Prognostic Value of Interlukine-1 Receptor Antagonist and Tumor Necrosis Factor Gene Polymorphism in Systemic Inflammatory Response Syndrome

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Abstract

**Objective:** The aim of this work was to evaluate the prognostic value of TNF-α, IL-1β, and IL-1ra genetic polymorphism in SIRS patients.

**Patients:** In this work 120 patients with SIRS admitted to ICU were included, as well 30 age, sex, weight, and body mass index matched healthy control subjects. Patients were divided into two groups of 60 patients each, a group with sepsis and a group with non sepsis.

**Methods:** Clinical parameters, routine laboratory investigations, and a prognostic scoring system (APACHE II) were studied. Serum levels of TNF-α, IL-1β, and IL-1ra as well as their corresponding genetic polymorphism were also examined. The relation between the cytokine serum levels, their different alleles and parameters of disease outcome were studied.

**Results:** All studied cytokines were significant higher in non survivors more than survivors in total SIRS patients while in sepsis group TNF-α, and IL-1ra were significant higher in non survivors than survivors. In non sepsis group on the other hand IL-1ra was not significant different between survivors and non survivors while the other two cytokines were significant higher in non survivors. There was statistical significant positive linear correlation between serum levels of TNF-α, IL-1β, and IL-1ra cytokines on one hand and APACHE II scoring and mortality on the other hand in total SIRS patients and sepsis group. A ratio of pro/anti inflammatory cytokine (TNF-α x IL-1β/ IL-1ra) was significantly higher in non survivors than survivors in total and non sepsis group while in sepsis group no significant difference in this ratio between survivors and non survivors. TNF-α serum levels was significant higher in patients possessing allele 2 while no significant relation between other cytokine serum levels and their corresponding genetic polymorphism. There was no difference in frequency of distribution between control, sepsis group, and non sepsis group. TNF-α allele 2 was significant associated with mortality in sepsis group only, but not in total SIRS patients or non sepsis group.

**Conclusion:** The present results demonstrate that higher serum cytokine levels is related to mortality and a balance towards the anti-inflammatory response might be potentially protective in non septic patients but not in septic condition. TNF-α allele 2 polymorphism is associated with higher levels of TNF-α cytokine and poor outcome. In addition, Both TNF-α allele 2 and IL-1ra allele A2 were associated with poor outcome in septic but not in non septic patients, both TNF-α allele 2 and IL-1ra allele A2 may be viewed as predictor of poor outcome in sepsis.

**Key Words:** Interlukine-1 – Tumor necrosis factor gene (SIRS).

Introduction

MORTALITY from sepsis is the most common cause of death in the critical care unit. It has not decreased dramatically in the past decade. Genetic epidemiologic studies suggest a strong genetic influence on the outcome from sepsis, and genetics may explain the wide variation in the individual response to infection that has long puzzled clinicians [1].

Molecular biology has revolutionized medicine by increasing our understanding of the pathophysiological mechanisms of diseases and ability to assess genetic risks. Individual differences in disease manifestation and course in intensive care medicine often cannot be explained by known phenotypic risk factor alone [2]. Recent data suggested an association between specific genotypes and the risk of adverse clinical outcome. This included inflammatory responses (TNF-α, IL-1β, IL-1ra, IL-10), infectious diseases such as pneumonia or meningitis, sepsis, ARDS, as well as the mortality of critically injured patients (polytrauma, severe brain trauma). It may potentially decrease morbidity and mortality through improved risk
assessment and the administration of prophylactic therapy [2].

For clinical practice, gene polymorphism of specific host immune defence elements appeared to be of major importance. These genetic variants, which modify the regulation or function of mediator, have been associated with susceptibility and/or outcome of severe sepsis and shock [3]. Polymorphism of cytokines genes (TNF-α, IL-1β, IL-1ra) have been reported to influence the level of secreted mediators and to unbalance the inflammatory cascade. The impact of these finding on the understanding of SIRS pathogenesis and on design of future preventive and therapeutic strategies awaits more clarification [3].

