TNF-α, VEGF, and Procalcitonin Levels Dynamic Changes During Severe Traumatic Brain Injury

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Abstract

BACKGROUND: The variety of traumatic brain injury (TBI) creates difficulty in evaluating its level and the clinical outcome correctly. This study aimed to analyze the level variations and dynamic of serum biomarkers, such as tumor necrosis factor (TNF)-α, vascular endothelial growth factor (VEGF), and procalcitonin (PCT) in response to severe TBI.

METHODS: Intravenous blood samples were collected from 20 TBI subjects at different time points: 0, 12, 24, and 48 hours. The serum levels of TNF-α, VEGF, and PCT were measured using specific monoclonal antibodies by quantitative sandwich enzyme-linked immunosorbent assay (ELISA).

RESULTS: In 0, 12, 24, and 48 hours, the serum levels were significantly higher for TNF-α ($p<0.0001$), VEGF ($p<0.0001$) and PCT ($p<0.0001$) compared to the healthy control. In comparison to admission time point, TNF-α had elevated significantly ($p<0.001$) at 24 hours. PCT showed a significant increase after 48 hours ($p<0.02$) and VEGF showed no significant differences. Comparing the 3 biomarkers dynamic changes at 0, 12 and 24 hours, PCT level showed to be lower than VEGF and TNF-α levels, while VEGF level showed to be higher than PCT and TNF-α levels. However after 48 hours, PCT level (0.25 ng/mL) had elevated more than VEGF (0.21 ng/mL) and TNF- α (0.18 ng/mL) levels.

CONCLUSION: Monitoring PCT in comparison to VEGF and TNF-α can be used to assist the progress of severe TBI, since PCT level progressive changes were associated with time increase.

KEYWORDS: trauma, TNF-α, VEGF, PCT, Glasgow Coma Scale

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Introduction

Predicting the outcome of patients with a life-threatening illness is a concern of intensive care unit physicians, it is important to recognize whether the patient has a highly favorable or unfavorable outcome. Traumatic brain injury (TBI) is an injury caused by an external force that traumatically injures the brain. Annually, an estimated of 10 million people suffered from TBI worldwide.(1)

Glasgow Coma Scale (GCS) score is the most important tool in predicting the outcome after TBI, and the most studied classification on the admission of TBI patients that reflects the severity of the trauma.(2) Nevertheless, GCS has its disadvantages such as the accuracy differences between the experienced and highly trained users compared to the inexperienced users (3), it was found that the agreement percentage for exact total GCS between physicians was 32% (4) GCS classified TBI into three groups: mild TBI (GCS 13-15), patients in this category usually have concussion

with full neurological recovery; moderate TBI (GCS 9-13), patients in this category are lethargic or stuporous; and severe TBI (GCS 3-8), patients classified into this category are in comatose or unable to open their eyes or follow commands. (5) GCS scoring system was proven to be successful (80%) in predicting a favorable outcome in patients with TBI that had a GCS score <8 at admission but improved to a ≥8 score within the first week (6), unfortunately, the one-week waiting time for predicting the outcome makes it a relatively slow prediction tool.

TBI is described by pathology progress, the time-dependent changes almost consistently after the primary injury.(7) Thereby, prognosis and diagnosis approaches based on biomarkers can only be interpreted from the perspective of pathology progression. The kinetic response for biomarkers expression and transfer across the blood-brain barrier (BBB) to the blood behave, represent indirect relation to TBI pathology.

Within minutes after the primary injury, numerous immune mediators will be released, leading to the acute post-traumatic period of TBI. These events cause the BBB to increase its vascular permeability causing the entry and accumulation of leukocytes in the damaged tissue, initiating an increase in the production of pro-inflammatory cytokines, anti-inflammatory cytokines, inflammatory biomarkers, and growth factors (8,9), such as tumor necrosis factor-α (TNF-α), vascular endothelial growth factor (VEGF), and procalcitonin (PCT).

