Interleukin-6 and interleukin-10 gene polymorphisms and their plasma level after polytrauma

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1. Background

Three modal periods of death have been acknowledged by trauma surgeons. The first peak of this pattern occurs during the first few hours after injury. The second peak happens after the first 24 h wherein haemorrhagic shock contributes highly to mortality. The third peak occurs after a few days of injury, and the cause of death during this phase is multiple organ dysfunction syndrome (MODS) to failure (MOF) [1–5].

Response to trauma on one hand amplifies immune system activation with its end result being cell–mediated organ destruction, and on the other hand generates an immunosuppressive environment. Many clinical studies have shown that within hours after trauma or heavy bleeding, patient condition may deteriorate to MODS-MOF [3]. Immune mediators and cellular elements function in coordination and maintain homeostasis for survival; cytokines are the main regulators among these molecules. Many studies have clearly identified the molecular events occurring after trauma.

Based on the pathogenesis of complications after trauma at the cellular and molecular level, a better understanding is beneficial to verify the degree of severity of immune responses to major trauma [6–9]. Genetic predisposition is another challenging matter in this context. With advanced molecular diagnostics, the genetic role in inflammatory responses can be elucidated. Single nucleotide polymorphisms (SNPs) are proven to play a role in several morbidities. Genetic polymorphisms are distinct biomarkers used to diagnose and determine poor prognoses after trauma. Some studies have defined the correlation between genetic polymorphisms in sepsis and MODS after trauma [6,10,11].

The goal of this study is to examine whether genomic variance might affect individual phenotype in terms of gene expression and protein production capacity related to susceptibility to complications after trauma.

2. Methods

This was a prospective cohort study conducted at 4 academic trauma centres at Kandou hospital, Universitas Hasanuddin hospital, Kariadi hospital, and Sanglah general hospital. The study included 54 polytrauma patients with Injury Severity Scores (ISS) ≥ 16 during 1 year period. The plasma levels and gene expression levels of the cytokines interleukin-6 (IL-6) and interleukin-10 (IL-10) were measured over 5 days after polytrauma. The polymorphisms in IL-6 and IL-10 genes were also examined. The patient outcomes such as multiple organ dysfunction syndrome (MODS) and survival rate were observed. MODS evaluation was performed using sequential organ failure assessment (SOFA) methods.

The severity of trauma (ISS), the plasma level and gene expression level of IL-6 and IL-10, and the polymorphisms in IL-6 and IL-10 genes were analysed by correlation tests.

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3. Results

We collected 54 eligible participants who had completed follow-up with an average observation time about 16.5 days. A correlation between the plasma levels of IL-6 and IL-10 was observed in a cuboid curve (see Fig. 1) with R² value being 0.983 for the MODS group and 0.990 for the non-MODS group. In patients who progressed to MODS, elevation of the IL-6 plasma level was followed by the IL-10 plasma level. This pattern also occurred in the non-MODS group.

A correlation between the IL-6 and IL-10 plasma concentrations was observed as a cuboid curve (see Fig. 2) with the R² value being 0.979 for surviving patients and 0.983 for patients who did not survive. In the surviving group, elevation of IL-6 plasma level is followed by the IL-10 plasma levels; whereas in the non-surviving group, the IL-10 plasma level increased only if the IL-6 plasma level <50 pg/mL. If the IL-6 plasma level elevated >50 pg/mL, the concentration of IL-10 was decreased.

The gene expression level of IL-6 and IL-10 in each outcome group was diverse (see Table 1). The mean IL-6 expression level was the highest in outcome group 4 (ISS low and surviving) and the lowest in outcome group 1 (high ISS and not surviving). The IL-6 gene expression in outcome group 2 (high ISS and surviving) was significantly higher than that in outcome group 1 but not significant compared to that in outcome group 3. The IL-6 gene expression in outcome group 2 was not significantly different compared to that in outcome group 3. Comparison of the IL-10 gene expression levels in these outcome groups showed the same pattern as that of IL-6 gene expression.

