

Short Communication

Serum IL-18 Is Increased at Early Postburn Period in Moderately Burned Patients

Y. Kenan Coban¹ and Murat Aral²

¹Department of Plastic Surgery, School of Medicine, Sutcu Imam University, Kahramanmaraş 46100, Turkey

²Department of Microbiology and Immunology, School of Medicine, Sutcu Imam University, Kahramanmaraş 46100, Turkey

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A severe systemic inflammatory response is usually seen after burn injury. IL-18 enhances the Th1 immune responses in bacterial and viral infections. In order to evaluate the IL-18 serum levels as well as IL-6 and TNF- α at the 48th hour postburn, serial serum samples of 8 burned patients were analyzed. 8 moderately burned patients were included into the study. Serum samples were taken at admission at the 48th hour of postburn. IL-6, IL-18, and TNF- α serum levels were analyzed. Total mean burned surface area (TBSA) was $24.6 \pm 5.7\%$ and mean BMI (body mass index) was 24.5 ± 3.4 . The patients' age ranged from 17 to 38 (mean 26.3 ± 7.4) years. An increase in sera IL-6, IL-18, and TNF- α was detected at the 48th hour postburn ($P < .0001$). All patients survived. A marked increase in serum levels of IL-18 as well as the other cytokines evaluated was observed in the moderately burned patients. These three parameters were highly correlated with each other ($r > 0.9$ and $P < .001$). This is the first study that shows an increase in serum IL-18 levels at the early postburn period.

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INTRODUCTION

Serious thermal injury leads to increased vascular permeability and subsequently hypovolemic shock, skeleto-muscular proteolysis, lipolysis, gluconeogenesis, increased metabolic rate, and a severe systemic inflammatory response. This response is induced by local infections, surgical procedures, and inflammatory mediators such as histamine, serotonin, bradykinin, prostaglandins, leukotrienes, and platelet activator factor. Cytokines are the primary mediators of the inflammatory response to injury. Cytokines are a group of proteins with autocrine and endocrine activities which provide communication among cells of tissues of the man. Active in minute quantities, the cytokines activate and regulate homeostasis and cellular repair through effects on cell growth and differentiation and on receptor expression and cell-mediated immunity.

The trauma of burn injury induces a distinct inflammatory response which is characterized by activation of all inflammatory pathways, dysregulation of cell-mediated immunity, and alterations of mediators of the immune system involving cytokines, growth factors, vascular endothelium, and different immunocompetent cell populations. Various cytokines, such as interleukin IL-1, IL-6, and tumor necrosis factor alpha (TNF- α), have been reported as markers of

the severity of burn injury that might have prognostic value [1, 2]. Large degree of variability in hormone and cytokine plasma levels in a burn patient group has been reported [3]. The incidence of systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS) has been found as three times increased in patients with total burned surface area (TBSA) $> 30\%$ compared to patients with TBSA $< 30\%$ [4]. Some proinflammatory markers, such as IL-6, procalcitonin, and IL-2 serum levels were reported significantly higher in severely burned patients than moderately burned patients. C-reactive protein (CRP) concentrations in moderately burned patients decreased continuously during postburn healing period, while the levels of severely burned patients remained significantly elevated [5, 6]. Current literature shows that there is an extended inflammatory response in severely burned patients.

Among the cytokines, IL-6 was pointed as the main mediator in burn injury by the body response within days of 2–21 postinjury [6]. A lack or delay in the plasma IL-10 elevated levels and burn sepsis has been reported as closely related at the early postinjury period [5]. An increase in serum levels of TNF- α , IL-6, and IL-8 were detected at the early postburn period [7]. TNF- α belongs to a family inducing cytotoxic reactions, whereas IL-6 stimulates humoral immunity. Although the hypercytokinemia associated with burn injuries

TABLE 1: Demographics of patients, burn score (TBSA), causes of burns, body mass index (BMI), and IL-6, IL-18 as well as TNF- α serum levels at admission and at 48th postburn hour.

