigation to find a rapid as well as accurate serum marker indicative of sepsis seems necessary. Seum antithrombin III (AT III) level may predict sepsis occurrence in multi-trauma patients.

Methods: From January 2010 to June 2010, 50 patients with multi-trauma injuries were enrolled in our study. All of them were hospitalized in the intensive care unit (ICU) of neurosurgery department, Imam Reza university hospital, Tabriz University of Medical Sciences, Tabriz, Iran. Twenty-four patients demonstrated sepsis manifestations (sepsis group), but 26 patients did not (non-sepsis group). During ICU stay, blood levels of AT III were measured on days 0, 3, 7. Then, the obtained values were compared between the two groups.

Results: Mean (±standard deviation, SD) serum AT III level upon hospitalization was 95.00 (±15.55) μg/ml in sepsis group and 106.28 (±17.45) μg/ml in non-sepsis group (P= 0.02). Serum AT III level variations during an inter-group study was not significant in non-sepsis group (P= 0.74), but it was statistically significant in sepsis group (P< 0.001). Serum AT III level variations were studied in patients with and without sepsis at different time points with repeated measured ANOVA test. This analysis showed that the variations were statically significant in both groups (P< 0.001).

Conclusion: Serum AT III level was lower in trauma patients who developed sepsis. Inter-group study showed that serum AT III level variations were statistically significant in the sepsis group but not in the non sepsis patients. So AT III serum level measurement may predict the occurrence of sepsis in traumatic patients earlier.

Keywords: Antithrombin III; ; Sepsis; Trauma

Introduction

Patients with multi-trauma injuries may die during the first 60 minutes in the place, about 1-4 hours after accident in the operating room or one week after trauma (1).

Sepsis is one of the most important causes of mortality in patients with trauma. Sometimes its diagnosis is difficult and is delayed. So this may increase the mortality of multi-traumatic patients. It is clear that early diagnosis of sepsis especially with serum markers may decrease the rate of death in these patients (2).

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Plasma AT III level decreases in septic patients. It also decreases during trauma, especially in multi-traumatic events (2-4). The cause of AT III decrease is not obviously known but IL6 may play a role (3). IL6 is releaseed from damaged epithelial cells and aggravates inflammatory response as a cytokine. It increases acute phase reactants and fibrinogen production. On the other hand, AT III is decreased (3). During the treatment, serum levels of AT III increase regardless ofIL-6 blood level (4).

Sepsis is affected by coagulation system and inflammation. Inflammatory responses promote coagulation system and then disseminated intravascular coagulation (DIC) along with multi-organ failure (MOF) may lead to patient's death (5).

Some cytokines such as IL-6, IL-1, and TNF-α activate coagulation cascade and suppress antithrombin, protein C and protein S system. When coagulation factors are increased and anti-coagulation factors are decreased (like AT III), DIC occurs.. These events cannot be predicted using tests like PT (prothrombin time), PTT (partial thromboplastin time) , INR or d-dimer. For this reason we need another rapid and accurate method to predict sepsis at earlier stages. Seum AT III level may evaluate sepsis much better than other laboratory tests like PT, PTT, INR and d-

dimmer. In fact, severe decrease in AT III levels in advanced stages of sepsis happens earlier than the changes in the mentioned lab tests (3).
It must be emphasized that serum AT III level decreases significantly when a patient is critically ill, especially in traumatic and septic patients (6). It is shown that AT III is a potentially predicting factor in patients with sepsis but the comparison of serum AT III levels between multi-traumatic patients with and without sepsis syndrome has not been considered in the published studies (4, 6). In this study, serum AT III levels were measured in multi-traumatic patients during the first, third and seventh days of hospitalization. Thereafter, the role of AT III in prediction of sepsis was evaluated.