Aim of the work: Is to evaluate the prognostic value of TNF-α, IL-1β, and IL-1ra gene polymorphism in SIRS patients.

Patient and Methods

At Critical Care Medicine, Cairo University from 2003-2005, the present study was done including 120 patients with SIRS. Patients with SIRS was divided into two groups:

1- With sepsis.
2- Without sepsis (e.g. Trauma, hemorrhage, pancreatitis).

Inclusion criteria:
- Ages are more than 18 years.
- Inclusion criteria in SIRS with sepsis:
  - Clinical evidence of infection, as suggested by, but not limited to, the presence of one or more of the following signs:
    - Presence of polymorphonuclear cells in a normally sterile body fluid.
    - Culture or gram stain of blood, sputum, urine, or normally sterile body fluids is positive for pathogenic microorganism.
    - Chest radiograph is consistent with a diagnosis of pneumonia.
    - Focus of infection is identified by visual inspection (wound with purulent drainage, radiograph or computed tomography evidence of an abscesses or osteomyelitis).
    - Patients has an underling diseases or condition that is likely to be associated with infection (e.g ascending cholangitis, ischemic bowel).

w Fulfillment of a systemic response to infection (SIRS) was included, as established by the consensus statement from the American College of Chest Physicians and Society of Critical Care Medicine.

It required that the patients had two of the following:
- Fever or hypothermia (core temperature of ≥38.0°C or ≤36.0°C).
- Tachycardia (heart rate of ≥90 beats/min), except in patients were receiving a β adrenergic receptor blocking agent or a rate of pace maker.
- Tachypnea (respiratory rate of ≥20 breaths/min while spontaneously breathing or patients requiring mechanical ventilation) or arterial CO2 pressure <32mm Hg.
- Leukocytosis or leukopenia (white blood cell count >12,000 cells/mm³ or <4,000 cells/mm³).
- Inclusion criteria in patients with SIRS without sepsis:
  - Poly trauma.
  - Evidence of hemorrhage.
  - Evidence of pancreatitis.
  - Major burn.

For every patients in the study, the following was dove:

Brief history taking, Clinical examination, Routine investigations (laboratory and radiological).

APATCH II: (Acute Physiology and Chronic Health Evaluation) is prognostic score of patients in ICU.

Survival: (Survivor or non survivor) and hospital stay were recorded.

Genotyping of patients: Tumor necrosis factor -alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-1 receptor antagonist (IL-1ra), genotypes and allele frequencies in patients and control were detected. Venous blood was drawn into sterile vacutainers containing Ethylene Diamine Tetra Acetic acid (EDTA) on the day of admission to hospital for genotypic analysis, and stored at -80°C until processed. DNA was extracted from the blood samples using QIA amp® DNA blood Mini Kit (50) (Germany). Polymerase chain reaction
(PCR) was used to amplify DNA candidate genes of interest using thermal cycle, Gene Amp PCR System 2400 (Perkin, Elmer, CA, USA).

I- TNF polymorphism: Genomic DNA were extracted as described before. The 5’ region of TNF gene (-331 to 14) was amplified by polymerase chain reaction (PCR) similar to that described by Wilson, et al. [4] with some modification: 5’ primer was.

5'- AGGCAATAGGGTTGGAGGCCCAT and the 3’ primer was 5’GAGCGTCTGCTGGGTGGTG (Perkin, Elmer, Taiwan). PCR conditions were as follows: Genomic DNA was amplified using 0.2 M primers and Taq PCR master Mix Kit (QIAGEN, Germany). Cycling, 94°C for 1min, 60°C for 1min and 72°C for 1min for 30 cycle, followed by 60°C for 1min and 72°C for 5min. The PCR product was ethanol-precipitated and digested with Nco1 (Roche Diagnostic, Germany) and analyzed on 2%. Metaphor agarose gel. DNA products were visualized by Etidium bromide staining. The TNF 1 allele would be digested into two fragments (325 and 20 base pairs) whereas TNF2 allele would not be digested (345 base pairs).

