Postmortem Cytokine Levels and the Cause of Death

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MIMASAKA, S. Postmortem Cytokine Levels and the Cause of Death. Tohoku J. Exp. Med., 2002, 197 (3), 145-150 — We investigated the usefulness of cytokine measurement in the field of forensic medicine. In this study, the levels of Interleukin (IL)-1β, IL-6, IL-8, IL-10 in serum collected within 2 days after death were used to estimate the cause of death. In seventy-one victims, mean age 55.5 ± 17.7 (s.d.) years, the cases were classified as traumatic death (24 cases, trauma group), unnatural deaths by other than traumatic causes (31 cases, unnatural death group), and deaths due to natural causes (16 cases, natural death group). The Kruskal-Wallis test showed that IL-6 and IL-8 were good indices of trauma. According to the Scheffé test, IL-6 and IL-8 levels of the traumatic death group were significantly higher than those of the unnatural death group (p<0.05), but there was no significant difference in IL-6 levels between the traumatic death and natural death groups. Further, IL-6 levels showed considerable variability even among similar cases. However, IL-8 measurement of post-mortem samples is useful to evaluate non-quantitative damage received before death. ——— autopsy case; cause of death; cytokine; forensic medicine; trauma

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In forensic practice there are sometimes criminal or accidental death cases with multiple injuries, but without a notable fatal injury. Conventionally, the cause of death is diagnosed as "traumatic shock," "haemorrhagic shock," or "bruises in the whole body." However, in these cases evaluating the severity of injury from the body is often difficult.

In recent years there have been several studies on inflammatory markers in postmortem examinations (Mimasaka et al. 2001; Tsokos et al. 2001; Uhlin-Hansen 2001). CRP is a helpful pointer to the cause of death in those cases which a definite cause is not immediately determined (Uhlin-Hansen 2001). We previously reported that postmortem Interleukin (IL)-6 levels were helpful in the diagnosis of traumatic shock (Mimasaka et al. 2001). IL-6 is a major

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mediator of the acute-phase protein response, and is available to gauge the severity of trauma and the index of surgical stress (Sakamoto et al. 1994; Biffi et al. 1996; Gebhard et al. 2000). We have already used IL-6 as an index of inflammation in postmortem cases of trauma and infectious disease. Besides IL-6, several cytokines are known as inflammation markers, with the relationship between trauma and the cause of death having been examined using IL-1β, IL-6, IL-8 and IL-10 levels (Baigrie et al. 1992; Miller-Graziano et al. 1995; Carlstedt et al. 1997; Nast-Kolb et al. 1997; Neidhardt et al. 1997).

**MATERIAL AND METHODS**

The subjects consisted of 71 autopsy cases, 50 men (mean, 56.2 ± 17.6 [s.d.] years) and 21 women (mean, 53.4 ± 18 [s.d.] years), ranging in age from 15 to 90 years. Postmortem intervals were within 2 days in all cases. The cases were divided into three groups: deaths resulting from physical trauma (traumatic death group), deaths due to unnatural causes but in which trauma did not participate (unnatural death group), and deaths due to natural causes (natural death group). The traumatic death, unnatural death, and natural death groups contained 24, 31 and 16 victims respectively with mean ages of 53.4 ± 19.9 (s.d.), 57 ± 18.6 (s.d.) and 55.9 ± 12 (s.d.) years. The most common cause of traumatic death was head injury (8 cases), followed by haemorrhagic shock (4 cases), cervical injury (4 cases), traumatic shock (3 cases), and others (5 cases). The causes of the unnatural deaths were drowning (12 cases), mechanical asphyxia (9 cases), hypothermia (3 cases), intoxication (2 cases), and others (5 cases). The natural causes of deaths were heart disease (9 cases), intracranial hemorrhage (3 cases) and others (4 cases).

Blood samples were collected from the right atrium at autopsy. The serum obtained by centrifugation was stored at −30°C until analysis. Markedly hemolytic samples were excluded from the study. Cytokines were identified by enzyme-linked immunosorbent assay (ELISA). IL-1β, IL-6, IL-8 and IL-10 concentrations were determined using human IL-1β, IL-6, IL-8, IL-10 ELISA kits (Cytoscreen™, BIOSOURCE), respectively. The absorbance was measured using a microplate reader (BIO-RAD Model 550) set at 450 nm. When the reader failed to read the absorbance because of high sample concentrations, they were diluted with the kit diluent buffer. The minimum detectable doses of IL-1β, IL-6, IL-8 and IL-10 in these assays were 1 pg/ml, 2 pg/ml, 5 pg/ml and 1 pg/ml, respectively.

For multiple group comparisons, the Kruskal-Wallis test was performed. Comparisons between groups were evaluated by using the Scheffe test if the Kruskal-Wallis test was significant.

**RESULTS**

The average levels of serum IL-1β, IL-6, IL-8 and IL-10 of five healthy volunteers, as measured by the kits, was 19 ± 5 (s.d.) pg/ml, not detectable, not detectable and 3 ± 1 (s.d.) pg/ml, respectively.