The gene expression level of IL-6 and IL-10 was the highest in patients who had a relatively low ISS score and had survived (see Fig. 3) and was the lowest in patients who had a high ISS score and did not survive. The gene expression of IL-10 is higher than that of IL-6 in every outcome group. The gene expression of IL-6 and IL-10 in patients who had low ISS and had not survived was lower than that in patients who had high ISS and did not survive.

The next step was to analyse the correlation between host factors such as sex, age, trauma load (ISS), and poor outcome as the number of organ system dysfunctioning during MODS, the plasma levels of IL-6 and IL-10, and the occurrence of cytokine gene polymorphisms (see Table 2). In all the polytrauma patients in this study, we found that the IL-10 gene polymorphism (1082 A/G) could only be found as the AA genotype and no AG or GG genotypes were observed. For the IL-6 gene polymorphism (174G/C), we did not encounter the CC genotype, but the AC genotype was found in only 1 participant, and the rest were of the GC genotype (not normally distributed). For the IL-10 gene polymorphism (592-C/A), the genotype was distributed vastly between two kinds as CC (24 samples) and CA (30 samples), and no instance of AA genotype was found. Because of the correlation analysis of these factors i.e. age, sex, ISS, MODS, and plasma level of IL-6 and IL-10, genotype, with the exception in IL-10 polymorphism (592 C/A) CC and CA genotype.

4. Discussion

4.1. IL-6 and IL-10 plasma level

Concerning the correlation between IL-6 and IL-10 plasma levels in patients enduring MODS, elevation of IL-6 plasma levels is followed by IL-10 levels; this pattern is also observed in patients who did not have MODS (see Fig. 1). This result is supported by several studies reporting that secretion of the proinflammatory cytokine IL-6 induces upregulation of the anti-inflammatory cytokine IL-10.
The role of this anti-inflammatory cytokine during the acute phase has a clear impact on the clinical presentation in trauma patients [12–14]. In surviving patients, elevation of IL-6 plasma levels is followed by the elevation of IL-10 plasma levels, however, in non-survivors, elevation of IL-10 is only encountered if the IL-6 plasma level is less than 50 pg/mL. If the IL-6 plasma levels exceed 50 pg/mL, the plasma levels of IL-10 were found to be decreased (see Fig. 2). This condition does not clarify whether the elevation and depression of IL-10 plasma levels is associated with complications or poor outcomes or whether these biomarkers indicate the host struggle for survival. IL-10 is an important component of the negative feedback against proinflammatory cytokines. IL-10 production depends on the trauma mechanism, and the changes in IL-10 plasma levels may help restore the inflammatory response [1,15–17].

Some cytokines play a double role as pro-inflammatory and anti-inflammatory cytokines, but these mediators work in orchestration to re-establish homeostasis. An imbalance among these mediators after being triggered by heavy trauma may result in morbidity and death. In this study, the threshold of severe inflammation is a plasma IL-6 level of 50 pg/mL followed by a decline in the anti-inflammatory response demonstrated by the reduced IL-10 plasma level. If this threshold is breached, poly-trauma patients may progress to poor outcomes and eventual death. The correlation test between IL-6 and IL-10 plasma levels

<table>
<thead>
<tr>
<th>Outcome group</th>
<th>IL-6 gene mRNA expression</th>
<th>IL-10 gene mRNA expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n – 3)</td>
<td>7.06/714 (0.044)</td>
<td>10.06/10.22 (0.083)</td>
</tr>
<tr>
<td>2 (n – 9)</td>
<td>11.36/14.93 (155)</td>
<td>17.616 (1.506)</td>
</tr>
<tr>
<td>3 (n – 6)</td>
<td>9.54/12.74 (1.669)</td>
<td>13.479 (1.936)</td>
</tr>
<tr>
<td>4 (n – 3)</td>
<td>16.00/16.71 (1.6241)</td>
<td>19.24/20.07 (1.661)</td>
</tr>
</tbody>
</table>

Outcome group 1 – high ISS and no surviving; 2 – high ISS and surviving; 3 – low ISS and surviving; 4 – low ISS and surviving.
with an odds ratio of 3.7. This heavy immune response may progress to immune paralysis-tired-rest state and produce no outcome and established a threshold of new injury severity score (NISS) expression levels. Brumen et al. (2014) obtained a different result. Brumen studied 20 multitrauma subjects and used the NISS score expression levels. IL-6 was balanced by its anti-inflammatory mediators. If during minor trauma, IL-10 mRNA expression is higher than normal, the host succeeds in achieving homeostasis after trauma [18–22]. Our study supports this concept in which elevation of IL-10 (592C/A) and MODS occurrence with an odds ratio of 4.7.