TNF-α is a cytokine that is initially expressed as a trimeric membrane-bound cytokine. It is suggested that TNF-α plays a role in mediating post-traumatic inflammation and contributes to neuronal injury.(9,10) TNF-α is used as a marker of heart failure progression indicator (11) and insulin resistance (12). It was found that in TBI patients the TNF-α showed a stereotyped temporal peak on day one which was at least twice the median value for the TNF-α over 5 days monitoring period.(13) Which justifies using TNF-α as a predictor of the outcome of patients with TBI.

VEGFs are potent pro-angiogenic cytokines that stimulate vasculogenesis, angiogenesis, and neurogenesis. The VEGF family is the downstream of hypoxia-inducible factor (HIF)-1α activation, and comprises in mammals 5 members: VEGF-A, placenta growth factor (PLGF), VEGF-B, VEGF-C , and VEGF-D, with 3 types of receptors (VEGFR-1, VEGFR-2, and VEGFR-3).(14) VEGF and brain derived neurotropic factor levels are used as an indicator of ischemic stroke.(15) Infused TBI mice with externally VEGF for 1 week after TBI have improved proliferating cell number in the perilesional cortex, these

changes were associated with significant improvement in the recovery parentage and functional outcome.(16) Most activity of VEGF in the brain involves VEGF-A and VEFGR-2, however VEFG-B, PIGF, and VEGFR-1 appear to be involved to a lesser degree.(17)

PCT is composed of 116 amino acids and is physiologically synthesized by thyroid C cells. Many studies showed that PCT could be used as a strong indicator tool of inflammation and sepsis.(18,19) PCT level measurements were higher in multiple-trauma patients that associated with further severe trauma and a higher incidence of several complications, counting inflammation and sepsis.(19)

This study aimed to investigate the time-dependent associations between serum biomarkers concentration levels of TNF-α, VEGF, and PCT in severe TBI patients, and to evaluate their prognostic sensitivity value in association with the severity of injury and mortality in the first 48 hours which can help to determine the TBI patient's outcome in addition to GCS score.

Methods

Subjects Recruitment
A total of 20 male patients aged 18-52 with TBI who were admitted to the Intensive Care Unit (ICU) of Jordanian Royal Medical Services, Amman, Jordan were recruited in this study. During the admission, the following information was obtained from each patients: type of injury (accident, or attack, etc.), chronic history, GCS classification (severity of trauma), gender, and age.

The inclusion criteria for the subjects were: admitted within 12 hours of injury, age ≥18 years old, have severe TBI (GCS 3-8). Subjects who didn't get admitted within 48 hours after injury and subjects on antibiotics or receiving immune repressing therapy for more than 3 days before the admission, were excluded from the study. Approval from the Ethics Committee of the Jordanian Royal Medical Services (No.: 2011–281) was obtained. Every subjects also have given their approval and informed consent.

Blood Samples Collection
Intravenous blood samples for the measurement of TNF-α, VEGF, and PCT diagnosis were collected from subjects after admission at 0, 12, 24, and 48 hours. Samples were analyzed in the Department of Biochemistry, Biolab Diagnostic Laboratories, Amman, Jordan. Samples were centrifuged (10 minutes at 1000x g) for serum separation and stored at -20°C to -70°C before analyzed.

Measurement of TNF-α, VEGF and PCT Levels

The serum levels of TNF-α, VEGF, and PCT were measured using specific monoclonal antibodies by quantitative sandwich enzyme-linked immunosorbent assay (ELISA) (ABclonal Biotechnology, Woburn, MA, USA). Marker assays measurement was done based on the datasheet instructions supported with the commercial kits as follows: TNF-α (Human Tumor Necrosis Factor Alpha, Cat. No: RK00030, ABclonal Biotechnology), VEGF (Human VEGFA ELISA Kit, Cat. No: RK00023, ABclonal Biotechnology), and PCT (Human Procalcitonin ELISA Kit, Cat. No: RK02032, ABclonal Biotechnology). The cut-off points used were <0.0028 ng/mL for TNF-α, <0.0962 ng/mL for VEGF, and <0.05 ng/mL for PCT, as suggested by ABclonal Biotechnology

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 9.1.1.225 (GraphPad Software, La Jolla, CA, USA). All data were represented as means±standard error (SEM). One-way analysis of variance (ANOVA) with Dunnett`s analysis was used to find out statistical differences of markers level between time points.

