Int J Burn Trauma 2016;6(2):37-43
www.IJBT.org /ISSN:2160-2026/IJBT0025342

Original Article

Pattern of cytokine (IL-6 and IL-10) level as inflammation and anti-inflammation mediator of multiple organ dysfunction syndrome (MODS) in polytrauma

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Received January 31, 2016; Accepted April 29, 2016; Epub June 1, 2016; Published June 15, 2016

Abstract: Massive injury remains the most common cause of death for productive age group globally. The current immune, inflammatory paradigm, based on an incomplete understanding of the functional integration of the complex host response, remains a major impediment to the development of effective innovative diagnostic and therapeutic effort. This study attempt to investigate the pattern of inflammatory and anti-inflammatory cytokines such as interleukin-6 and 10 (IL-6 and IL-10) and their interaction in severe injury condition with its major complication as multiple organ dysfunction syndrome (MODS) and failure (MOF) after polytrauma. This is multicenter study held at 4 academic Level-1 Trauma center included 54 polytrauma participants. Inclusion criteria were age between 16-60 years old, had new acute episode of polytrauma which defined as injury in ≥2 body region with Injury Severity Score (ISS) ≥16, and the presence of Systemic Inflammation Response Syndrome (SIRS). Serum level of IL-6 and IL-10 were taken on day 2, 3, and 5 after trauma. During hospitalization, samples were observed for the occurrence of MODS or MOF using Sequential Organ Failure Assessment (SOFA) and mortality rate were also noted. Participant were mostly male with mean of age of 35, 9 years old, endured polytrauma caused by traffic accident. Elevation of cytokines (IL-6, IL-10, and IL-6/IL-10 ratio) had directly proportional with MODS and mortality. Threshold level of compensation for severe trauma is IL-6 of 50 pg/mL and trauma load of ISS ≥30. Inflammation reaction greater than this threshold level would result in downhill level of IL-6, IL-10, or IL-6/IL-10 ratio which associated with poor outcome (MODS and death). The elevation of these cytokines level were represent as compensation/adaptive immune system and its fall represent decompensating/failure of immune system after severe trauma. The pattern of IL-6 and IL-10 after polytrauma represent immune system effort to restore homeostasis. Besides cytokines interaction, there must be other factors that contribute to mortality and poor outcome after major trauma. Further study is needed to investigate genomic variant or polymorphism related to trauma.

Keywords: Multiple organ dysfunction syndrome, polytrauma, IL6, IL10

Introduction

Several experimental and clinical studies revealed that trauma and major surgery has significant impact on immune mechanism that consists of specific and nonspecific immune system [1]. Several mediator and cellular elements works in harmony to restore homeostasis for host survival and among these molecules, the main regulator is cytokines [2].

Trauma occurs sporadically with its morbidity and mortality outcome. Studies have been done to identify molecular events occurs after trauma. Based on WHO report, injury/trauma is amongst the main cause of deaths world widely