Materials and Methods

From January 2010 to June 2010, fifty patients with multi-trauma injuries were enrolled in our study. All of them were hospitalized in the ICU of neurosurgery department, Imam Reza university hospital, Tabriz University of Medical Sciences, Tabriz, Iran. The patients were entered into one of the two study groups. In "sepsis" group (24 cases), the patients developed sepsis criteria (systemic response to infection when it was associated with proven infection). In "non-sepsis" group (26 cases), the patients did not show any signs or symptoms of sepsis.
During ICU stay, blood AT III levels were measured on days 0, 3, and 7. The measurement was done using affinity purified polyclonal anti-AT III for coating ELISA plate and polyclonal affinity purified IgG for detecting antibody. Technically the captured antibody was diluted as 1/100 in coating buffer and immediately 100 µl was added to every well in the microplate. It was incubated for 2 hours at room temperature (22°C). After coating, blocking of the plate was not required under the described conditions. The plate was washed with PBS-Tween and that was sufficient to block non-specific interactions. Plates were washed 3 times with wash buffer. Dilutions of standard plasma were made in a range from 1/2000 (100%) down to 1/64000 (3.13%). Test plasma samples were diluted as 1/4000, 1/8000 and 1/16000. All dilutions were made in PBS-Tween. Then 100 µl/well was applied and the plates were incubated at 22°C for 90 minutes. Being washed 3 times with wash buffer, 100 µg of pre-diluted detecting antibody was applied to every well. Plates were incubated at 22°C for 60 minutes. Again it was washed 3 times with wash buffer and 100 µl of freshly prepared OPD substrate was applied to every well. The color was allowed to develop for 10-15 minutes then color reaction was stopped by addition of 50 µl/well of 2.5 M H_2S0_4. The plate could be read at a wavelength of 490 nm.
The obtained values were compared between the studied groups using the student t test. We also used multivariate analysis with repeated measured test for comparing the results at each time point. P values less than 0.05 were considered to be significant. Descriptive studies were reported as mean ± standard deviation (SD).. SPSS version 16.0 was used for this study.

Results

The mean age of the patients in sepsis group was 42.91 (±20.06) years and for the patients without sepsis it was 43.26 (±19.55) years (P= 0.88). Male:female ratios in sepsis and non-sepsis groups were 11/13 and 12/14, respectively (P=0.46).
Both systolic and diastolic blood pressures were lower in sepsis group compared to non-sepsis group (P= 0.01, P= 0.001, respectively).

At the time of hospitalization, mean (±SD) serum AT III level was 95.00 (±15.55) µg/ml in sepsis group and 106.28 (±17.45) µg/ml in non-sepsis group (P= 0.02). On day 3, mean serum AT III level was lower in sepsis group in comparison to non-sepsis group (P= 0.001). Likewise, serum AT III level was also lower in septic patients compared to non-sepsis group on the 7th day of hospitalization (P= 0.001) (Table 1).

Table 1: Comparison of mean (SD) blood antithrombin III levels between sepsis and non-sepsis groups at different time points.

	Sepsis	Non-sepsis	P value
Day 0	95.00 (±15.55)	106.28 (±17.45)	0.02
Day 3	75.22 (±15.79)	103.74 (±16.74)	0.001
Day 7	64.06 (±13.72)	101.80 (±16.76)	0.001

Inter-group study showed that serum AT III level variations were not significant in the patients without sepsis (P= 0.74). But it was statistically significant in sepsis group (P< 0.001) (Figure 1).

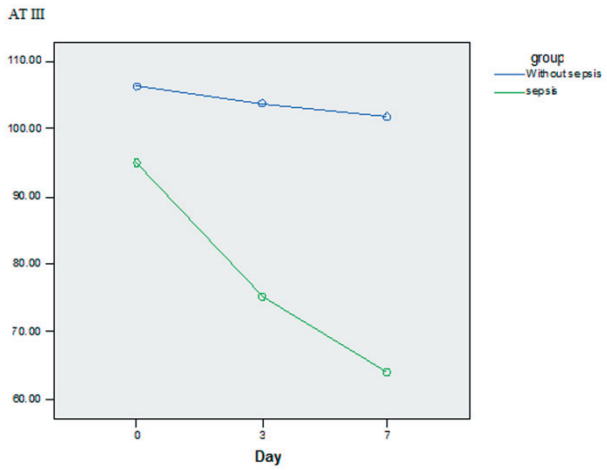

Figure 1: AT III changes in both groups during the study.

Serum AT III level variations were studied in patients with and without sepsis at different time points with repeated measured ANOVA test. It was shown that serum AT III level variations were statically significant in both groups (P< 0.001) (Figure 2).

