Int J Legal Med (2006) 120: 265–270
DOI 10.1007/s00414-006-0081-2

ORIGINAL ARTICLE

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Postmortem cytokine levels and severity of traumatic injuries

Received: 13 May 2005 / Accepted: 6 February 2006 / Published online: 4 April 2006

Abstract The relationship between postmortem serum cytokine levels and severity of traumatic injuries was studied. The postmortem serum levels of interleukin-6 (IL-6) and interleukin-8 (IL-8) of 131 victims who died from traumatic injury were measured by an enzyme-linked immunosorbent assay method and compared with scores of total abbreviated injury scale (total AIS) and injury severity score (ISS) calculated from detailed autopsy reports. A significant positive correlation was observed between IL-6 and total AIS (rs=0.4508, $p<0.0001$), between IL-6 and ISS (rs=0.3337, $p<0.0001$), between IL-8 and total AIS (rs=0.6593, $p<0.0001$), and between IL-8 and ISS (rs=0.5305, $p<0.0001$). The significant correlation between cytokine levels and anatomical traumatic severity indicated that the cytokine levels are useful objective indexes of traumatic severity. In addition, the total AIS is a suitable marker to evaluate traumatic severity as the coefficient of correlation between the cytokine levels and the total AIS was higher than that for the ISS values.

Keywords Cytokine · Interleukin-6 · Interleukin-8 · Forensic pathology · Traumatic severity score

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Introduction

Forensic pathologists often diagnose traumatic death by the severity of organ injuries, fractures and/or, bleeding. It is difficult, however, in some cases with multiple traumatic injuries to objectively evaluate the damage of some organs and soft tissues. It is also difficult to measure precise amounts of extensive subcutaneous bleeding and muscular hemorrhages. Furthermore, all injuries observed during a forensic autopsy may not have been sustained whilst the victim was alive and it is sometimes difficult to determine which of these injuries were sustained before death. As a result, forensic pathologists have usually adopted a standard where a traumatic injury deemed to be severe enough to be lethal is considered to be the cause of death. Therefore, studies to develop a new objective method evaluating the severity of trauma seem to be essential in the field of forensic pathology.

Cytokines have recently been used for the quantification of inflammation caused by elective surgery, traumatic injury, infectious disease, tumors, and pancreatitis [1–7]. Furthermore, a postoperative measurement of cytokine levels has proven to be useful in both the management of surgical patients and the prediction of their convalescence [1–3]. Cytokines are useful markers of tissue inflammation and are suitable for the evaluation of surgery-associated stress. The cytokines interleukin-6 (IL-6) and interleukin-8 (IL-8) are circulating throughout the whole body and, therefore, systemically transmit localized inflammation. Therefore, IL-6 and IL-8 are said to be useful markers of inflammation [2, 8].

In the forensic field, several studies have been made on chemical mediators in skin wounds [9–12]. Furthermore, postmortem cytokine levels in systemic inflammation have been studied extensively by other researchers [13, 14]. However, these studies discussed the association between postmortem cytokine levels and sepsis. In the present study, we investigated the correlation between postmortem cytokine levels and the anatomical trauma severity score to find out if cytokine levels can indicate

the severity of the traumatic injuries that the victim sustained before death and, therefore, be helpful for diagnosing the correct cause of death.

Materials and methods

Study subjects

The subjects were 156 autopsy cases (traumatic death group: 131 cases; non-traumatic death group as a post-mortem control: 25 cases) where the age ranged from 15 to 90 years at the time of death. The duration between trauma and death was not always ascertained precisely; however, from the information available, we selected traumatic death cases for our study where the time of death was within 6 h after sustaining the traumatic injury. Forensic autopsies were performed within 2 days after death in all cases. The exclusion criteria included chronic infectious diseases, tumors, pregnancy, pancreatitis (these conditions were diagnosed by macroscopic examination and/or histological examination), and cases where the traumatic injury had been medically treated. In each of these situations, the cytokine levels may have increased due to various factors unrelated to the traumatic injury.

Samples

Blood samples were collected from the right atrium at autopsy and centrifuged immediately for 5 min at 3,000×*g* with the serum frozen at −30°C until analysis. Hemolytic samples were obviously excluded from the study.