Table 2
Distribution of age, sex, ISS, number of organ systems dysfunctioning during MODS occurrence, IL-6 and IL-10 plasma levels, and polymorphism location and genotype. IL-10: 592-C/A group was distributed vastly while other polymorphism groups were not normally distributed.

<table>
<thead>
<tr>
<th>Polymorphism genotype</th>
<th>N</th>
<th>Age (years)</th>
<th>sex (M/F)</th>
<th>ISS score</th>
<th>MODS</th>
<th>IL-6 (pg/mL)</th>
<th>IL-10 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10: 1082-A/G</td>
<td>54</td>
<td>16–54</td>
<td>42/12</td>
<td>16–50</td>
<td>1–4</td>
<td>15.5–177.4</td>
<td>21–340.7</td>
</tr>
<tr>
<td>(36.4 ± 14.7)</td>
<td></td>
<td></td>
<td></td>
<td>(24.1 ± 7.7)</td>
<td>(1.8 ± 1.1)</td>
<td>(47.30 ± 26.18)</td>
<td>(83.70 ± 47.17)</td>
</tr>
<tr>
<td>AG</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>GG</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IL-10: 592-C/A</td>
<td>24</td>
<td>17–54</td>
<td>21/3</td>
<td>17–35</td>
<td>1–4</td>
<td>16.5–62.2</td>
<td>30.4–112.2</td>
</tr>
<tr>
<td>(38.1 ± 14.3)</td>
<td></td>
<td></td>
<td></td>
<td>(23.3 ± 6.2)</td>
<td>(2.0 ± 1.1)</td>
<td>(41.18 ± 9.81)</td>
<td>(73.53 ± 17.32)</td>
</tr>
<tr>
<td>CA</td>
<td>30</td>
<td>16–54</td>
<td>21/9</td>
<td>16–50</td>
<td>1–4</td>
<td>15.5–112.2</td>
<td>21–340.7</td>
</tr>
<tr>
<td>(35.0 ± 15.2)</td>
<td></td>
<td></td>
<td></td>
<td>(24.8 ± 8.7)</td>
<td>(1.7 ± 1.1)</td>
<td>(52.19 ± 33.47)</td>
<td>(91.84 ± 60.62)</td>
</tr>
<tr>
<td>AA</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IL-6; 174 G/C</td>
<td>53</td>
<td>16–54</td>
<td>41/12</td>
<td>16/41</td>
<td>1–4</td>
<td>15.5/177.4</td>
<td>21.0–340.7</td>
</tr>
<tr>
<td>(36.0 ± 14.6)</td>
<td></td>
<td></td>
<td></td>
<td>(23.6 ± 6.8)</td>
<td>1.1 ± 0.2</td>
<td>(46.24 ± 25.23)</td>
<td>(81.62 ± 45.04)</td>
</tr>
<tr>
<td>GC</td>
<td>1</td>
<td>54.0</td>
<td>1/0</td>
<td>50.0</td>
<td>1</td>
<td>103.4</td>
<td>14.15</td>
</tr>
<tr>
<td>CC</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 3
Cross tabulation between the IL-10 gene polymorphism (592C/A) and MODS

<table>
<thead>
<tr>
<th>IL10 gene polymorphism-592C/A</th>
<th>MODS</th>
<th>Non-MODS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>% MODS</td>
<td>60%</td>
<td>30%</td>
<td>45%</td>
</tr>
<tr>
<td>CA</td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>% MODS</td>
<td>40%</td>
<td>70%</td>
<td>55%</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>29</td>
<td>54</td>
</tr>
<tr>
<td>% MODS</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Next, we further cross tabulated the incidence of MODS and the IL-10 gene polymorphism (592C/A) (see Table 3). The χ² test is considered to be qualified if the expected value < 20%. This χ² test resulted in χ² = 5.247 with p = 0.022 (p < 0.05) indicating a significant association between IL-10 (592C/A) and MODS occurrence with an odds ratio of 2.7.

with mortality (see Fig. 1) showed that the inflammatory aspect of IL-6 was balanced by its anti-inflammatory counterpart until the IL-6 threshold at 50 pg/mL, followed by a reduction in IL-10 plasma levels. This heavy immune response may progress to immune paralysis-tired-rest state and produce no outcome as a defeat of the anti-inflammatory side according to the Bone theory (SIRS > CARS), eventually resulting in death [1,14].