II- IL-1β polymorphisms: The region contains the Taq I polymorphic site within exon 5 of the IL-1β gene was amplified by PCR. The oligonucleotides 5’-GGTGATGCTACTCGTACGCTGG-3’ and 5’-TTCAGTTCATATGGACC-3’ were used as primers flanking this region to amplify the region which contains the Taq I polymorphic site within exon 5 of the IL-1β gene (Fang, et al. 1999). Amplification was performed by the following PCR protocol, an initial denaturation was conducted at 95°C for 3 mins, followed by 35 cycles of a three temperature PCR segment consisting of denaturation (95°C, 30 secs), annealing (62°C, 30 secs), and extension (72°C, 30 secs), followed by one cycle at 72C for 10 min and cooling at 4C. The PCR products (alleles A1, A2, A3, A4, and A5). 410 bp (allele 1, four repeats of the 86 bp region) 240 bp (allele 2, two repeats), 500 bp (allele 3, five repeats) 325 bp (allele 4, three repeats) and 595 bp (allele 5, six repeats) were analyzed by electrophoresis on 2% agarose gel stained with ethidium bromide.

Pro/anti inflammatory cytokines ratio: In the present study there were pro-inflammatory cytokines (IL-1β, TNF-α) and anti-inflammatory cytokine (IL-1ra). The ratio between pro and anti inflammatory cytokines was utilized to study the balance between the pro and anti inflammatory cytokine effect. Pro/anti-inflammatory cytokines ratio was computed by multiply IL-1β and TNF-α then dividing by IL-1ra. (IL-1β x TNF-α/IL-1ra). TNF α cytokine and IL-1β cytokine were assessed by Immunoassay and the IL-1ra cytokine was assessed biosource IL-1ra cytoscreen assay.

Control group: 30 Healthy control, for each one the following was done:
- History and examination including age, gender, weight, height were done.
- Serum cytokine levels were taken (TNF-α, IL-1β, IL-1ra).
- Cytokine genotyping were done (TNF-α, IL-1β, IL-1ra) as describe before.

Statistical analysis: The statistical analysis was run on IBM compatible personal computer by using the statistical package for social scientist (SPSS) program for windows version 10.00 (SPSS Inc, IL, USA). Test of normality of data distribution was done first (one-sample kolmogorov-Smirnov test). Independent-samples t test used for compare parametric two variables while one way Anova (F-test) was used to compare between more than two group. Qualitative data were presented as number, and percent. Comparison between groups was done by Chi-Square. In non parametric variables Mann-Whitney test was used to compare between two groups variable while Kruskal-wallis test used for multiple variants. Quantitative data were expressed as mean ± SD for parametric variables, median and
range for non parametric variables. Pearson’s correlation coefficient was used to test linear correlation between variables. Spearman’s correlation coefficient was used to test linear correlation between variables. Partial correlation was done to control the effect of some variables during study linear correlation between another variables. A statistical index of the degree of linear dependence between the pair of values taken by observation of two variables. By definition. This must lie between +1 and -1, being positive if the two variables increase or decrease together. A zero correlation coefficient (r) implies a complete absence of correlation. p<0.05 was considered to be mild statistically significant. p<0.01 was considered to be moderate statistically significant. p<0.001 was considered to be high statistically significant.

