Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4995 - 5001

Received 11 May 2021; Accepted 15 May 2021.

Cytokines (TNF-α, IL-1Ra), CortisolResponse in Patients with Severe Thoracic Trauma

Mamur M. Abdurakhmanov, NutfulloKh. Rakhmonov, Zufar M. Abdurakhmanov*
Bukhara State Medical Institute, Bukhara, Uzbekistan

***Correponding author:** MD, PhD Zufar M. Abdurakhmanov, Department of Surgical Diseases and Intensive Care, Bukhara State Medical Institute, Bukhara, Uzbekistan; Mob.: +998907180051; email: z_abdurakhmanov@yahoo.com; **ORCID ID** http://orcid.org/0000-0002-0444-9791

ABSTRACT

Background: Severe thoracic trauma (TT) is often associated with severe infection and multiple organ dysfunction syndrome burdened with even lethality. This study was aimed to assess the concentration of immune markers in the early posttraumatic phase hours in order to comprehend immune response to severe TT. **Materials and methods:** From 2015 to 2020, patients with severe TT (aged 18-74 years) and 30 healthy volunteers were enrolled in this prospective study. **Results:**A total of 249 patients with TT were treated, of those, 47 patients met the inclusion criteria.There was a male prevalence with male to female ratio of 6,8:1.The mean ICU stay was 12,3±8,8 days. The meanhospital stay of TT patients was 20,4±15,4 days. The mean concentration of TNF-αwas significantly higher on hours 3, 6 and 24, respectively, was 243,65±37,57 ($p<0,001$), 169,71±34,49 ($p<0,001$), 138,54±25,28 ($p<0,001$), whereas that of IL-1Ra - 6247,87±834,37 ($p<0,001$), 5829,42±385,58 ($p<0,001$)1229,42±257,47 ($p<0,05$).On day 1 of admission, the cortisol concentration in serum of patients with later inflammatory complications after severe TT was significantly lower compared to that of patients without complications and healthy volunteers ($p<0,05$).**Conclusion:** Early immunological diagnostics within the first three hours of hospitalization can facilitate a modulated immunotherapy to balance physiological and pathological immune responses to severe TT. Referring to evidence in terms of relationship of low cortisol level and later complications, future studies should focus on analysis of cortisol-based immune system disorder to extend our knowledge and understanding of TT.

Keywords
immune response, thoracic trauma, cytokines, TNF-α,IL-1Ra, cortisol.

INTRODUCTION

One-third of all trauma cases present with severe TT, two thirds of which associated with severe infections, multiple organ dysfunction syndrome and a high incidence of mortality[1-3]. In this regard, it has been known that an excessive systemicpro-and anti-inflammatory mediators play a crucial role in the response to innate immune disorders in the first post-traumatichours [4]. Nevertheless, the mechanism of the immune disorders is still poorly studied because of its multifactorial complex nature that still contributes to a high post-traumatic mortality rates with an increasing tendency and significant peak in morbidity, length of hospital stay [5]. The detection of the concentration of immune markers in peripheral blood at early hours after TT may have clinical and practical significance in the early proper diagnosis and adequate treatment. Therefore,this study was aimed to better clarify the mechanisms of early immune response to TT by mean of evaluating changes in the level of tumor necrosis factor-α (TNF-α), interleukin-1 receptor antagonist (IL-1Ra) and as well as cortisol in the serum of patients who have suffered severe TT and acute blood loss.

MATERIALS AND METHODS

A prospective observational study conducted on patients with severe TT, aged between 18 and 74 years, who were hospitalized in surgical unit of Bukhara branch of Republican specialized emergency center between December 2015 and December 2020. Evaluation of severity of chest injury was carried out using injury severity score (ISS). The inclusion criterion of TT patients was the ISS≥16. The informed consent was obtained from the patient's next of kin. None of the patients died.

