

*Full Length Research Paper*

# Assessment of inflammatory cytokines and soluble adhesion molecules in patients with systemic inflammatory response syndrome in an intensive care unit of a Saudi tertiary hospital

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**Cytokines are endogenous inflammatory mediators, which play a central role in the pathophysiology of sepsis and in the expression of the adhesion molecules. The aims of this study are to analyze the levels of cytokines and the soluble adhesion in serum of infected (N = 68) and non-infected (N = 41) patients with systemic inflammatory response molecules (SIRS). 109 patients in the intensive care unit (ICU) of a tertiary hospital were included. IL-6, TNF- $\alpha$ , IL-10, IL-13, sICAM-1 and VCAM-1 were measured using enzyme-linked immunosorbent assay (ELISA). Patients with infectious SIRS, the levels of IL-6 varied between 27.65 to 39.6 pg/L (mean = 33.4 pg/L); the levels of sVCAM-1 varied between 543 and 1079 ng/ml (mean = 782 ng/ml) and the levels of sICAM-1 varied between 320 and 664 ng/ml (mean = 458 ng/ml). In patients with non-infectious SIRS the levels of IL-6 varied between 18.2 to 20.3 pg/L (mean 19.2 pg/L); the levels of sVCAM-1 varied between 251 and 635 ng/ml (mean = 286 ng/ml) and the levels of sICAM-1 varied between 98 and 351 ng/ml (mean = 168 ng/ml). The levels of IL-6, sVCAM-1 and s ICAM-1 were significantly higher in septic patients than in non-septic patients ( $p = 0.002$ ;  $p = 0.003$  and  $p = 0.0002$ , respectively). There was no statistically significant difference in the levels of TNF- $\alpha$ , IL-10 and IL-13 between infectious and non-infectious SIRS patients. Measurement of pro-and anti-inflammatory cytokines and soluble adhesion molecules may be useful in the follow up of ICU patients and in providing a point of care tests that will help in decision making and in management of ICU patients.**

**Key words:** Cytokines, adhesion molecules, enzyme-linked immunosorbent assay (ELISA), intensive care unit (ICU) patients.

## INTRODUCTION

Systemic inflammation is a highly organized response to infectious and noninfectious threats to homeostasis (Shubin et al., 2011). The main effectors of systemic inflammation are inflammatory cytokines, such as tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\beta$ , and IL-6; chemokines and other mediators of inflammation (Shubin et al., 2011; Kibe et al., 2011).

Cytokines (including chemokines) are endogenous

inflammatory mediators, which play a central role in the pathophysiology of sepsis (Nagai et al, 2011). TNF- $\alpha$  is a principal pro-inflammatory cytokine that induces systemic inflammatory response against the infectious insult (Nagai et al., 2011). Other pro-inflammatory cytokines include IL-1 $\beta$ , IL6, IL-8, interferon (IFN)  $\gamma$ , and macrophage migration inhibitory factor (MIF) (Eggimann and Pittet, 2001; Martin et al., 1994; Goldie et al., 1995; Pinsky et al., 1993). Excessive production of pro-inflammatory cytokines by immunocompetent cells may induce systemic inflammatory response syndrome (SIRS) (Damas et al., 1992; Oda et al., 2005).

Sepsis also activates the production and release of

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specific anti-inflammatory substances, including the cytokine receptor antagonists, the soluble cytokine receptors and the anti-inflammatory cytokines (Makhija, 2005; Delsesto and Opal, 2011; Tamayo et al., 2011). IL-10, IL-13 and transforming growth factor  $\beta$  (TGF- $\beta$ ) are anti-inflammatory cytokine, which probably have an important down-regulatory function in decreasing the production of various pro-inflammatory cytokines, such as TNF- $\alpha$  and IL-6 (Marchant et al., 1994; Martin et al., 1997).

Nosocomial infections (NIs) are today by far the commonest complications affecting hospitalised patients. Currently, 5 to 10% of patients admitted to acute care hospitals acquire one or more infections, and the risks have steadily increased during recent decades (Esposito and Leone, 2007; Jarvis, 2001). Although representing only 5 to 15% of hospital beds, intensive care units (ICUs) account for 10 to 25% of healthcare costs, corresponding to 1 to 2% of the gross national product of the United States (Esposito and Leone, 2007).

Vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) are mediators of endothelial-leukocyte adhesion in inflammatory states (Shapiro et al., 2010; Gando et al., 2005; Figueras-Aloy et al., 2007). They mediate tight binding and extravasation of leukocytes through endothelial cell junctions (Brenner et al., 2010). Low levels of adhesion molecules are detected in serum of normal individuals and several investigators have recently documented increased levels in patients with sepsis and other critical illnesses (Brenner et al., 2010; Cumming et al., 1997). There is interest in examining serum levels of adhesion molecules in sepsis and other inflammatory conditions and relating their measurement to outcome from critical illness (Newman et al., 1993). Up-regulation of membrane-bound and soluble forms of adhesion molecules and their corresponding ligands on endothelial cells is induced by inflammatory mediators, such as TNF- $\alpha$  and IL-6. Soluble isoforms of adhesion molecules are critical for the early events of leukocyte recruitment (Jaber et al., 2009).

The published data on the relationship between the serum levels of soluble adhesion molecules and SIRS is sparse. Serum levels of cytokines and adhesion molecules could be different in different groups of SIRS patients because of the differences in the underlying cause. The purpose of this study was to assess the level of cytokines and soluble adhesion molecules in different groups of SIRS patients in the ICU of a tertiary hospital.

## MATERIALS AND METHODS

### Study population

All patients admitted to the ICU with SIRS over a period of 12 months (2007 to 2008) were included (N = 109). The study was conducted in a tertiary hospital in Eastern Saudi Arabia (Alzahrani et al., 2009). Patients were categorized into the following groups:

patients with sepsis (N = 68) and those with SIRS without infection (N = 41). Clinically suspected infection was defined by the attending physician including the suspicion of an ongoing infection, combined with the initiation of a diagnostic work-up. Patients with SIRS were defined as having two or more of the following criteria (Rosengart, 2006): fever (body temperature  $>38^{\circ}\text{C}$ ) or hypothermia (body temperature  $<35.5^{\circ}\text{C}$ ), tachycardia (heart rate  $>90$  beat  $\text{s}^{-1}$ ), tachypnea ( $>20$  breaths  $\text{min}^{-1}$ ) and leukocytosis or leukopenia (white blood cell count  $>12\,000$  or  $<4000/\text{mm}^3$ ). The infection was confirmed if the patient has an identifiable site of infection which is confirmed by positive microbiological cultures. Bacterial cultures were obtained from trachea, urine, abdominal or mediastinal drains or from perioperative or percutaneous bacteriological samples. A written consent was obtained.

### Measurements

For cytokine assays, peripheral blood samples were collected from the subjects by venepuncture in plain tube, left to coagulate and fresh serum samples were separated and aliquots and stored at  $-70^{\circ}\text{C}$  prior to analysis. Aliquots were frozen within 8 of collection. The samples were taken within 24 h of admission to the ICU (day 1). Cytokine and soluble adhesion (IL-4, IL-6, IL-10, IL-13, TNF- $\alpha$ , sVCAM-1 and sICAM-1) analyses were performed with commercially available enzyme-linked immunosorbent assays (Quantikine<sup>®</sup>, R and D Systems, Minneapolis, Minn., USA; Biosource International, Camarillo; Pelikine Compact<sup>™</sup>, Central Laboratory of the Netherlands Red Cross Transfusion Service, Amsterdam, the Netherlands) according to the manufacturer's recommendations. In addition, complete blood counts were obtained on all patients including a full white blood cell (WBC) differential. Bacterial, viral and candida cultures/molecular detection of cytomegalovirus, respiratory syncytial virus and adenovirus were done using established assays.

### Statistical analysis

Data was entered in a personal computer and statistical analyses were performed using the statistical package for social sciences (SPSS-PC version 16). The descriptive statistics were reported as mean and range values. The patient groups were compared using the unpaired Student's t-test, the chi-squared test and the Friedman's test as indicated. P-values below 0.05 was considered significant in the SPSS software.