Results

Subjects' Characteristics

Total 20 male subjects with median of age 30 (18-52) years old were included in this study. All subjects had injury severity score between 3-8 (severe TBI) and stayed at the hospital for more than 2 days. The cause of the injury mostly was from traffic accident (60%) and followed by fall (25%) and assault (15%).

TNF-α, VEGF, and PCT Levels

Serum TNF-α, VEGF, and PCT levels were quantified and calculated (Table 1). First, the comparison among different time points for each marker was analyzed. The serum levels of TNF-α were significantly higher ($p<0.0001$) in all-time points (0, 12, 24, and 48 hours) when compared to the control group, especially more noticeable in 12, 24, and 48 hours. Significant difference was also found between 0 hours and 24 hours time points ($p<0.001$) (Figure 1A).

Serum levels of VEGF were significantly higher ($p<0.0001$) at 0, 12, and 48 hours time points when compared to the control group. Meanwhile, the comparison of VEGF level between 0 hour time points to at 12, 24 and 48 hours time points showed no differences (Figure 1B).

However, the result of PCT level showed different pattern than TNF-α and VEGF levels. The PCT were significantly higher ($p<0.0001$) in all-time points (0, 12, 24, and 48 hours) when compared to the control group, and higher upregulation at 48 hours was seen. Incremental elevation in the PCT level was in line with the duration of hospital stay. The 12 and 24 hours time points showed a stable increase of PCT level compared to 0 hour time point, while 48 hours had shown significant upregulation ($p<0.02$) (Figure 1C).

We also analyzed the variations between targeted markers and their dynamic in each time point. At 0, 12 and 24 hour, PCT level was lower than VEGF and TNF-α. However, at 48 hours, PCT level (0.25 ng/mL) had increased more than VEGF (0.21 ng/mL) and TNF-α (0.18 ng/mL) (Figure 2).

Discussion

Serum biomarkers are associated with TBI, therefore it is essential in monitoring TBI patients' condition. Biomarkers may support in identifying patients at great risk in the first 48 hours and also during follow-up.

In this study, it was found that TNF-α level was significantly elevated at the time of admission and remained high in the next time points whether compared to the healthy controls. It got more notable at 24 hours time point as compared to the admission. TNF-α showed to be expressed less than VEGF, but higher than PCT in each time point, except at 48 hours.

Our results are compatible with previous studies, in which continual and significant elevation of TNF-α levels are recognized 4 hours after trauma.[20] TNF-α peak levels are identified 24 hours after injury. Additionally, TNF-α levels remains significantly high for up to three days after trauma.[21] TNF-α has been described to be secreted faster than other pro-inflammatory cytokines.[22] Increased TNF-α levels following trauma are described to be negative to the body.[23] TNF-α levels upregulation has been displayed

Table 1. TNF-α, VEGF, and PCT levels in each time point.