Sample collection and measurement. Blood samples were collected on hours 3, 6, 24 and days 7, 14following severe chest trauma with acute blood loss. 30 healthy volunteersin similar range and gender were enrolledsimultaneously as controls for the blood samples.The cytokines (IL-1β, TNF-α) and cortisol were assayed in serum, and the estimation was done by enzyme-linked immunosorbent assay (ELISA)using reagents of test systems of "Proteinovy

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4995 - 5001

Received 11 May 2021; Accepted 15 May 2021.

contour Ltd" (St. Petersburg).The sample of venous blood was centrifuged at a speed of 3000/min for 15 minutes, then plasma for further tests was stored at -20˚C for further assay using ELISA kit. According to the results in the control group, the assumed normal range for TNF-α was on average 49,25±5,7pg/ml, and 1088,07±385,48pg/ml for IL-1Ra.

Statistical analysis. Data were processed by using SPSS 19.0 statistical analysis software. The quantitative data were expressed as the meanand standard deviation (SD) and median (min-max) for normal and skewed data, respectively.Continuous variables were compared using t test and the rank-sum tests. P<0,05 was considered statistically significant, whereas P<0,001 was highly significant.

RESULTS

During the study period, 249 patients of TT were admitted and managed in our department.Of them, 47 patients with severe TT required ventilatory support and were included in the study. The male-female ratio was 6,8:1.Mean age was comparable between cases and controls (38,4±11,3 years vs. 39,7±10,1 years, p=0,57).The mean intensive care unit stay was 12,3±8,8 days with a median of 11 days.The meanhospital stay of TT patients was 20,4±15,4 dayswith a median of 19 days.

The mean values of TNF-α and IL-1Rawas compared between the controls and baseline values of the patientat all follow-up periods(Table 1). Serum TNF-α were high significantly raised within 1 day (p<0,001), which generally reached peak on hour 3, whereasthe current marker decreased daily throughout days 1, 7 and 14 of observation after TT. On day 7 after trauma, the levels of the TNF-α were still higher than the normal limits (p<0,05).

Table 1. Comparison of cytokines in serum samples of thoracic trauma cases vs. control.

Post-trauma time (hours/days)	Group	TNF-α (pg/ml)	IL-1Ra (pg/ml)
	Control (n=30)	39,25±5,73	1088,07±385,48
3h	TT (n=47)	243,65±37,57***	6247,87±834,37***
6h		169,71±34,49***	5829,42±385,58***
24h		138,54±25,28***	1229,42±257,47*
7d		55,58±14,18*	1095,39±207,58
14d		45,74±5,47	1084,28±104,37

TNF - α tumor necrosis factor, alpha, *IL-1Ra*- interleukin receptor antagonist, *TT* - thoracic trauma.
*** Indicates statistically high significant from healthy volunteers at p<0,001, whereas **-at p<0,01 and * -at p<0,05.

IL-1Ra high significantly reached peak on hour 3 (p<0,001), which gradually dropped throughout hours 6 and 24 (still higher than the normal limits at p<0,001 and p<0,05, respectively), but failed to reach a statistically significant increase as compared with that of control values on days 7 and 14 of observation after TT.

In addition, at 3 hours of observation, the level of IL-1Ra in patients with infection and acute bleeding significantly higher compared to the group without complications (7750,74 [857,94-17400,37] pg/ml vs. 880,2 [75,81-12900] pg/ml(p<0,01), respectively, (Figure 1). In the group with complications, the highest increase was observed at 12 hours of the study (9005,48 [1737,25-17559,15] pg/ml, while in the group without complications at six hours of observation(p<0,001). Onhour 6, concentrations of IL-1Ra in both groups were not at

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4995 - 5001

Received 11 May 2021; Accepted 15 May 2021.

similar levels and averaged in complicated and no complicated patients 5545,9 (735,78-15700,58) pg/ml vs 1890,45 (454,83-15900,37) pg/ml($p<0,05$), respectively.