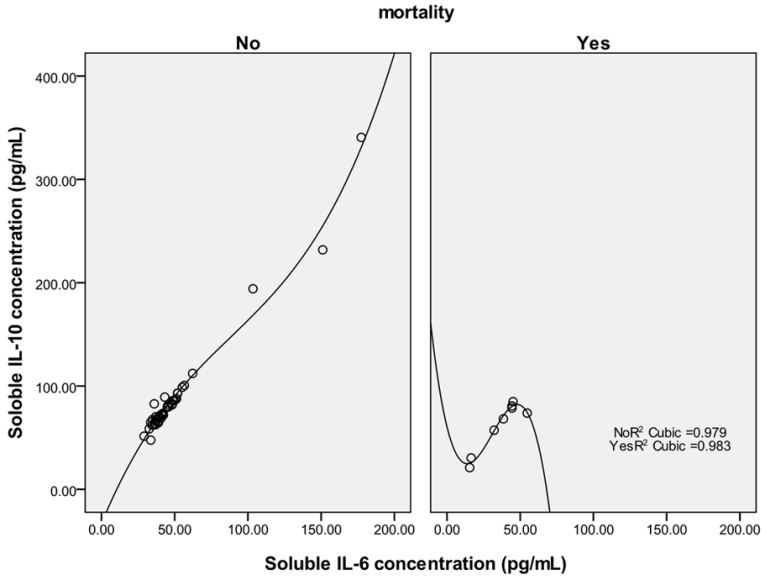

Figure 1. Correlation between IL-6 and IL-10 level among survivor and nonsurvivor group. There was a correlation between IL-6 level and IL-10 in cuboid curve, with R^2 of 0.979 for survivor group and 0.983 for nonsurvivor.

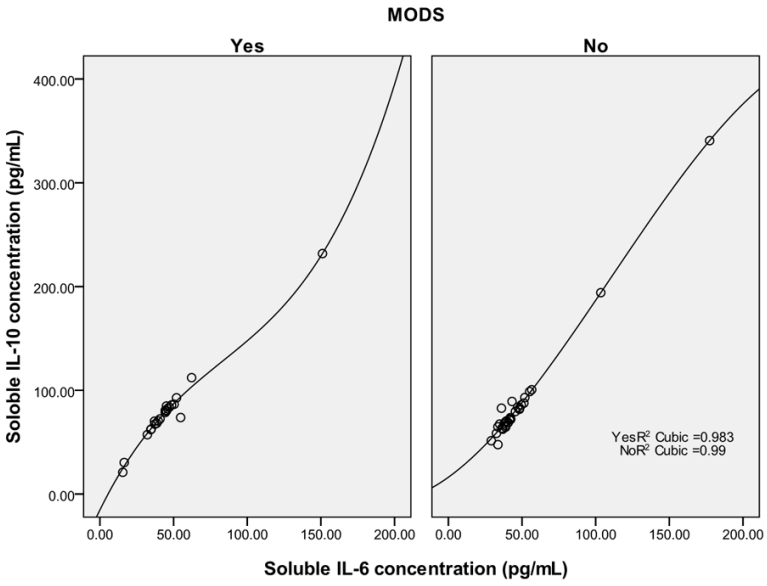

Figure 2. Correlation between IL-6 and IL-10 level among MODS and non-MODS group. There was a correlation between IL-6 and IL-10 level in cuboid curve, with R^2 of 0.983 for MODS group and 0.990 for non-MODS group.

that is (9% cause of mortality) and became number one cause of deaths in 15-29 years old group [3]. In year 2004, traffic accidents ranked ninth as cause of death and will climb up to fifth if there are no significant efforts within the next few years and in 2020 will be in third place [3, 4]. Patients who survived within first few hours have high risk to endure further deadly complications caused by immune reaction. Based on trimodal of death theory, first peak occurs within seconds to minute of injury. Second peak occurs within next 24 hours when hemorrhagic shock gives high number of mortality rate. Third peak occurs after 48 hours or weeks and the cause of death during this periode are multiple organ dysfunction syndrome (MODS) and multiple organ failure (MOF) [5].

Body response in trauma has two side of coin. In one side there will be hyperactive immune system with cell-mediated organ damage as its end result while in other side is immunosuppression state. Immunoinflammation reaction occurs within hours after trauma and hemorrhage event may developed to MODS-MOF with high mortality rate [6]. The induction of inflammation response and proinflammation cytokines may manage tissue injuries, infectious microorganism, and restore body function back to normal, although hyperactive reaction of these proinflammation cytokines will result in unstable hemostasis and disarray metabolism. To balance proinflammation cascade, anti-inflammation cytokines is released. This anti-inflammation mediator will depress immune cells and result in immunosuppression [7].

Several cytokines have a role in inflammation response and one of them is interleukin-6 (IL-6). IL-6 is released as a response to tissue injury or inflammation stimulus, has local and systemic effect to create physiological responses as needed. IL-6 concentration increases after trauma and in chronic disease. IL-6 has a role as proinflammatory mediators and also anti-inflammation regulator which stimulates potent anti-inflammation cytokines such as IL-10 [8].