Results

1-Demographic data (Table 1):

Table (1): Demographic data of total SIRS patients versus control.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control (N=30)</th>
<th>SIRS (N=120)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE (years) Mean ±SD</td>
<td>45.93±10.79</td>
<td>44.93±13.08</td>
<td>0.785</td>
</tr>
<tr>
<td>WT (kg) Mean ±SD</td>
<td>71.33±14.73</td>
<td>73.62±15.6</td>
<td>0.610</td>
</tr>
<tr>
<td>BMI Mean ±SD</td>
<td>24.43±3.1</td>
<td>25.56±5.9</td>
<td>0.476</td>
</tr>
<tr>
<td><strong>Gender:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male N (%)</td>
<td>20 (66.67%)</td>
<td>66 (55%)</td>
<td>0.562</td>
</tr>
<tr>
<td>Female N (%)</td>
<td>10 (33.33%)</td>
<td>54 (45%)</td>
<td></td>
</tr>
</tbody>
</table>

Independent-samples t test for Age, Weight (wt), and body mass index and Pearson chi square test for gender.

2- Serum cytokines (Table 2):

In control group, TNF-α serum cytokine ranged from 1.8 to 3.1 pg/dl with median value of 2.13 pg/dl while in total SIRS, it ranged from 6.1 to 74.2 with median value of 13 pg/dl. TNF-α serum cytokine was significantly higher in total SIRS patients than control group (p<0.001). IL-1β ranged from 1.8 to 2.4 pg/dl with median value of 2.14 pg/dl in control group while IL-1β ranged from 5 to 19 with median value of 5 pg/dl in total SIRS patients. IL-1β was significant higher in total SIRS than control group (p<0.001). IL-1ra ranged from 205 to 250 pg/dl with median value of 230 pg/dl in control group while IL-1ra ranged from 120 to 5460 with median value of 640 pg/dl in total SIRS patients. IL-1β was significant higher in total SIRS than control group (p<0.001). TNF-α IL-1β and IL-1ra cytokines were positively correlated to each other.

3- Pro/anti inflammatory ratio (Table 2):

Ratio ranged from 1.5 to 2.6 with median 2 In control group while in total sepsis group, it ranged from 2.98 to 133.21 with median 20.38. There was significant increase in ratio in total sepsis group (p<0.001).

4- Frequency off alleles (Table 3):

In the present study, in control group there were 26 (86.7%) TNF-α allele 1 patients while TNF-α allele 2 patients were 4 (13.3%). In sepsis group, there were 40 (66.7%) TNF-α allele 1 patients while TNF-α allele 2 patients were 20 (33.3%). There were 40 (66.7%) TNF-α allele 1 patients in non sepsis group while TNF-α allele 2 patients were 20 (33.3%). There was no statistically significant difference (p=0.314). In control group, there were 24 (80%) IL-1β allele 1 patients while IL-1β allele 2 patients were 6 (20%). In sepsis group, there were 52 (86.7%) IL-1β allele 1 patients while IL-1β allele 2 patients were 8 (13.3%). There were 52 (86.7%) IL-1β allele 1 patients in non sepsis group while IL-1β allele 2 patients were 8 (13.3%). There was no statistical significant difference (p= 0.808). In control group, IL-1ra allele A1 gene polymorphism was in 28 (93.3%) patients while IL-1ra allele A2 was only in 2 patients (6.7%). In sepsis group, there were 54 (90%) IL-1ra allele A1 patients and 6 (10%) IL-1ra allele A2 patients. In non sepsis group, there were 54 (90%) IL-1ra allele A1 patients, 4 (6.7%) IL-1ra allele A2, and 2 (3.3%) IL-1ra allele A4 patients. There was no allele A3, or A5 in the present study. There was no statistically significant difference between groups in frequency of IL-1ra gene polymorphism (p=0.779).