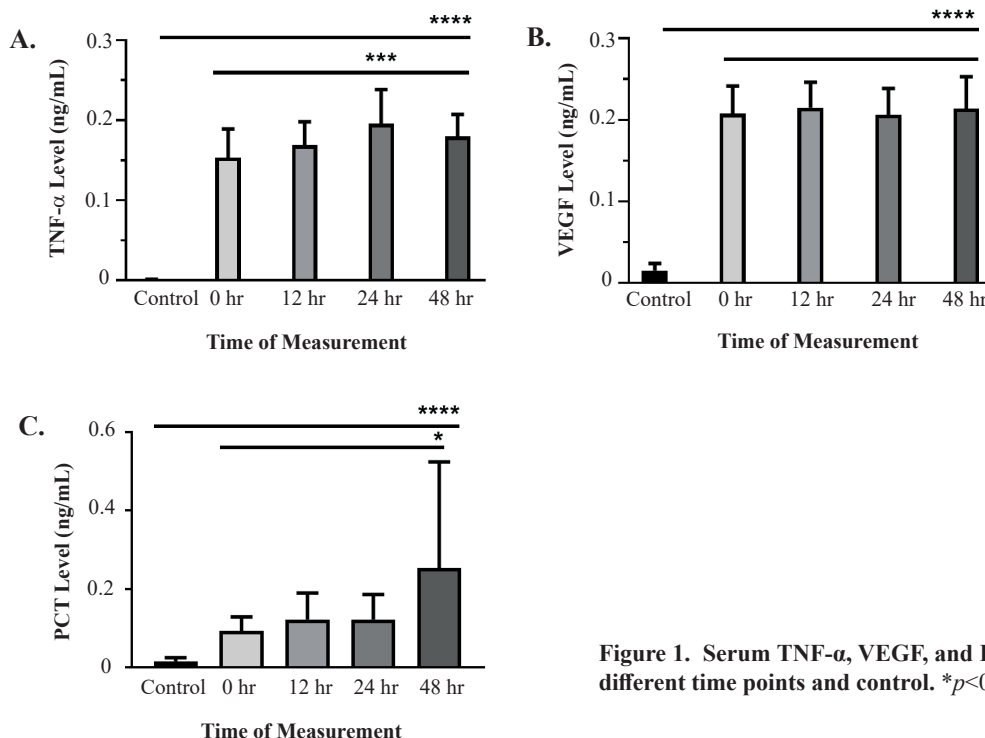| Time Point of Measurement	TNF-α (ng/mL)	VEGF (ng/mL)	PCT (ng/mL)
0 hour	0.15	0.21	0.09
12 hours	0.17	0.21	0.12
24 hours	0.20	0.20	0.12
48 hours	0.18	0.21	0.25

Figure 1. Serum TNF-α, VEGF, and PCT levels comparison between different time points and control. *p<0.02, ***p<0.001, ****p<0.0001.

to promote hyperalgesia and central sensitization.(24) Nonetheless, after brain traumatic injury, TNF-α utilizes a neuro defensive contribution through neuroanatomical plasticity.(25)

In the brain of mild TBI-challenged mice, the time-dependent rise of TNF-α level is highest at 12 hours and decrease to baseline level by 18 hours.(26) Several studies of TBI models have described a correspondence of TNF-α elevation in the brain within a few hours of trauma.(27) In the CSF of TBI patients, TNF-α levels up-regulation have been identified 22 days after injury and showed a fluctuation in brain samples from TBI patients taken initially follow injury less than 20 min compared with later following injury 6-120 hours.(28,29) TNF-α levels are shown to be higher in both initially and late follow injury groups in comparison with controls.(28)

In the present study, VEGF level was significantly increased at the time of admission and this elevation continued in the next time points in comparison with healthy controls, however, there were no obvious changes on its level among different time points as compared to admission. VEGF expression was higher than TNF-α and PCT in all-time points except for 48 hours.

Studies indicate that VEGF levels are elevated around the injury site in chronic stages of TBI (30), as it also shows an elevation in VEGF expression following TBI (31). The early secretion of VEGF is shown in mild TBI mouse model, whereas VEGF level is initially elevated significantly 6

hours after injury in comparison to pre-injury level, then declined at 24 hours which show no difference than baseline values.(32) Moreover, the highest number of neutrophils secreting VEGF is observed between 8 hours and 24 hours after TBI.(33)

VEGF stimulates repair and angiogenesis following TBI.(34) VEGF is also recognized as a vascular permeability factor, based on its potential to cause leakage of vessels (35). VEGF elevation has been connected by its involvement in the BBB breakdown, thus inducing the brain edema formation.