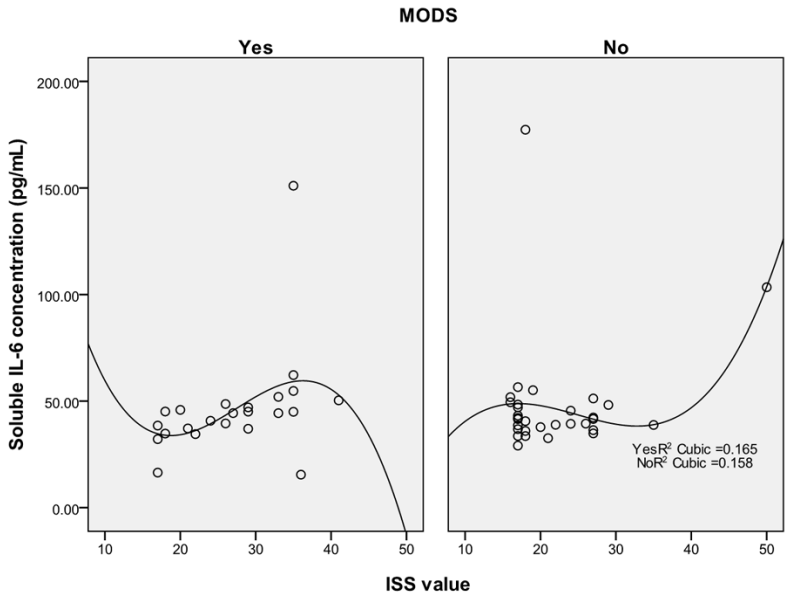

Figure 3. Correlation between ISS and IL-6 level in patients with and without MODS after trauma. ISS has correlation with IL-6 level in cuboid curve shape with R^2 of 0.165 for MODS group and 0.158 for non-MODS group.

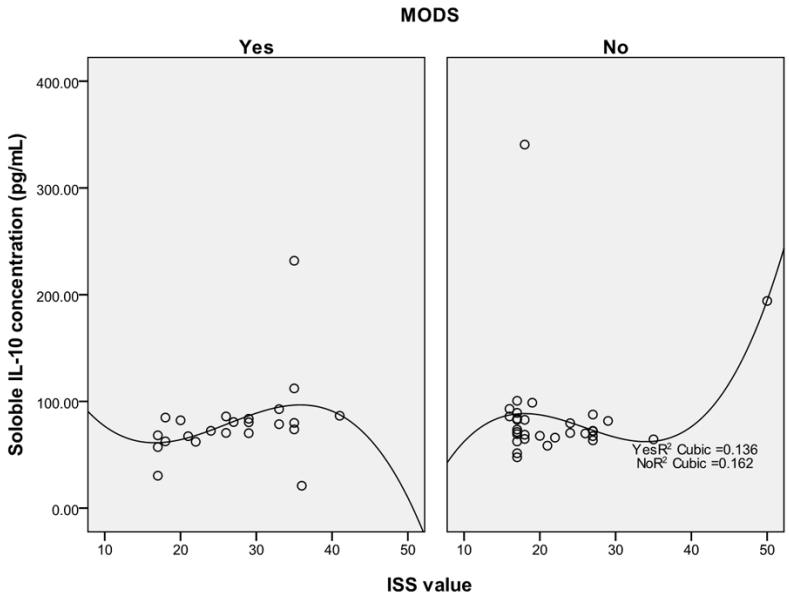

Figure 4. Correlation between ISS and IL-10 level in patients with and without MODS after trauma. ISS has correlation with IL-10 level in cuboid curve shape with R^2 of 0.165 for MODS group and 0.158 for non-MODS group.

The aim of the study is to investigate severity degree of immune system after trauma based on IL-6 and IL-10 level pattern.

Material and methods

The ethics committee at each of the participating hospitals approved the study protocol with registration number UH150-50350.