Table 1 shows the serum cytokine values in the traumatic death, unnatural death, and natural death groups. From a comparison of the mean values of the postmortem and control materials, IL-6 and IL-8 showed marked increases in all groups. IL-1β and IL-10 of unnatural death group and IL-1β of the natural death group were within normal ranges. In three groups, the mean values of IL-1β, IL-6 and IL-8 of the traumatic death group indicated higher than those of the other two. As the samples did not show a Gaussian distribution (Fig. 1), the nonparametric (Kruskal-Wallis) test was applied. The test indicated significant differences among the groups on values of IL-1β (p < 0.05), IL-10 (p < 0.01), IL-6 and IL-8 (p < 0.001). The significance of the individual differences was evaluated by the Scheffe test, with a significant difference between each group.
Postmortem Cytokine Levels

Table 1. Serum cytokine values in all groups

<table>
<thead>
<tr>
<th></th>
<th>Traumatic death group</th>
<th>Unnatural death group</th>
<th>Natural death group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbers (n)</td>
<td>24</td>
<td>31</td>
<td>16</td>
</tr>
<tr>
<td>Age (years)</td>
<td>53 ± 20</td>
<td>57 ± 19</td>
<td>56 ± 12</td>
</tr>
<tr>
<td>IL-1β (pg/ml)</td>
<td>180 ± 687a</td>
<td>4 ± 8b</td>
<td>8 ± 18c</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>14 834 ± 17 771</td>
<td>3195 ± 9660</td>
<td>7362 ± 13 777</td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>1111 ± 1873</td>
<td>166 ± 250</td>
<td>194 ± 217</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>13 ± 24a</td>
<td>4 ± 5b</td>
<td>14 ± 43c</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± s.d.

*a Not detectable cases were 7*, 19*, and 11*.

*b Not detectable cases were 1*, 14*, and 7*.

Fig. 1. Distribution of cytokine values of each group. *p < 0.05.

a, Traumatic death group; b, Unnatural death group; c, Natural death group.

being shown only for IL-8 (p < 0.05). Although the IL-6 level of the traumatic death group was higher than that of the unnatural death group (p < 0.05), the difference between the traumatic death and natural death groups was not significant.
**DISCUSSION**

Damage to tissue leads to the production of a wide variety of chemical mediators. Cytokines are polypeptide messenger molecules and are produced by all types of cells that are involved in cellular immunological responses (Baron 2000). Cytokines are used to evaluate inflammation induced by surgery, injury, heat or infection (Faist et al. 2000). Clinically, IL-6 and IL-8 are applied as the indices of inflammation seen in patients with trauma, surgical stress, infectious disease (particularly sepsis), and tumours. Ohzato et al. (1992) reported that IL-6 concentrations correlated with the extent of surgical trauma, duration of surgery and amount of blood loss. Lowry et al. (1995) reported that IL-6 concentration showed the most constant increase with surgical trauma, as compared with the other cytokines. Hack et al. (1992) reported that IL-8 increased in most patients with sepsis and correlated with levels of IL-6, elastase-alpha1-antitrypsin, and C3a.

The present study shows significant increases of IL-6 and IL-8 in the traumatic death group, suggesting that a postmortem interleukin examination might be useful to assess the severity of physical stress. In some instantaneous death cases, such as death due to severe brain lacerations, IL-6 was markedly increased. A recent paper on the relationship between IL-6 and trauma suggested that the increase in IL-6 occurred in serum more rapidly than had previously been thought (Taniguchi et al. 1999). However, the values of the cases in the present study cast doubt on the notion that all of the cytokines are released into the blood before death. Surviving cells around injured tissue may play a role after cardiac arrest (individual death) in increasing the serum cytokine level. Tsokos et al. (2001) reported that a significant postmortem increase in IL-6 serum levels which correlated well with progression of time after death, was observed in five out of eight sepsis-related fatalities. They hypothesized that the excessive postmortem increase in IL-6 levels in some of the septic patients could be to some extent a phenomenon of systemic autolysis of cells such as monocytes, macrophages, lymphocytes and endothelial cells, in response to inflammatory tissue injury. However, they found no correlation between in vivo hematological parameters and IL-6 serum concentrations, and they also reported that a postmortem decrease of IL-6 serum levels, associated with increasing time after death, was observed in the other three of the eight sepsis-related fatalities. Correlation between the postmortem period and the cytokine levels is thus still controversial.

IL-1 induces the secretion of IL-6 and TNF, and is an essential stimulatory agent required for activation of cellular mediated immune responses (Houssiau et al. 1989). IL-1β is known to increase in local lesions where physical stress was present and to decrease rapidly (Baigrie et al. 1992). In the present study, IL-1β was detected in just 34 of 71 cases. The mean value of IL-1β in the traumatic death group was higher than that of the other two groups, but the differences between each group were not statistically significant. IL-1β was therefore not seen as an effective marker of trauma.

IL-10 is a cytokine whose biological activity inhibits the elevation of other cytokines (Cassatella et al. 1993; Klava et al. 1997), and the elevation of IL-10 follows that of IL-6 and IL-8. Neidhardt et al. (1997) reported that increased plasma concentrations of IL-10 correlated with morbidity and mortality of injured patients. A significant elevation of IL-10 level was not observed in many traumatic death cases whose IL-6 and IL-8 levels had increased. Statistically, IL-10 values in the autopsy samples were not effective.

Among four types of cytokines, IL-6 and IL-8 were useful as postmortem indices of physical trauma. However, IL-6 showed considerable variability among similar traumatic death
cases, and this variability might invalidate the use of statistical differences. We therefore recommend IL-8 as the index of traumatic death.

References


fatalities during the early postmortem period. 