The results of current study revealed that PCT level was significantly increased at the time of admission and this elevation continued progressively in the next time points in comparison with healthy controls, reaching the highest level at time point 48 hours as compared to admission. PCT showed to be expressed less than VEGF and TNF-α in each time point except 48 hours where its level was higher than both.

The progression of sepsis and infections in patients with operating circumstances and trauma have been assessed by using PCT.(36) Moreover, PCT level fluctuations in response to medical therapy have been described, which proposes prognostic importance of it in different clinical aspects.(37) PCT level insistent elevation is linked with a longer period of staying at the ICU and mortality.(38) However, there is a shortage of significant data concerning the use of PCT in the therapy treatment of TBI.(39)
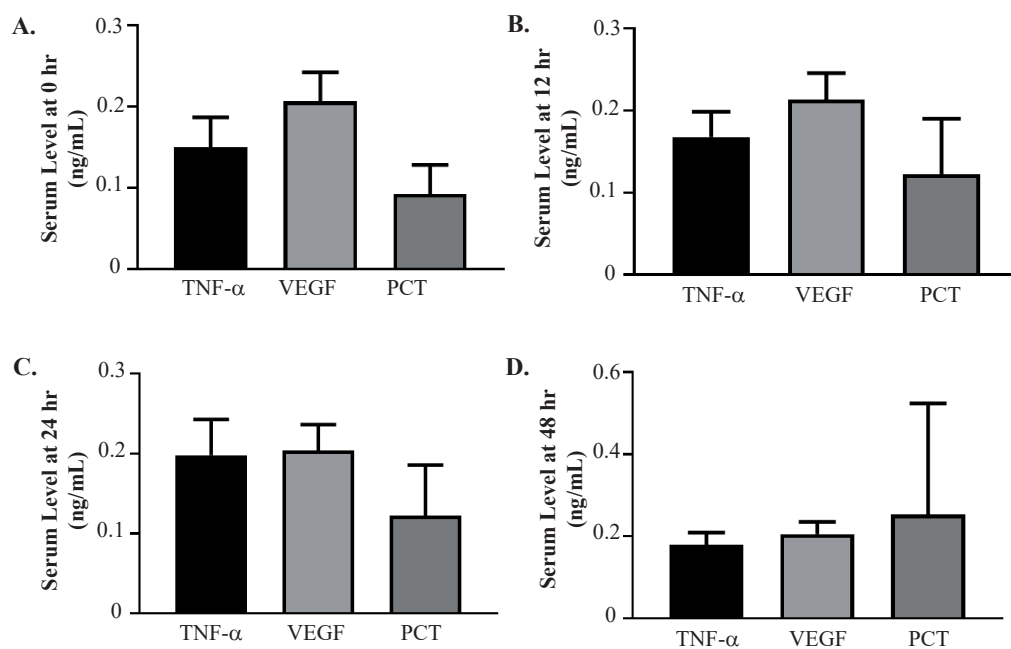
Figure 2. Serum level comparison at different time points.

This study had small sample size due to the ICU restrictions caused by Covid-19 pandemic, which decreases the study potential. However, the result of our study will guide to further analysis of the relationship between biomarkers levels and severe TBI patient outcomes.

Conclusion

From a clinical point of view, the results of the current study are significant, since one of the main problems of TBI patients follow-up in Jordan is the dependence on GCS score for their case evaluation and monitoring. PCT showed a correlation with time progress and to be more sensitive in comparison to VEGF and TNF-α. Therefore, setting up a delicate and reliable serum biomarkers system for monitoring during the first 48 hours can help to determine the TBI patient's outcome in addition to GCS score, decrease the rate of mortality and improve treatment excellence.

Acknowledgments

Authors Contribution

DH was involved in analysis and interpretation of the results, draft manuscript preparation, and visualization; YI was involved in the study design and data collection; AT performed experiment and data processing; MZ was involved in the manuscript preparation and visualization; AA was involved in the critical revision of the article.

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