Subjects

This study was held at 4 academic tertiary level-1 trauma center included 54 participants, with range of age of 16-60 years old, had new episode of polytrauma defined as multiple body region injuries with Injury Severity Score (ISS) ≥16, and endured SIRS. ISS was determined based on highest Abbreviated Injury Scale (AIS) at three body region according to latest edition of AIS manual [9]. Polytrauma defined as injury on at least two body region with AIS ≥3 and presence of SIRS for at least 24 hours within 72 hours after trauma [10, 11].

Enzyme linked immunosorbent assay (ELISA)

IL-6 and IL-10 level were taken during acute phase of injury (48, 72, 120 hours after trauma). Patients were treated according to Advanced Trauma Life Support (ATLS) and Definitive Surgery Trauma Care (DSTC) protocol. During hospitalization, patients were observed for the presence of MODS-MOF according to sequential organ failure assessment (SOFA) score and mortality rate was also noted [12].

Sera IL-6 and IL-10 were determined triplicate by enzyme-linked immunosorbent assay (ELISA) according to manufacture procedure [Abcam, ab46027-IL-6 (Interleukin-6) Human ELISA Kit and ab46034-IL-10 (Interleukin-10) Human ELISA Kit]. Concentration and ratio of IL-6 and IL-10 and its correlation with MODS-MOF and mortality were analyzed.

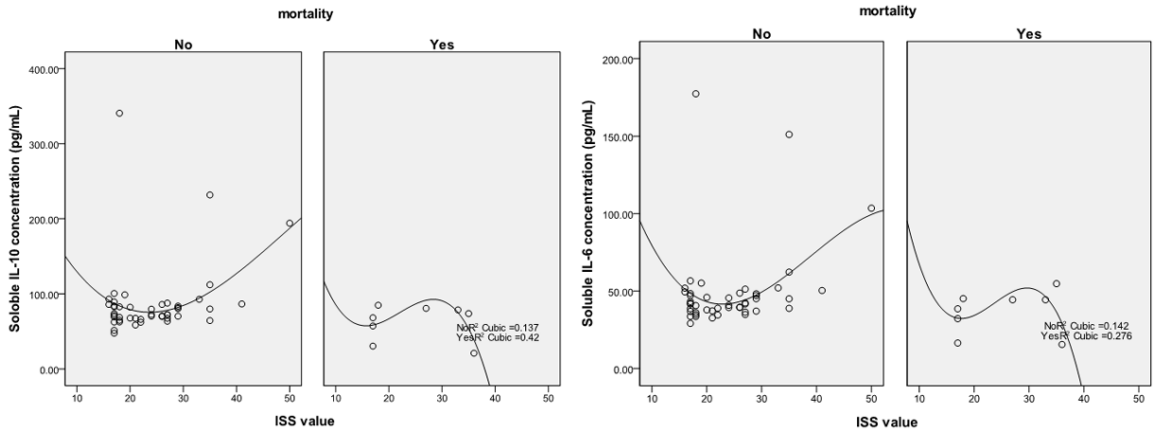

Figure 5. Correlation between ISS, mortality, IL-10 (*left picture*) and IL-6 level (*right picture*). ISS had a correlation with ISS and cytokines (IL-6 and IL-10) level in cuboid curve shape, R^2 of 0.142 for survivor group and 0.276 for nonsurvivor group.