Cytokine assay

The cytokines IL-6 and IL-8 were determined by an enzyme-linked immunosorbent assay (ELISA) using human IL-6 and IL-8 ELISA kits (Cytoscreen, BIO-SOURCE, Camarillo, CA, USA). Absorbance at 450 nm was measured with a microplate reader (BIO-RAD Model 680). When the absorbance could not be read because of high concentrations, the samples were diluted with the kit buffer. The minimum detectable doses of IL-6 and IL-8 in these assays were 2 and 5 pg/ml, respectively.

Scoring of the anatomical trauma severity

The abbreviated injury scale (AIS) for each traumatic injury that had been sustained before death was determined using the 1985 protocol of the American Association for Automotive Medicine [15, 16]. The AIS score ranges from 1 to 6, and the total AIS and the injury severity score (ISS) was calculated by adding each score of the AIS. The total AIS is the sum of all AIS scores in each of the following six body regions: head and neck, face, chest, abdomen, extremities, and the exterior skin. For example, a victim of a traffic accident with the following injuries will be scored as: skull fracture (AIS: three points), brain contusion (AIS: three points), three rib fractures (AIS: two points), pulmonary contusion (AIS: four points) and extensive abrasions to the head and chest (AIS: two points), and small abrasions to the leg (AIS: 1 point), giving a total of 15 points. Although the total AIS is not generally a standardized indicator of the severity of trauma, it seems to reflect the severity of anatomical traumas. The ISS is the most popular scoring system of anatomical trauma and is defined as the sum of the squares of the single highest AIS score in each of the three most severely injured body regions [17]. The ISS scoring system consequently works with values from 0 to 75. The ISS of the sample case described is, therefore, $3^2+4^2+2^2=29$ points.

Statistical evaluation

A non-parametric analysis was performed. The differences in cytokine levels between traumatic death vs non-traumatic death were compared using Mann–Whitney *U* test. Spearman rank correlation coefficients (two-tailed) were used to evaluate the correlation between cytokine levels and traumatic severity score (total AIS or ISS).

Results

Age, sex, and cause of death

Of the 156 autopsy cases (mean age 52.1±19.9 years), 109 were male (mean age 51.4±19.1 years) and 47 were female (mean age 53.8±21.7 years). The most common cause of traumatic death was head injury (37 cases) followed by exsanguination (35 cases), hemorrhagic shock (19 cases), traumatic shock (18 cases), multiple trauma (nine cases), chest injury (seven cases), and cervical injury (six cases). The total AIS values ranged from 4 to 57 points (mean 21.34±12.02 points) and the ISS from 8 to 75 points (mean 41.99±23.85 points). The most common cause of non-traumatic death was drowning (nine cases) followed by ischemic heart disease (seven cases), asphyxia (five cases), intracranial hemorrhage (two cases), and hypothermia (two cases).

Postmortem cytokine levels

Although serum IL-6 and IL-8 from five healthy volunteers were undetected by the kits used in our study, serum IL-6 levels range from 11 pg/ml to 3.3 μg/ml in the traumatic death group and range from 9 pg/ml to 2.7 ng/ml in the non-traumatic death group; serum IL-8 was from 7 pg/ml to 198 ng/ml in traumatic deaths and from 1 pg/ml to 1.3 ng/ml in non-traumatic death group. The cytokine level of traumatic death group was significantly higher than that of non-traumatic death group ($p<0.0001$), and the average