Causes of burns	Age year/gender	TBSA	BMI	IL-6 (pg/mL)		TNF- α (pg/mL)		IL-18 (pg/mL)			
				Admission	48th hour	Admission	48th hour	Admission	48th hour		
Scalding	23/male	16	23	12.81	36.82	4.63	14.65	14.6	58.6		
Scalding	20/female	20	22	19.41	40.27	5.57	21.52	16.6	57.9		
Flames	34/male	24	27	20.42	42.82	6.28	23.89	16.1	64.2		
Contact	21/male	26	28	22.44	44.64	6.83	27.44	23.6	73.1		
Explosion	17/male	22	19	22.94	64.36	7.07	33.36	33.0	94.5		
Electricity	26/female	32	23	23.44	68.03	7.31	34.54	38.1	109.4		
Scalding	31/male	33	25	35.44	83.70	7.79	48.75	41.1	117.4		
Scalding	38/female	24	29	51.72	87.97	8.84	78.34	43.5	125.4		
Mean \pm standard deviation (SD)				24.5 \pm 3.4	24.6 \pm 5.7	26.1 \pm 12.1	58.5 \pm 20.2	6.8 \pm 1.3	35.3 \pm 20.2	28.3 \pm 12.0	87.6 \pm 27.6

Control group values for BMI, IL-6, TNF- α , and IL-18 were as follows: 23.3 \pm 4.3, 24.1 \pm 8.1 pg/mL, 4.6 \pm .5 pg/mL, and 25.1 \pm 8.4 pg/mL, respectively.

is a well-known clinical entity, the plasma levels of IL-18 were not studied in burned patients before. Development of an extended inflammatory response which covers not only proinflammatory but also immunoinhibitory aspects is observed as different in moderately and severely burned patients. IL-18 is required for facilitating neutrophil-dependent injury through suppressing anti-inflammatory cytokine expression during ischemia/reperfusion injury [8]. The inclusion of IL-18 to study depends on the fact that burn injury is accompanied by tissue hypoxia, reperfusion injury, induction, and activation of leukocytes. Endogenous IL-18 plays an important role in the early antibacterial host response [9]. This study aimed to evaluate the early changes of IL-18, IL-6, and TNF- α in serum after burn injury in moderately burned patients.

MATERIALS AND METHODS

The informed consent of the patients or their families and the approval of the Ethics Committee of Sutcu Imam University, School of Medicine, were obtained before the study. 8 patients admitted within 2 hours of burn injury were segregated based on burn size. The first serum sample was obtained from each patient at time of admission to the KSU Medical Research Hospital. The second serum sample was obtained at the 48th hour of postburn injury. The diagnoses of systemic inflammatory response syndrome (SIRS), sepsis, septic shock, and multiple organ dysfunction syndrome (MODS) were based on the criteria of the College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) [10]. A control group consisting of 8 healthy persons was chosen from the volunteers which matched the study group. Any patient who had chronic obstructive pulmonary disease or an inhalation injury was excluded from the study. And any patient with cigarette smoking history was also excluded from the study.

Measurement of cytokine levels in sera

Blood (10 mL) was collected, allowed to clot at room temperature for 30 minutes, and was then centrifuged for 10 minutes, at 1000 xg. Sera were quickly frozen at -70°C and

stored until the day of analysis. Serum IL-6 levels were measured by ELISA (enzyme-linked immunosorbent assay) using IL-6 EASIA kit (enzyme amplified sensitivity immunoassay, BioSource Europe S A, Nivelles, Belgium). This assay detected only human IL-6 and the minimum detectable concentration in our laboratory was 4.6 pg/mL.

Serum TNF- α levels were measured by ELISA using TNF- α EASIA kit. This assay detected only human TNF- α and the minimum detectable concentration in our laboratory was 4.6 pg/mL.

Serum IL-18 levels were measured by an ELISA IL-18 kit (Biosource Europe S A, Nivelles, Belgium). This assay detected only human IL-18 and the minimum detectable dose in our laboratory was 14.6 pg/mL.

Statistical analysis

All the data were expressed as mean \pm standard deviation (SD). The Students t test was used to test for significant differences, the Spearman equation for correlations. In all tests, P values of less than .05 were considered significant.

RESULTS

The subjects were 8 burn patients with a TBSA of 15% or more, from whom blood specimens could be repeatedly obtained beginning immediately after the injury. All the patients survived. TBSA was mean 24.6 \pm 5.73% and mean BMI (body mass index) was 24.5 \pm 3.38. The patients ages ranged from 17 to 38 (mean 26.3 \pm 7.4). Table 1 shows the burn demographics of the study group and the causes of the burn injury of the cases. TBSA of the burn injury ranged from 16 to 33%. Among these 8 patients, 6 recovered within 4 weeks and 2 patients who needed a skin graft operation recovered within 5 weeks. In these patients, skin grafting was applied for third degree burns at the third week of the admissions. An uneventful healing were achieved in all the patients within three weeks. Two of the patients in which wound cultures were positive for *Staphylococcus aureus* and *Pseudomonas aureginosa* had septic events during the healing course (Table 1).