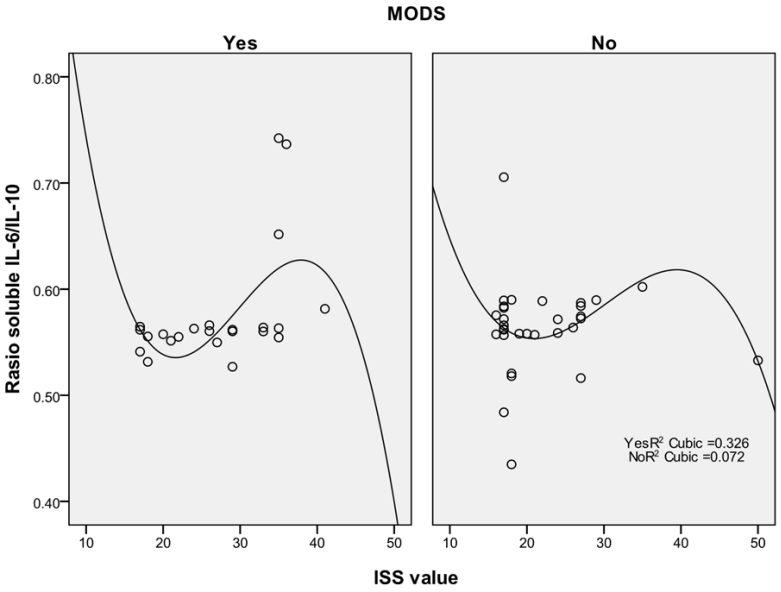

Figure 6. Correlation between ISS and IL-6/IL-10 ratio among patients with and without MODS. ISS had a correlation with IL-6/IL10 ratio in cuboid curve shape, with R^2 as 0.326 for MODS group and 0.072 for nonMODS group. Both curve had similar shape.

Statistical analysis

Data were analyzed with Statistical Package for The Social Sciences (SPSS) version 17.0 (SPSS Inc, Chicago, IL), and the significance level was set at $p<0.05$. The results of quantitative pathological evaluation were scatter graph and Pearson Correlation test.

Results

Most samples were male (59.25%, 32:12) with range of age between 16-64 years old (mean 35.98), mostly had blunt trauma mechanism (94%) and caused by traffic accident (79.63%). IL-6 level concentration range was 15.5 to 177.4 pg/mL (mean 47.3 pg/mL) and IL-10 level 21.0 to 340.7 pg/mL (mean 83.71 pg/mL).

Correlation between IL-6 and IL-10 level and mortality

Among survivor group, the elevation of IL-6 level followed by elevation of IL-10 level, while in nonsurvivor group IL-10 level were increase only if IL-6 level <50 pg/mL. If IL-6 level rose >50 pg/mL, IL-10 level fell (**Figure 1**).

Correlation between IL-6 and IL-10 level and MODS

Among patients who developed MODS, the elevation of IL-6 level were followed by IL-10. This pattern also encountered at non-MODS group (**Figure 2**).

Correlation between IL-6, ISS, and MODS

Among patients had not underwent MODS, ISS ≥35 elevated IL-6 level while in MODS group IL-6 level was decline (**Figure 3**).

Correlation between IL-10, ISS, and MODS

Among patients had not underwent MODS, patients with very severe injury (ISS ≥35) had

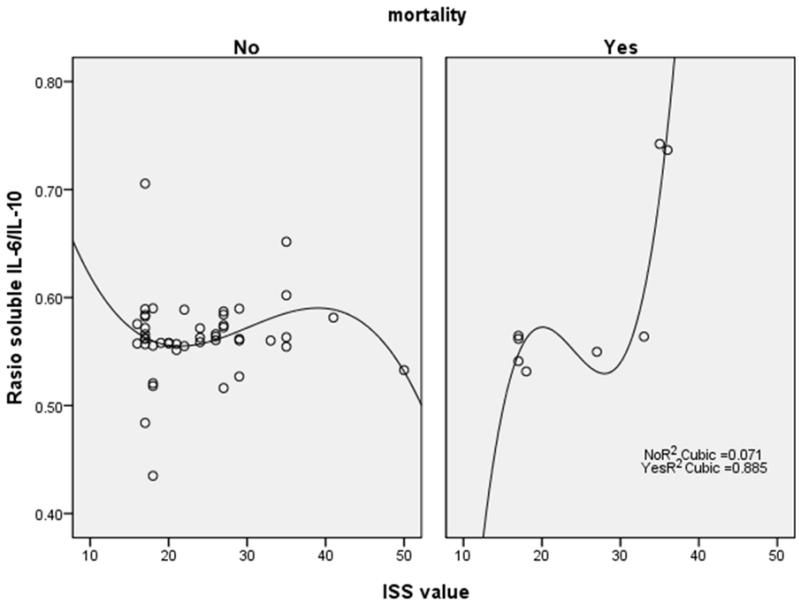

Figure 7. Correlation between ISS and IL-6/IL-10 ratio among patients who survived and not survived. ISS had a correlation with IL-6/IL10 ratio in cuboid curve shape, with R^2 as 0.371 for survivor group and 0.885 in nonsurvivor group.

elevation of IL-10 level while in MODS group IL-10 level was decline (**Figure 4**).