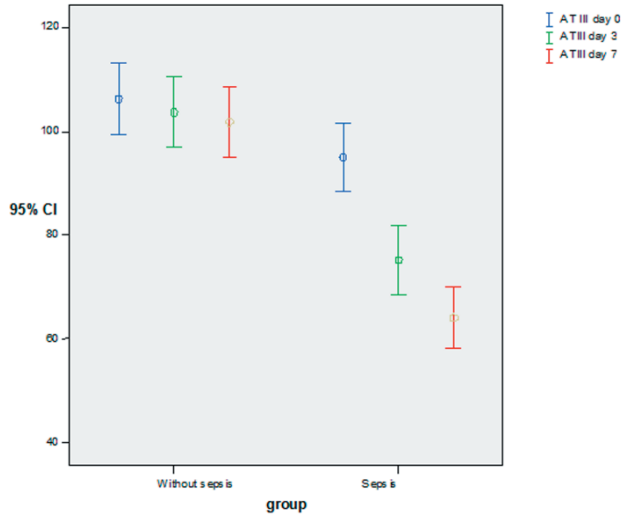

Figure 2: Repeated measured ANOVA of serum AT III level at different time points.

Discussion

DIC is seen in different medical conditions such as shock, trauma, burns, infection, cancer and pre-eclampsia. Protease activity is uncontrolled during this syndrome, so a lot of events occurs in the coagulation system and eventually it leads to multiple organ damages. Tissue factors which are released from damaged tissues lead to PT prolongation, thrombocytopenia, hypofibrinogenemia and increased fibrin and fibrinogen degradation products. AT III level also decreases during DIC.

In our study it was shown that serum AT III level decreased in traumatic patients with or without sepsis but it did not significantly decrease in non-sepsis group (P= 0.74).On the other hand, the decrease was statistically significant in sepsis group (P< 0.001).

Blood AT III level decreases during catastrophic events. In some studies, this marker has been suggested as a predicting factor of outcome especially in septic DIC (6, 7). Blood AT III level was measured in a group of 59 traumatic patients and its relationship with outcome and infection clarified that lower AT III level showed correlation with worse outcome (1, 3).

Here, AT III variations were studied at different time points. These variations were statistically significant in both groups (P< 0.001).

AT III concentration predicts outcome. Early detection of AT III reduction helps us to manage DIC. Administration of a natural anticoagulant like AT III (if available) can be very effective. It has been shown in several animal studies (4).

AT III concentration was studied in Schreiber study. It was shown that AT III activity reduces in traumatic patients, especially in severely injured patients. AT III spontaneously increases in all studied groups, so there is no clear impact of AT III activity on survival (5, 8).Several complex serial events affect coagulating system during trauma. Routine hematologic tests cannot adequately describe these events. With analysis of some new agents like AT III, survival may be improved (9). In an experimental study, serum AT III level was very low in a highly histoincompatible model of rat lung allograft rejection. Treatment with AT III improved parameters of acute inflammation in studied group. Serum AT III level was accompanied with worse outcome (10).

Plasma AT III level decreases during trauma and the decrease is profound in more severe traumas. Thrombo-embolic events in traumatic patients may be increased due to lower plasma AT III level (11, 12). High dose AT III administration interrupted coagulopathy and improved outcome in several septic patients (13).

In a prospective study, incidence of low AT III levels and its association with selected clinical variables in adult traumatic patients was investigated. AT III was about sixty percent lower in traumatic patients. It was associated with more severe traumas (14, 15). Plasma AT III levels were compared in lethal and nonlethal murine anthrax models. Plasma AT III level depletion is proceeding through anthrax cougulopathy (16, 17). In contrast to the aforementioned facts, Moubarak suggested that AT III therapy cannot improve prognosis in septic patients and plasma AT III level cannot predict the outcome (18).

According to our results, serum AT III levels decreased at different time points but this was more prominent in patients with sepsis. A lot of studies showed that sepsis has worse prognosis in traumatic patients (1, 3, 13), for this reason close measurement of serum AT III levels at different time points in traumatic patients may predict the sepsis occurrence.

In an experimental study AT III administration had many advantages in DIC management in pigs and decreased plasma AT III level predicted the worse prognosis (19).

According to the results of our study, serum AT III level was lower in traumatic patients who developed sepsis. Intergroup study showed that serum AT III level variations were not significant in patients without sepsis, but it was statistically significant in sepsis group. So serum AT III level measurement can predict the occurrence of sepsis in traumatic patients at earlier stages.

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