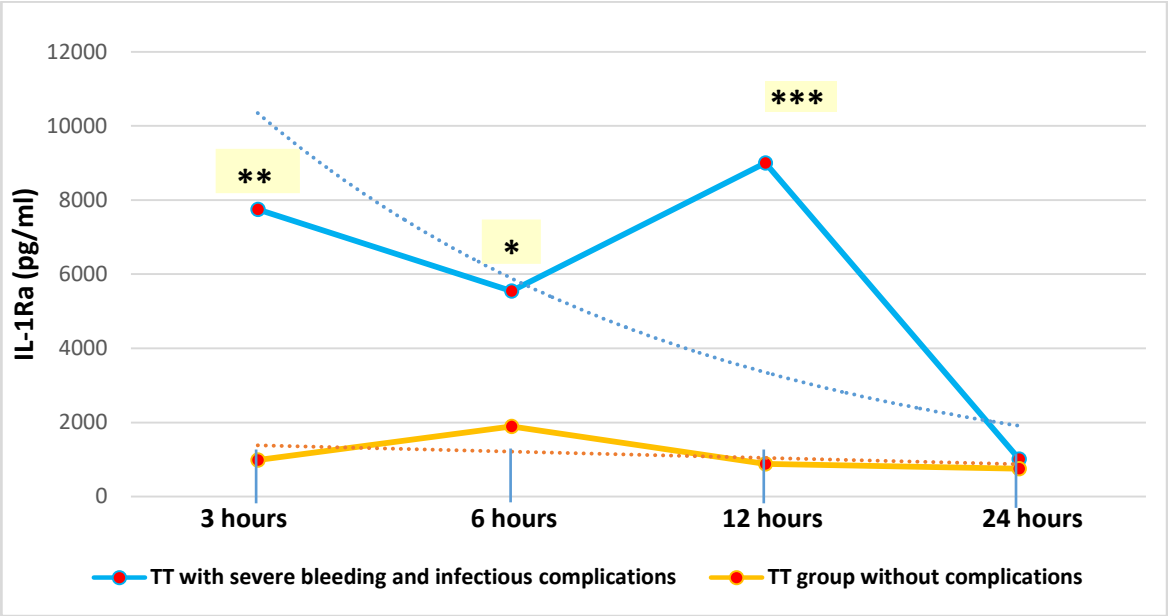
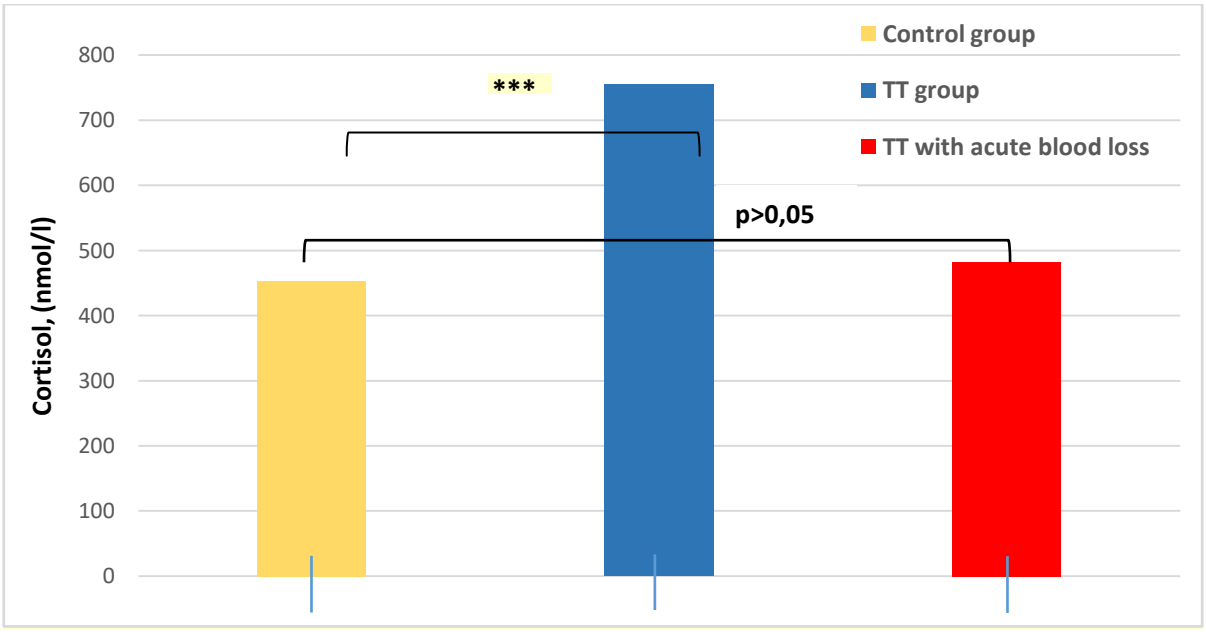


Figure 1. IL-1Raconcentrationin comparison to the complications.