Correlation IL-6 and IL-10 level with ISS and mortality

Among patients who survived, patients who had very severe injury (ISS ≥30) had a elevation of IL-6 and IL-10 level; while in nonsurvivor group these cytokines level was declining (**Figure 5**).

Correlation between IL-6/IL-10 ratio, ISS, and MODS

IL-6/IL-10 ratio will decline in patients with ISS >40 in MODS and nonMODS group (**Figure 6**).

Correlation between IL-6/IL-10 ratio, ISS, and mortality

In nonsurvivor patients with ISS >30, IL-6/IL-10 ratio increased while in survivor groups, IL-6/IL-10 ratio was decline (**Figure 7**).

Discussion

Each cytokine had a role as proinflammation or antiinflammation effect; however every cytokines works synergistically with end point to restore homeostasis. Imbalance among mediators caused by trauma load may progress to morbidity and mortality.

Based on this study, threshold for severe inflammation response is IL-6 level 50 pg/mL. More severe inflammation response greater than this threshold will lead to death. Correlation between IL-6 and IL-10 level to mortality (**Figure 1**) showed that IL-6 is an inflammation marker which had its counterpart as anti-inflammation tried to balance the situation until its limit at IL-6 level of 50 pg/mL. Immune response heavier than this limit would develop to immune system failure which explained by Bone theory as SIRS>CARS and eventually becomes multiple organ failure and death [13, 14]. Stensballe et al. had done a prospective cohort study from 265 adult trauma patients revealed that significant IL-6 and IL-10 level increased significantly during the first 24 hours [15] and similar with this study showed a correlation between IL-6 (also IL-10) and injury severity and mortality.

Threshold of trauma load for the occurrence of MODS is ISS 30. IIf trauma load heavier than ISS 30, host immune system which still have strength to compensate exemplified by elevation of IL-6 and IL-10 level (**Figures 3**-**5**). Decline of both cytokines illustrated "exhausted" immune system and host response which lead to MODS and death. Gebhard et al. had similar findings about ISS threshold. The study showed elevation of IL-6 level in ISS >32 [16]. From 4 groups (ISS <9, ISS 9-17, ISS 18-30, and ISS >32), highest IL-6 level was found in highest ISS and lowest IL-6 level was found in the most low ISS [16].

Interaction between IL-6 and IL-10 may also be illustrated as IL-6/IL-10 ratio. In major trauma with ISS 30, elevation of IL-6/IL-10 ratio exhibited more overactive systemic inflammation response and progression of this state may lead to MODS and death (**Figures 6**, **7**).

Reduction of IL-6/IL-10 ratio on severe trauma ISS >40 indicated that CARS more dominant than SIRS which one's again showed immune system failure. Failure of immune system may progress to sepsis in the presence of infectious focus or contamination [14, 17].

Interleukin-6 (IL-6) and IL-10 interaction on second to fifth day this study had the same pattern with study by Spindler-Vessel et al. who prospectively 30 multitrauma patients treated at intensive care unit. Serum samples were taken on day 2 and 4 after trauma. On day 2, median value of IL-6 in MOF group was higher compare with non-MOF group (145 pg/mL vs 61.9 pg/mL). There was significant correlation between IL-6 on fourth day and intestinal permeability on fourth day [18].

In conclusion of this study is pattern of IL-6 and IL10 cytokines after polytrauma on this study represent immune system struggle in order to cope with severe trauma and restore the condition to homeostasis. Besides these interactions of cytokines, there are other factors who have roles in determining mortality. Polymorphism and genomic variant affect individual phenotype including gene expression profile in trauma need further studies to obtain more clear understanding about body response to injury.

Disclosure of conflict of interest

None.

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