## RESULTS

The present study used the same study population of ICU patients that we used in previous study (Alzahrani et al., 2009). We evaluated a total of 109 SIRS patients admitted to ICUs in a tertiary hospital over the period of the study. Out of 109 patients, 68 fulfilled the criteria of infectious SIRS. The remaining 41 patients were classified as non-infectious SIRS, with no defined source of infection as proved by negative bacterial cultures. The mean age of the study population (45 males and 64 females) was  $49.8 \pm 20.9$  years. The clinical diagnosis included infection with varying degrees of sepsis (68), trauma (12), respiratory failure (10), post surgical (7), non infective complications requiring ICU (5), heart failure (3), sickle cell with crisis (3) and seizure disorder (1). Among

**Table 1.** Cytokines levels in infectious and non-infectious SIRS patients.

Cytokine	Level in infectious SIRS	Level in non-infectious SIRS
IL-6	27.65-39.6 pg/L(mean=33.4 pg/L)	18.2-20.3 pg/L(mean 19.2 pg/L)
TNF $\alpha$	2.5-5.6 pg/ml (mean 2.8 pg/ml)	2.3-4.2 pg/ml (mean 2.6 pg/ml)
IL-10	11.2-14.2 pg/L(mean=13.4 pg/L)	10.7-14.8 pg/L(mean 12.7 pg/L)
IL-13	22.5-39.2 pg/L(mean 31.5 pg/ml)	28.3-41.1 pg/L(mean=32.2 pg/ml)

**Table 2.** levels of soluble adhesion molecules (SAM) in infectious and non-infectious SIRS patients.

SAM	Level in infectious SIRS	Level in non- infectious SIRS
sVCAM-1	543-1079 ng/ml (mean=782 ng/ml)	251-635 ng/ml (mean=286 ng/ml)
sICAM	320-664 ng/ml (mean=458 ng/ml)	98-351 ng/ml (mean=168 ng/ml)

patients with defined source of infection, blood stream infection, lower respiratory tract infection, soft tissue infection and urinary tract infection constituted the most common infections.

The levels of proinflammatory cytokines were measured using enzyme-linked immunosorbent assay (ELISA) (Table 1). In patients with infectious SIRS, the levels of IL-6 varied between 27.65 to 39.6 pg/L (mean = 33.4 pg/l) and in patients with non-infectious SIRS the levels varied between 18.2 to 20.3 pg/L (mean 19.2 pg/L). In patients with infectious SIRS, the levels of TNF- $\alpha$  varied between 2.5 to 5.6 pg/ml (mean 2.8 pg/ml) and in patients with non-infectious SIRS the levels varied between 2.3 to 4.2 pg/ml (mean = 2.6 pg/ml). The levels of IL-6 was significantly higher in septic patients than in non-septic patients ( $p = 0.002$ ). There was no statistically significant difference in TNF- $\alpha$  levels between infectious and non-infectious SIRS patients.

The levels of anti-inflammatory cytokines were measured using ELISA (Table 1). In patients with infectious SIRS, the levels of IL-10 varied between 11.2 to 14.2 pg/L (mean = 13.4 pg/l) and in patients with non-infectious SIRS the levels varied between 10.7 to 14.8 pg/L (mean 12.7 pg/l). In patients infectious SIRS, the levels of IL-13 varied between 22.5 to 39.2 pg/L (mean 31.5 pg/ml) and in patients with non-infectious SIRS the levels varied between 28.3 to 41.1 pg/L (mean = 32.2 pg/ml). There was no statistically significant difference in IL-10 and IL-13 levels between infectious and non-infectious SIRS patients.

The levels of sICAM-1 and sVCAM-1 were measured using ELISA (Table 2). The levels of sVCAM-1 in patients with infectious varied between 543 and 1079 ng/ml (mean = 782 ng/ml) and in non-infectious SIRS the levels varied between 251 and 635 ng/ml (mean = 286 ng/ml). The levels of sICAM in infectious SIRS varied between 320 and 664 ng/ml (mean = 458 ng/ml) and the levels in non-infectious SIRS varied between 98 and 351 ng/ml (mean = 168 ng/ml). The levels of sVCAM and sICAM

were significantly higher in the infectious SIRS than non-infectious SIRS patients ( $p = 0.003$  and  $= 0.0002$  respectively).