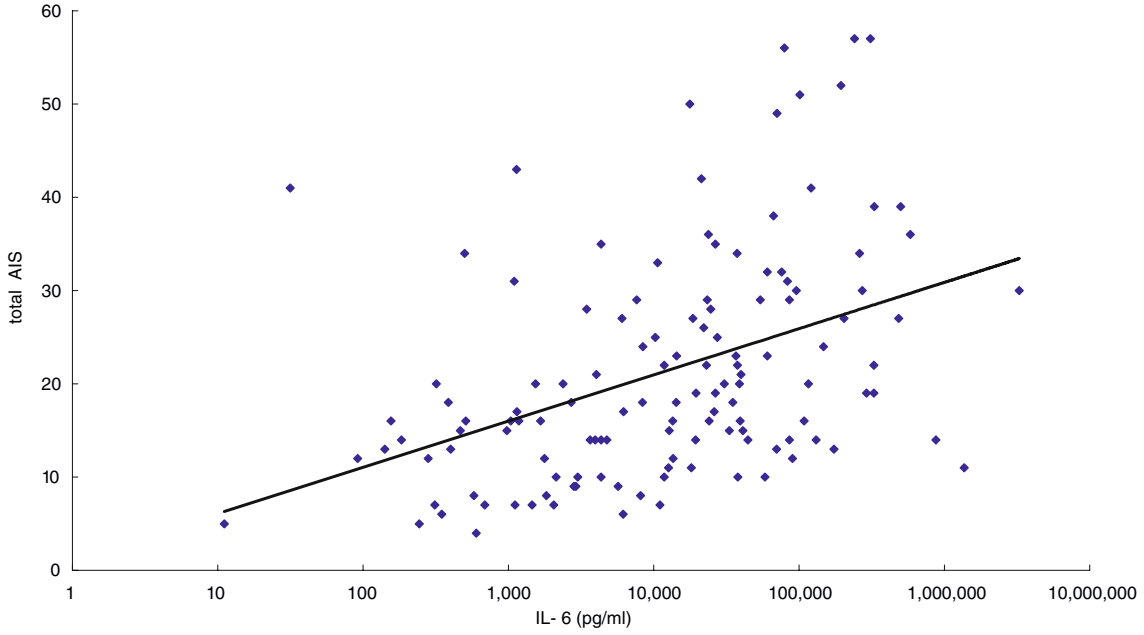

Fig. 1 Correlation between postmortem IL-6 levels and total AIS (rs=0.4508, $p<0.0001$)

level of non-traumatic death group as a postmortem control was 1.0 ng/ml in IL-6 and 152 pg/ml in IL-8.

Correlation between postmortem cytokine level and the anatomical trauma severity

In traumatic death group, there was a significant positive correlation between the postmortem IL-6 level and total AIS (Fig. 1; rs=0.4508, $p<0.0001$), postmortem IL-6 level and ISS (Fig. 2; rs=0.3337, $p<0.0001$), postmortem IL-8 level and total AIS (Fig. 3; rs=0.6593, $p<0.0001$), and postmortem IL-8 level and ISS (Fig. 4; rs=0.5305, $p<0.0001$). However, it should be noted that many samples in Figs. 2 and 4 reached the uppermost limit of 75 points of the highest ISS scores.

Discussion

Cells are destroyed by inflammation resulting from traumatic injury, while the inflammation, in turn, activates

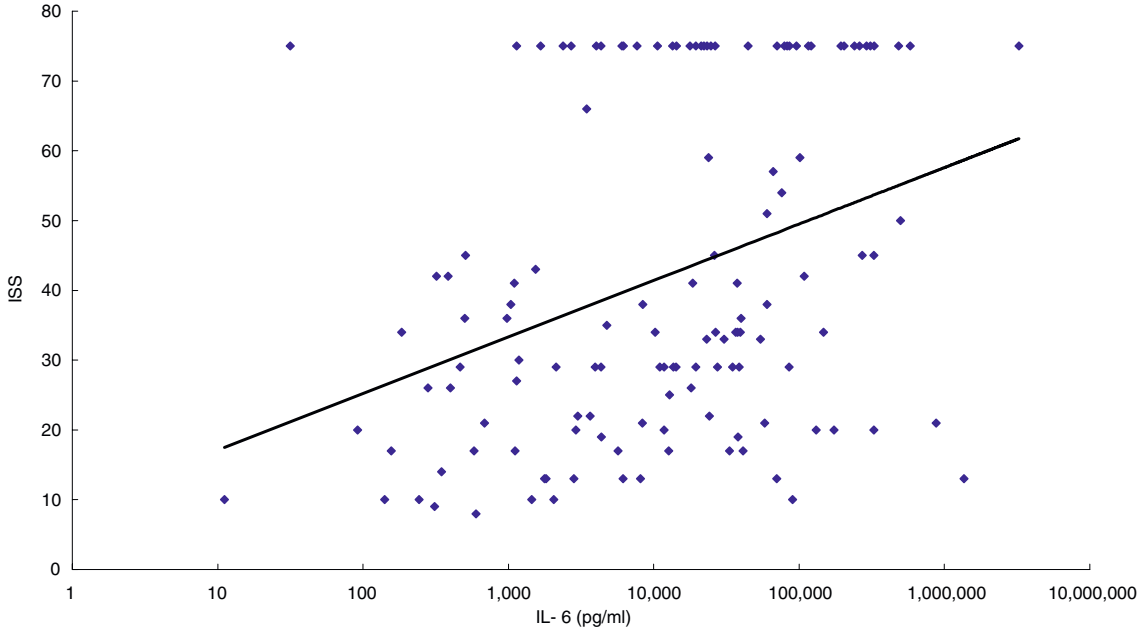

Fig. 2 Correlation between postmortem IL-6 levels and ISS (rs=0.3337, $p<0.0001$)