5- Cytokines with different alleles in total SIRS patients:

In TNF-α allele1 patients, TNF-α cytokine ranged from 6.1 to 74.2 with median value of 13 pg/dl, and it ranged from 7.4 to 78.5 with median

<p>| Table (2): Serum cytokines, and pro/anti inflammatory ratio in total SIRS patient versus control. |
|--------------------------------------------------|-----------------------------------|--------------|</p>
<table>
<thead>
<tr>
<th>Time (min-max)</th>
<th>Median (pg/dl)</th>
<th>SD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (pg/dl)</td>
<td>2.13 (1.8-3.1)</td>
<td>14.85 (6.1-78.5)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>IL-1β (pg/dl)</td>
<td>2.14 (1.8-2.4)</td>
<td>5 (5-19)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>IL-1ra (pg/dl)</td>
<td>230 (205-250)</td>
<td>640 (120-5460)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Pro/anti inflammatory ratio</td>
<td>2 (1.5-2.6)</td>
<td>20.38 (2.98-133.21)</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Mann-whitney test.
value of 20 pg/dl in TNF-α allele 2 patients, with significant higher in TNF-α allele 2 patients (p=0.046). IL-1β cytokine ranged from 5 to 18 with median value of 5 in IL-1β allele 1 patients, and it ranged from 5 to 15.3 with median value of 5 in IL-1β allele 2 patients, without difference between IL-1β with IL-1β allele 1 patients and IL-1β with allele 2 patients (p=0.816). In IL-1ra allele A1 patients, IL-1ra cytokine ranged from 120 to 1540 with median value of 615 pg/dl, and it ranged from 520 to 5460 with median value of 1010 pg/dl in IL-1ra allele A2 patients, without significant difference between IL-1ra allele A1 patients and IL-1ra allele A2 patients (p=0.113).

Table (3): Frequency of alleles in different groups.

<table>
<thead>
<tr>
<th></th>
<th>Control (30)</th>
<th>Sepsis (60)</th>
<th>Non sepsis (60)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α allele 1</td>
<td>26 (86.7%)</td>
<td>40 (66.7%)</td>
<td>40 (66.7%)</td>
<td>0.314</td>
</tr>
<tr>
<td>TNF-α allele 2</td>
<td>4 (13.3%)</td>
<td>20 (33.3%)</td>
<td>20 (33.3%)</td>
<td></td>
</tr>
<tr>
<td>IL-1β allele 1</td>
<td>24 (80%)</td>
<td>52 (86.7%)</td>
<td>52 (86.7%)</td>
<td>0.808</td>
</tr>
<tr>
<td>IL-1β allele 2</td>
<td>6 (20%)</td>
<td>8 (13.3%)</td>
<td>8 (13.3%)</td>
<td></td>
</tr>
<tr>
<td>IL-1ra allele A1</td>
<td>28 (93.3%)</td>
<td>54 (90%)</td>
<td>54 (90%)</td>
<td>0.779</td>
</tr>
<tr>
<td>IL-1ra allele A2</td>
<td>6 (20%)</td>
<td>4 (10%)</td>
<td>4 (6.7%)</td>
<td></td>
</tr>
<tr>
<td>IL-1ra allele A4</td>
<td>2 (3.3%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6- APACHE II score with different alleles:

In the present study, APACHE II score ranged from 18 to 35 with median 21 in TNF-α allele 1 while it ranged from 19 to 35 with median 25 in TNF-α allele 2, there was no statistically significant difference (p=0.218). APACHE II score ranged from 18 to 35 with median 23 in IL-1β allele 1 patients while it ranged from 19 to 27 with median 20 in IL-1β allele 2 patients, there was no statistically significant difference (p=0.336). APACHE II score ranged from 18 to 35 with median 22 in IL-1ra allele A1 patients while it ranged from 19 to 35 with median 28 in IL-1ra allele A2 patients, there was no statistically significant difference (p=0.234).

7- Pro/anti inflammatory ratio and outcome:

In survivor subgroup, ratio ranged from 2.98 to 113.83 with median value of 12.82 while it ranged from 3.53 to 133.21 with median 34.13 in non survivor subgroup, this ratio was significant higher in non survivor subgroup (p=0.001).