## DISCUSSION

Excessive production of pro-inflammatory cytokines by immunocompetent cells can induce SIRS and that these cytokines may play an important role in the development of acute respiratory distress syndrome (ARDS) or multiple organ dysfunction syndromes (MODS) (Oda et al., 2005; Delsesto and Opal, 2011). It has been reported that blood levels of these pro-inflammatory cytokines are elevated in patients with ARDS and septic shock, and that measurement of blood levels of these cytokines is useful in evaluating the severity and in predicting the outcome of the patients with these pathophysiological conditions (Delsesto and Opal, 2011). Among these pro-inflammatory cytokines, IL-6 has a longer half-life than TNF- $\alpha$  and IL-1 $\beta$  and its blood level remains consistently elevated in the presence of various diseases (Tamayo et al., 2011). For these reasons, the measurement of cytokines such as IL-6 blood levels is potentially useful in severity assessment and outcome prediction in patients with septic shock, trauma, severe acute pancreatitis, and cardiogenic shock (Martin et al., 1997). The rapid measurement system that allows blood IL-6 levels to be measured within about 30 min using chemiluminescent enzyme immunoassay (CLEIA) has recently been reported. This system can yield results of IL-6 measurement on approximately real-time basis when incorporated into the clinical laboratory test menu. The longer half-life of IL-6 in comparison with TNF- $\alpha$  explains why we can demonstrate a difference in the level of IL-6 and not in the level of TNF- $\alpha$ .

IL-6 levels are significantly elevated in the majority of patients with sepsis and the circulating IL-6 levels have correlated with the severity of sepsis in most studies

(Damas et al., 1992; Oda et al., 2005). The persistently high levels of IL-6 have been associated with the development of multiple organ failure (MOF) and poor prognosis (Tamayo et al., 2011). It has been suggested that elevated IL-6 levels reflect the activation of inflammatory response in sepsis, although the exact role of IL-6 in the pathogenesis of sepsis is not clear.

There was no statistically significant difference in IL-10 and IL-13 levels between infectious and non-infectious SIRS patients. This could be explained by the short half-life that leads to transient increase in their level in the plasma which makes their measurement in the plasma more difficult. Although many cell types express IL-10 mRNA, not all make detectable amounts of protein, and this variation can be accounted for by posttranscriptional mechanisms (Powell et al., 2000). Intra-cellular measurement of cytokines may provide more sensitive mean of cytokine analysis.

Theoretically, these anti-inflammatory substances may have an important regulatory function in controlling and attenuating the systemic inflammatory response in sepsis (Delsesto and Opal 2011). However, patients with an excessive compensatory anti-inflammatory response syndrome may be immunosuppressed and at increased risk of infection or death.

The published data on the relationship between the serum levels of soluble adhesion molecules and SIRS is sparse (Brenner et al., 2010). To assess the serum soluble adhesion molecules in the SIRS ICU patients, the levels of, soluble Inter-cellular adhesion molecule-1 (sICAM-1) and Soluble Form of Vascular Cell Adhesion Molecule 1(sVCAM-1) were measured using ELISA. The levels of sVCAM and sICAM were significantly higher in the infectious SIRS than non-infectious SIRS patients. This could be due to the vital role of adhesion molecules in the process of leukocyte adhesion and migration that occur during inflammation. ICAM-1 participates in the adhesion of leukocytes to the endothelium and may be crucial in regulating leukocyte activation at a very early inflammatory response. Expression of adhesion molecules is regulated by cytokine activation and recent studies examined the role of sICAM-1 in neonatal infections, but with controversial results.

Both mononuclear cells and endothelial cells secrete ICAM-1, and ICAM-1 expression on endothelial cells is up-regulated by cytokines such as IL-6. ICAM-1 is a ligand for lymphocyte function-associated antigen-1 (LFA-1) and is one of the receptors responsible for adhesion of T lymphocytes, monocytes and granulocytes to endothelium. VCAM-1 is expressed on dendritic cells and vascular endothelium activated by TNF, IL-1 and IL-4. It plays a major role in the adhesion of leucocytes to the endothelium by interaction with its ligand very late activation antigen-4 (VLA-4), which is expressed by lymphocytes and monocytes (Jaber et al., 2009).

In conclusion, measurement of IL-6, sVCAM-1 and sICAM-1 appear to correlate with the development of

infection and sepsis in SIRS patients. Measurement of pro-and anti-inflammatory cytokines and soluble adhesion molecules may be useful in the follow up of ICU patients and in providing a point of care tests that will help in decision making and in management of ICU patients. There is a need for more studies to establish a clear strategy for a diagnostic algorithm for cytokine and adhesion molecules measurements before such tests are eventually used in practice.

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