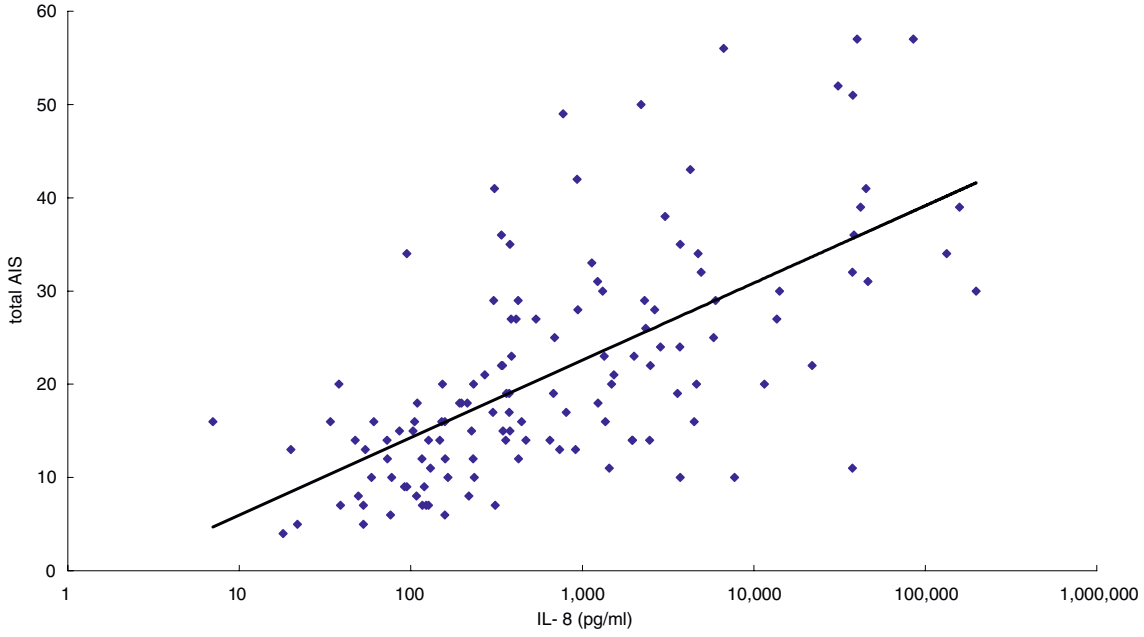

Fig. 3 Correlation between postmortem IL-8 levels and total AIS (rs=0.6593, $p<0.0001$)

immunocytes such as macrophages, monocytes, and lymphocytes. Once stimulated by the inflammation, immunocytes, fibroblasts, and vascular endothelial cells will discharge cytokines, which leads to inflammatory reactions in tissues such as discharging neutrophils, lymphoid migration, and cell differentiation [18–20]. The levels of cytokines discharged reflect the degree of tissue trauma [21] and can, therefore, be used as a measure of the scale of inflammation.

There were significantly higher cytokine levels in the traumatic death group than in the non-traumatic death group as a control. Although there were some cases where the cytokine levels were less than that of mean of postmortem control, this may have been influenced by some factors such as the time interval from trauma to death and individual differences of cytokine release [22]. Further studies are needed to elucidate the underlying mechanism of these factors.

We have previously reported that postmortem cytokine levels are useful for diagnosis of traumatic shock [23, 24]. However, the relationship between serum cytokine concentrations and anatomical traumatic severity has never