4.2. IL-6 and IL-10 gene expression levels

Animal studies show that mRNA expression is significantly higher during major trauma. Elevation of IL-10 mRNA expression might be beneficial to slow down the pro-inflammatory response, facilitate cellular and tissue repair, and attain a balance between the pro- and anti-inflammatory mediators. If during minor trauma, IL-10 mRNA expression is higher than normal, the host succeeds in attaining homeostasis after trauma [18–22]. Our study supports this concept in which elevation of IL-10 is an important buffer mechanism for the pro-inflammatory storm after major trauma and elevation of IL-6 plasma levels is proportionate with IL-6 gene expression levels. Brumen et al. (2014) obtained a different result. Brumen studied 20 multitrauma subjects and used the NISS score and established a threshold of new injury severity score (NISs) > 41 as a distinctive border to classify minor and major trauma, and an mRNA expression profile within polymorphonuclear cells showed that IL-6 plasma level are higher in less severe trauma (NISs < 40). The result of the Brumen study is in accordance with an animal study that investigated IL-6 and IL-10 mRNA expression levels in proportion with the severity of the injury (see Table 1 and Fig. 3).

4.3. IL-6 and IL-10 gene polymorphisms

Cytokine gene polymorphisms associated with inflammation may affect the physiological response and clinical manifestation after trauma [23–25]. Hidebrand et al. found that the incidence of polymorphisms is higher in SIRS patients who have the homozygous allele genotype IL-6-174GC. Schluter et al. found that patients suffering from severe sepsis after surgery have better chance of survival if they have the GC genotype. This study demonstrated that IL-6 caused a reduction in mortality rate and multiple organ dysfunction after SIRS. However, all the participants with this result have the same SNP, IL-6-174, and did not count trauma as early predictor factor. IL-6-174 SNP did not show a significant difference. A study of critically ill patients showed an association between lessening of the IL-10 polymorphism promoter at the allele position (−1082 to −819, and −592) and mortality risk. Several studies have demonstrated the functional implication of IL-10 polymorphisms, and its plasma levels during major trauma or sepsis have different results [6]. The highest level of IL-10 is found as the GCC/GGC haplotype. Patients with the −597AC genotype had a higher MODS score and had a 3.3-fold increased risk to develop MODS [26]. Schroder O et al. (2004) also found that patients with the −597AC genotype have a 3.3 higher risk of enduring multi-organ dysfunction. Huebinger et al. (2006) found that the −592A allele IL-10 promoter seemed to decrease the risk of death after burn injury. This study only included the −592 and −1082 alleles and only the −592A allele that has a significant association with MODS, and the IL-10 −592C/A SNP have an odds ratio of 3.7 (see Table 3).

Immune response is a kind of communication and interaction that may be upregulated or downregulated continuously to maintain balance. When this immune response is haulted, immune cells are considered to be in a “paralysis-tired-rest” state and produce no response. Unresponsive cells may result from the failure to respond under the upregulation or downregulation stimuli. Moore explained the biphasic theory that SIRS > CARS may rapidly lead to MODS and MOF, and SIRS < CARS will lead to MARS and create immune paralysis and eventually also cause MODS and MOF slowly.

We did not observe an absolute balance in the immune response because the immune system is always under stimulation and buffering. We did not find any significance of both IL-6 and IL-10 genetic factors in disease outcome. Because long exposure is needed to change the genetic sequence while in trauma, exposure occurs in a short time and so, the exact correlation between gene polymorphisms and plasma level patterns cannot be observed. Under trauma conditions, mRNA expression levels are the main countable factors.

References