IL-1Ra- interleukin receptor antagonist, *TT* - thoracic trauma.
*** Indicates statistically high significant from healthy volunteers at $p<0,001$, whereas ** -at $p<0,01$ and * -at $p<0,05$.

A significantly higher level of the cortisol in serum was observed in patients on the 1st day after suffering a severe chest injury compared with control values in healthy people (755,78±103,49 vs 453,25±105,47 nmol/l, respectively, $p<0,001$). Although the concentration of cortisol in the blood serum of patients after TT with acute blood loss at all periods of observation did not significantly differ from that of control group (482,25±93,37 vs 453,25±105,47 nmol/l, respectively, $p>0,05$) (Figure 2).



Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4995 - 5001

Received 11 May 2021; Accepted 15 May 2021.

Figure 2. Comparison of cortisol serumE level (nmol/l) of thoracic trauma cases vs. control on 1st day.

TT - thoracic trauma.*** Indicates statistically high significant from healthy volunteers at P<0,001.

Of particular interest, by the 1st day after admission, the cortisol concentration in serum of patients with later inflammatory complications after severe TT was significantly lower compared to that of patients without complications and healthy volunteers (p<0,05).

DISCUSSION

As previously reported, post-traumatic respiratory system injury has been considered as a trigger in the development of multiple organ dysfunction syndrome, even death.

The results of Ayan et al. experimental TT model [2] showed that a severe inflammatory response develops during the first 24-48 hours in patients blunt TT, but normal rangeswerereached at 72 hours. So, in order to clearly identify patients burdened with high risk of severe complications in patients with TT, early immune response mechanisms to trauma should be better investigated, which may predict the further outcome of the disease and be useful in designing future therapeutic intervention. To the best of our knowledge, previous studies are scarce regarding the evaluation of immunologic mechanisms and early cytokine responses to isolated TT and mostly have focused on severe multiple traumas. Thus, our goal in this study is to facilitate research onassociation of early inflammatory cytokines with severity of disease and final clinical prognosis in patients with isolated TT.

The results of the present study suggest that the evaluation of concentrations of selected pro- and anti-inflammatory cytokines (TNF-α and IL-1Ra) in the serum of posttraumatic patients can be helpful markers handling the identification of patients with increased risk of complications.

TNF-α showed to be released immediately and peaked on hour 3 after TT that confirmed the study of Binkowska et al [5]. The peak value of TNF-αon early post-traumatic houralso acknowledges the consideration of other study authors that TNF-α is primary mediator and inductor in the activation of other cytokines [2,6]. We established that the concentration of TNF-α dropped daily throughout days 1, 7 and 14 of observation after TT and was still higher than the normal limitswithin 7 days. We did not encounter any death case that is probably due to the gradual decrease of TNF-α level which consistent with suggestion of Barnett et al. that persistently elevated plasma level of TNF-α in chest trauma patients is a predictor of mortality [7]. Similarly, Ayan et al. obtained the results in terms of TNF level in the TT patients which were higher than those of the control group at 24 hours [2]. Bagaria et al. revealed thatIL-1β, IL-6, and IL-10 were significantly raised in bronchoalveolar lavage of TT patients, whereas TNF-αfailed to reach a statistically significant increase as compared with that of controls.In contrast to our work, one should take into account that the latter study did not control time-basedfluctuations in cytokine concentrations during the first hours of treatment, and only correlated with TT severity score (TTSS) [1].The discrepancy between the authors of different groups may be also interpreted in relation to time-based variation of TNF-α as the latter is the primary mediator in the activation of other cytokines [8, 9] that indicates a higher usefulness of TNF-α assessment in the early period after TT. So, similarly as by us,the suggestions have been givenby other group of authors that a persistent increase in the level of TNFα in severe TT is an unfavorable prognostic sign indicating a high probability of developing septic complications and death [10-13].