Mean IL-6 serum level at admission was 26.1 ± 12.1 pg/mL (min: 12.8 and max: 51.7). Mean serum IL-18 level at admission was 28.3 ± 11.9 pg/mL (min: 14.6 and max: 43.5). Mean serum TNF- α level was 6.8 ± 1.3 pg/mL (min: 4.6 and max: 8.8).

Mean serum IL-6 level at the 48th hour of postburn period was 59 ± 20 pg/mL (min: 36.8 and max: 88). Mean serum IL-18 level at the 48th hour of postburn period was 87.6 ± 27.6 pg/mL (min: 57.9 and max: 125.4). Mean serum TNF- α level at the 48th hour of the postburn period was 35.3 ± 20.1 pg/mL (min: 14.65 and max: 78.34).

Significant increases in sera IL-6, IL-18, and TNF- α were detected at the 48th hour postburn ($P < .0001$). The IL-6, IL-18, and TNF- α levels at admission did not show any difference from the control group ($P > .05$). Table 1 shows the results at first admission and 48th hour in postburn period.

With using Spearman test, it was seen that these three parameters were highly correlated with each other ($r > 0.9$ and $P < .001$). There was no MODS event in any patient who experienced sepsis during healing course in the study group.

DISCUSSION

IL-18 enhance the IL-12 driven Th1 immune responses in bacterial and viral infections and is therefore essential for host defence [11]. IL-18 is a multifunctional cytokine that affiliates to the IL-1 cytokine family and is produced by immunocompetent and nonimmunocompetent cells such as keratinocytes, intestinal and airway epithelial cells (entry of microbes and allergens to body) even under normal physiological conditions [12, 13]. The aim of this study was to investigate the course of a proinflammatory cytokine IL-18 in burned patients at the first 48 hours of postburn period. Previous studies have shown that increased serum IL-6 and TNF- α levels are found postburn [5–7]. IL-18 (also known as interferon-gamma inducing factor, or IGIF), a new molecule has now been added to the very short list of interferon regulators. Either independently or in synergy with IL-12, the effects of IL-18, through its induction of IFN-gamma, can lead to a rapid activation of the monocyte/macrophage system with an upregulation of their innate immune capabilities [13–15]. IL-18 itself is induced by stressful stimuli (i.e., bacterial or neurogenic signals) [16]. IL-18 has also been implicated in killing mediated by the Fas ligand (FasL). FasL is a tightly regulated 40 kDa member of the TNF superfamily of molecules [17]. IL-18 upregulates both FasL and IFN-gamma production in T cells and the IFN-gamma produced may well induce Fas antigen on a variety of cell types. Thus IL-18, via IFN-gamma induction, could be considered a molecule that provides both the means (FasL) and the opportunity (Fas) for instigating apoptotic cell death [18].

This study is the first to show that serum IL-18 levels increases within 48 hours postburn. It is well known that cigarette smoking may affect IL-18 levels by amplifying the airway inflammation associated with asthma [19]. We exclude such a confusing comorbid situations, as no patient who had chronic obstructive pulmonary disease or

inhalation injury included in the study. The purpose of measuring BMI depends on the fact that higher circulating concentrations of the proinflammatory cytokines interleukin-6, IL-8, and IL-18 as well as C-reactive protein were found in obese men [20]. Although BMI of the study group ranged between normal and obese (mean 24.5 ± 3.38), we do not think that this parameter had a profound effect on IL-18 levels at the postburn period, as the IL-18 levels of first blood samples did not show any heterogeneity. TNF- α production has been previously shown to correlate with the release of lipopolysaccharide and IL-6 [21]. In the present study, this was confirmed, because both TNF- α and IL-6 levels were found as elevated. A further implication of this study is that detection of increased serum IL-18 after burn injury significantly correlates with TNF- α and IL-6.

Experimentally, it was shown that IL-18 therapy decreased the mortality of burn-injured mice followed by a severe infection, whereas it unexpectedly increased the mortality of burned mice with a mild infection [22]. Neutralisation of IL-18 during experimental lethal endotoxemia protects mice against lethal effects of lipopolysaccharide which is a composition of some bacteria [23]. Burn sepsis usually occurs by bacteriemia. IL-18 has also been shown to improve the early defence system against influenza virus infection [24]. IL-18 also exerts proinflammatory properties by inducing the production of IL-1 β , TNF- α , IL-6, and IFN- γ [25]. The observed elevation of IL-18 serum levels may be related to bacteriemic and/or neurogenic stimuli in clinical scenario of burned patient. With these findings obtained from this study, it is too early to draw conclusions about the relationship between IL-18 and other parameters evaluated. Future studies will delineate the role of IL-18 in the pathophysiology of postburn period.

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