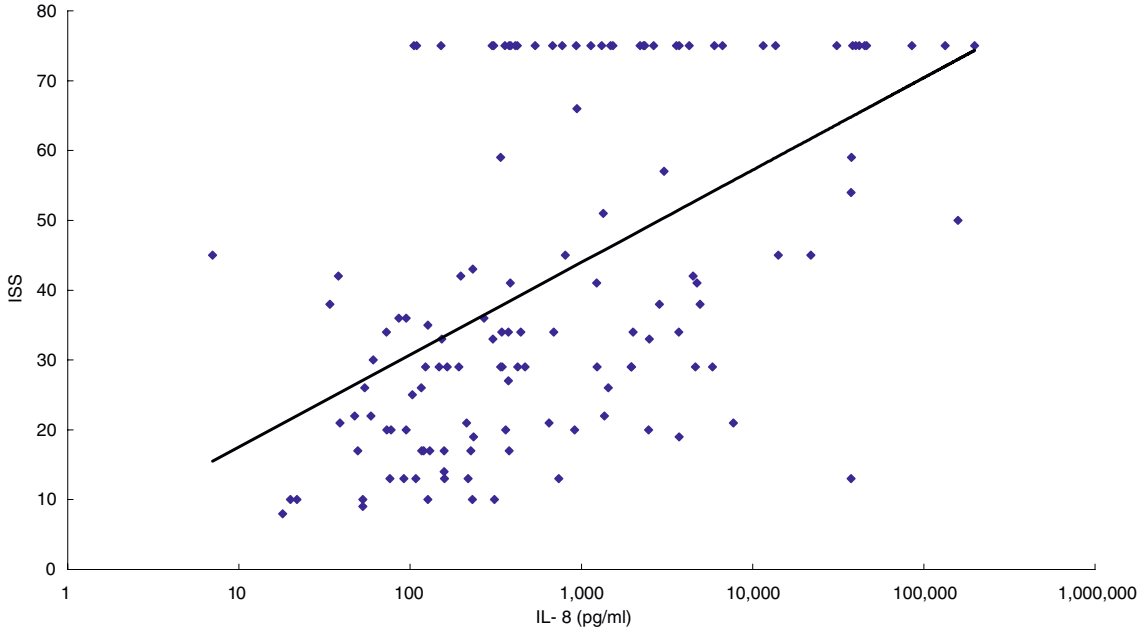

Fig. 4 Correlation between postmortem IL-8 levels and ISS (rs=0.5305, $p<0.0001$)

been investigated. Although some scoring methods for traumatic severity are used in clinical practice, most of them include physiological variables such as blood pressure, heart rate, coma level, respiratory rate, etc., and are, therefore, unsuitable for postmortem applications. As the valuation method of traumatic severity in postmortem tissues and organs is limited, the simple AIS scoring system seems to be the most appropriate system. In the present study, the postmortem levels of IL-6 and IL-8 showed meaningful equilateral correlations both with the total AIS and the ISS; there were, however, differences in the values of the Spearman rank correlation coefficients. The coefficient values of the Spearman rank correlation between the cytokine levels and the ISS were lower than those values between the cytokine level and the total AIS. It seems quite probable that these differences in values are due to the ISS scoring system being a clinical scoring system for multiple trauma and not a postmortem scoring system. One possibility is to assume that the ISS is defined as the sum of the squares of the single highest AIS score in each of the three most severely injured body regions, based on clinical experiences for multiple trauma. Another possibility is that the ISS automatically reaches 75 points if the AIS score is six points in one region. In contrast, total AIS is simply the addition of the individual regional AIS scores. Therefore, the total AIS was deemed to as a suitable scoring system for the evaluation of forensic traumatic severity.

Cytokine levels will change within the time that passes between sustaining an injury, dying, and postmortem examination. These changes posed considerable problems, as it was often difficult to accurately determine these time factors in forensic cases. Therefore, we selected the cases where the victims had clearly died less than 6 h after sustaining the fatal injury and an autopsy had been carried out within 2 days. It is interesting that the values of IL-6 and IL-8 were found to be higher in a victim who died instantly from his injuries. It has formerly been recognized that IL-6 and IL-8 reach a peak value 3–48 h after injury [1, 3, 25] so that the finding of high cytokine levels in the case of instantaneous death, therefore, remains unexplained. However, Taniguchi et al. [18] reported that IL-6 increased within 2 h after traumatic injury and other researchers have reported that IL-6 and IL-8 increased considerably within 30–60 min after the start of surgery [21, 26, 27]. Perl et al. [28] found that IL-6 and IL-8 levels were consistently present in human skin, subcutaneous fat, muscle, cancellous bone, and lung tissue and that the very early systemic occurrence of IL-6 or IL-8 after trauma is likely to be due to direct release from damaged cells. Furthermore, Muenhlstedt et al. [29] stated that IL-6 and IL-8 reached peak values within 2 h after injury and were associated with the reactions of the hypothalamus–pituitary–adrenal gland system to stress. These findings, therefore, present a gray area in the timing of IL-6 and IL-8 release after traumatic injury.

In the present study, we concluded that the postmortem levels of IL-6 and IL-8 are useful markers of traumatic severity and that the total AIS is more suitable than the ISS for the evaluation of traumatic severity in postmortem cases. We believe that this present report is the first report which presents a correlation between anatomical trauma severity and postmortem cytokine levels.

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