In the last years, the evidence of the increased concentration of TNF-α at early 1-3 hours after TT was confirmed via the changes in the coagulation system. It has been shown, that the plasminogen activator urokinase receptor significantly increases that to be

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Received 11 May 2021; Accepted 15 May 2021.

upregulated by the increased level of TNF-α [14] early following clinical and experimental blunt TT [15].

In our study, the values of IL-1Ra concentration was significantly higher than that of the control group up to 12 hours of observation thatcoincides with judgement of other centers that the assessment of IL-1Ra concentrations can be meaningful in the early determination of post-traumatic complications [16].Similarly as for TNF-α, the highest concentrations of IL-1Ra were observed in the third hour of the study, which may indicate a higher usefulness of IL-1Ra assessment in the early period after TT.The significantly eight-fold higher level of IL-1Ra at 12 hours of the study relative to that at 3 hours in patients with later complications compared with the group with no complications may be interpreted in relation with the cumulative effect of such complications as acute bleeding and infection. As in our study, the highest value of IL-1Rawas recorded three hours after admission in patients with later complications compared with the group without complications in the examination of Binkowska et al [5], who found the diagnostic value of the IL-1Ra by the determination of a statistically significant positive correlation between baseline concentrations of IL-1Ra and the values of the ISS. Moreover, basing on the ROC analysis, the author of the latter study observed the high sensitivity and specificity of the indicators (0,8/0,83 for IL-1Ra), indicating that the studied cytokine may be considered as a high sensitive and specific marker of serious complications, including sepsis, death in post TT patients.

Of particular interest, Ehrnthaller et al. studied transcriptional programs of cytokines in response to TT and found that gene expression for IL-1α is upregulated about threefold 1 hour after trauma while the genes forthe TNFα-receptor displayed a robust upregulation1 h through 6 h after blunt TT[17].It should be emphasized that the latter work confirms enough precisely the results of the current study at the molecular level.

The results of the current study found a lower concentration of cortisol in the blood serum of patients on the 1st day after severe TT that may be an unfavorable sign indicating a high probability of developing sepsis or death. These data confirm the results of clinical observations, which established a correlation between low cortisol levels and the risk of developing sepsis in patients with TT[18]. A high concentration of cortisol in the blood serum of people with severe TT, on the contrary, is a typical, natural response of the body's neuroendocrine system to an extremely strong stressful effect caused by TT [19]. Thus, it has been needed to unravel the cortisol-based mechanisms of innate immune disorders opening up the possibilities for the development of highly informative prediction of the course of TT with acute blood loss and its purposeful immune-oriented therapeutic correction.

CONCLUSION

To summarize, the functional activity of immunocompetent cells changes following severe TT at various times after hospitalization of patients. Thus, our findings suggest that at three hours of surveillance, the inflammatory response by means of measuring the levels of cytokines, one can determine patients burdened with high risk of later complications (severe infection, multiple organ dysfunction syndrome, sepsis and death) after TT. In order to clearly observe a relationship between subsequent complications and cytokine response, one should conduct a complex evaluation of the level of cytokines at early post-traumatic period and their correlations with ISS or TTSS.Early immunological diagnostics within the first three hours of hospitalization (“obvious diagnostic window”)can facilitatea modulated immunotherapy to balance physiologicaland pathological immune responses. Nevertheless, confirmation of the clinical usefulness of measuring the concentration of markers (TNF-α and IL-1Ra) requires further studies in a larger cohort of TT patients. Besides, basing on evidence in terms of relationship of low cortisol level and later complications, future studies should focus on

Received 11 May 2021; Accepted 15 May 2021.

analysis of cortisol-based immune system disorder to extend our knowledge and understanding of TT.

CONFLICTS OF INTEREST

The authors have declared no conflicts of interest.

ACKNOWLEDGMENT

We are grateful to the staff members of Bukhara State Medical Institutefor the cooperation and support in our research.

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