8- Cytokines and outcome:

TNF-α ranged from 6.1 to 37.2 with median value of 11.15 pg/dl in survivor subgroup while it ranged from 8.9 to 78.6 with median value of 37.6 pg/dl in non survivor subgroup, there was significant higher of TNF-α cytokine in non survivors (p<0.001). In survivor subgroup IL-1β ranged from 5 to 15.3 with median value of 5 pg/dl while it ranged from 5 to 19.5 with median value of 10.5 pg/dl in non survivor subgroup, there was significant higher of IL-1β cytokine in non survivors (p<0.001). IL-1ra ranged from 120 to 1450 with median value of 500 pg/dl in survivor subgroup while it ranged from 320 to 5460 with median value of 1067 pg/dl in non survivor subgroup, there was significant higher of IL-1ra cytokine in non survivors (p=0.001) (Table 4).

Table (4): APACHE II score, serum cytokines levels and pro/anti inflammatory ratio in relation to survival in total SIRS patient.

<table>
<thead>
<tr>
<th></th>
<th>Survivor (84)</th>
<th>Non Survivor (36)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>APACHE II score</td>
<td>21 (18-29)</td>
<td>29 (23-35)</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td>TNF-α (pg/dl)</td>
<td>11.15 (6.1-37.2)</td>
<td>37.6 (8.9-78.5)</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td>IL-1β (pg/dl)</td>
<td>5 (5-15.3)</td>
<td>10.5 (5-19.5)</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td>IL-1ra (pg/dl)</td>
<td>500 (120-1450)</td>
<td>1067 (320-5460)</td>
<td>0.001 *</td>
</tr>
<tr>
<td>Pro/anti inflammatory ratio</td>
<td>12.82 (2.98-113.83)</td>
<td>34.13 (3.53-133.21)</td>
<td>0.001 *</td>
</tr>
</tbody>
</table>

Chi square test.

Mann whitney test.

9- Mortality:

Mortality was linear positively correlated with APACHE II score, TNF-α, IL-1β, IL-1ra, and pro/anti inflammatory ratio, TNF-α alleles, and hospital stay while survival was not correlated with IL-1β alleles, and IL-1ra alleles. When correlation between mortality and TNF-α alleles had been controlled for APACHE II score, there was positive correlation.

When correlation between mortality and TNF-α alleles was controlled for TNF-α cytokine, there was positive correlation between mortality and TNF-α alleles. When correlation between mortality and TNF-α cytokine controlled for IL-1β and IL-1ra, positive correlation was persistent. Also positive correlation was persistent when controlled for APACHE II score and when controlled for TNF-α alleles. When correlation between mortality and IL-1β & IL-1ra was controlled for TNF-α cytokine, correlation was disappeared.
10- Gene polymorphism and outcome:

There were 80 patients had TNF-α allele 1, 64 (80%) patients survived while 16 (20%) patients died. 20 (50%) patients from TNF-α allele 2 patients survived while 20 (50%) patients died. TNF-α allele 2 was significantly associated with mortality ($p=0.018$). 70 (67.3%) patients from IL-1β allele 1 patients survived while 34 (32.7%) patients died. In IL-1β allele 2 patients, there were 14 (87.5%) survivor patients while only 2 died. There was no association between any IL-1β allele and survival ($p=0.415$). There were 78 (72.2%) IL-1ra allele A1 patients survived while 30 (27.8%) patients died. There were 10 patients had IL-1ra A2, 4 (40%) survived while 6 (60%) died. There was only 2 patient had IL-1β allele A4 and survived. There was no significant association between any IL-1β allele and mortality.

11- Comparison between sepsis and non sepsis groups:

Total SIRS patients was divided into two groups sepsis group and non sepsis group, each group included 60 patients. In sepsis group, APACHE II score ranged from 19 to 35 with median value of 25 and ranged from 19 to 30 in non sepsis group with mean value of 22, without significant difference ($p=0.121$). TNF-α cytokine ranged from 6.5 to 63.5 pg/dl in sepsis group with median value 24.6 pg/dl and in non sepsis group TNF-α cytokine ranged from 6.1 to 78.5 pg/dl with mean value of 11.25 pg/dl, TNF-α cytokine significant higher in sepsis group ($p=0.014$). In sepsis group, IL-1β ranged from 5 to 18 with median value of 5 and ranged from 5 to 19.3 in non sepsis group with mean value of 5, without significant difference ($p=0.622$). In sepsis group, IL-1ra ranged from 120 to 5450 pg/dl with median value of 830 pg/dl and ranged from 250 to 1450 pg/dl in non sepsis group with mean value of 570 pg/dl, without significant difference ($p=0.509$). Pro/anti inflammatory ratio ranged from 2.98 to 113.83 in sepsis group with median value 24.83 pg/dl and in non sepsis group pro/anti inflammatory ratio from 3.69 to 133.21 with mean value of 13.96, without significant difference ($p=0.092$).

A- Sepsis group:

Cytokines with different alleles: In TNF-α allele 1 patients, TNF-α cytokine ranged from 6.5 to 63.5 with median value of 23.4 pg/dl, and it ranged from 8.9 to 59.2 with median value of 31.54 pg/dl in TNF-α allele 2 patients, with significant higher in TNF-α allele 2 patients ($p=0.042$). IL-1β cytokine ranged from 5 to 18 with median value of 5 pg/dl in IL-1β allele 1 patients, and it ranged from 5 to 15.3 with median value of 7.75 pg/dl in IL-1β allele 2 patients, without difference between IL-1β with IL-1β allele 1 patients and IL-1β with IL-1β allele 2 patients ($p=0.298$). In IL-1ra allele A1 patients, IL-1ra cytokine ranged from 120 to 1580 with median value of 640 pg/dl, and it ranged from 1010 to 5460 with median value of 1260 pg/dl in IL-1ra allele A2 patients, without statistically significant difference between IL-1ra allele A1 patients and IL-1ra allele A2 patients ($p=0.086$).

Cytokines and outcome:

TNF-α ranged from 6.5 to 37.2 pg/dl with median value of 17.6 pg/dl in survivor subgroup while it ranged from 8.9 to 63.5 with median value of 38 pg/dl in non survivor subgroup, there was statistically significant higher of TNF-α cytokine in non survivors ($p<0.001$). In survivor subgroup IL-1β ranged from 5 to 15.3 with median value of 10.2 pg/dl in non survivors subgroup, without statistically significant difference ($p=0.057$). IL-1ra ranged from 120 to 1295 with median value of 500 pg/dl in survivor subgroup while it ranged from 440 to 5460 with median value of 1080 pg/dl in non survivor subgroup, there was significant higher of IL-1ra cytokine in non survivors ($p=0.005$).

Correlation between cytokines, APACHE II score, hospital stay, and mortality:

APACHE II score was positively correlated to TNF-α, IL-1β, IL-1ra and hospital stay. Hospital stay was positively correlated to TNF-α IL-1β, and IL-1ra cytokines. TNF-α, IL-1β, and IL-1ra cytokines were positively correlated to each other. Mortality was positively correlated to APACHE, hospital stay, TNF-α, IL-1β, and IL-1ra.

Gene polymorphism and outcome:

There were 40 patients had TNF-α allele 1, 30 (75%) patients survived while 10 (25%) patients died. 8 (40%) patients from TNF-α allele 2 patients survived while 12 (60%) patients died. TNF-α allele 2 was significantly associated with mortality ($p=0.045$). 32 (61.5) patients from IL-1β allele 1 patients survived while 20 (38.5%) patients died. In IL-1β allele 2 there were 6 (75%) survivor patients while only 2 (25%) died. There was no association between any IL-1β allele and survival ($p=0.530$). There were 38 (70.4%) IL-1ra allele A1 patients survived while 16 (29.6%) patients died. There were 6 patients had IL-1ra A2, 3 (100%) died. There was no patient had IL-1ra allele A3, IL-1ra allele A4 or IL-1ra allele A5. There was significant association between IL-1ra allele A2 and mortality ($p=0.041$).
B- Non sepsis group:

Cytokines with different alleles: In TNF-α allele 1 patients, TNF-α cytokine ranged from 6 to 74 with median value of 10 pg/dl, and it ranged from 7 to 78.5 with median value of 16 pg/dl in TNF-α allele 2 patients, with significant higher in TNF-α allele 2 patients (p=0.015). IL-1β cytokine ranged from 5 to 19 with median value of 5 in IL-1β allele 1 patients, and it ranged from 5 to 5.1 with median value of 5 in IL-1β allele 2 patients, without difference between IL-1β with IL-1β allele 1 patients and IL-1β with allele 2 patients (p=0.498). In IL-1α allele A1 patients, IL-1α cytokine ranged from 280 to 1450 with median value of 590 pg/dl, and it ranged from 520 to 940 with median value of 730 pg/dl in IL-1α allele A2 patients, without significant difference between IL-1α allele A1 patients and IL-1α allele A2 patients (p=0.768).

Cytokines and outcome: TNF-α ranged from 6.1 to 19.1 pg/dl with median value of 10.3 pg/dl in survivor subgroup while it ranged from 15 to 78 with median value of 30 pg/dl in non survivor subgroup, there was significant higher of TNF-α cytokine in non survivors (p<0.001). In survivor subgroup IL-1β ranged from 5 to 11.1 with median value of 5 pg/dl while it ranged from 5 to 19.3 with median value of 15 pg/dl in non survivor subgroup. IL-1β cytokine was significant higher in non survivors (p=0.022). IL-1α ranged from 280 to 1450 with median value of 535 pg/dl in survivor subgroup while it ranged from 385 to 1360 with median value of 960 pg/dl in non survivor subgroup, without significant difference (p=0.135).

Correlation between cytokines, APACHE II score, hospital stay, and mortality: APACHE II score was positively correlated to TNF-α, and IL-1β while APACHE II score was not correlated to IL-1α cytokines and hospital stay. Hospital stay was not correlated to TNF-α IL-1β, IL-1α cytokines. TNF-α, and IL-1β cytokines were positively correlated to each other. IL-1α cytokine was positively correlated to TNF-α cytokine and not correlated to IL-1β cytokine. Mortality was positively correlated to APACHE II score, TNF-α, and IL-1β while mortality was not correlated to IL-1α cytokine and hospital stay.

Gene polymorphism and outcome: There were 40 patients had TNF-α allele 1, 34 (85%) patients survived while 6 (15%) patients died. 12 (60%) patients from TNF-α allele 2 patients survived while 8 (40%) patients died. Without significant difference (p=0.181). 38 (73%) patients from IL-1β allele 1 patients survived while 14 (27%) patients died. In IL-1β allele 2 there were 8 (100%) survivor patients. There was no association between any IL-1β allele and survival (p=0.548). There were 40 (74.1%) IL-1α allele A1 patients survived while 14 (27%) patients died. There were 4 patients had IL-1α allele A2, the 4 (100%) survived. There was only 2 had IL-1α allele A4 and survived. There was no patients contain IL-1α allele A3, or IL-1β allele 5. There was no significant association between different IL-1α alleles and mortality (p=0.602).

Discussion

In the present study, there was no significant difference between total SIRS patients and control in age, gender, weight, and body mass index. Also, in both sepsis group and non sepsis group there were no significant difference in age, gender, weight, and body mass index. So control, sepsis group, and non sepsis group were matched together.

Age was higher in non survivors versus survivors without significance in total SIRS patients (p value 0.056). It is known that higher age group is usually attended with different degrees of deterioration of system function and lower immune response [8]. In accordance to this finding, Huang and his associates [6] found that old age was associated with higher mortality. Martin and his colleague [7] sought to determine the independent effect of age on outcome of adult sepsis, they found that mortality rates increased linearly by age. Age was an independent predictor of mortality. Elderly non survivors of sepsis died earlier during hospitalization and elderly survivors more frequently required skilled nursing or rehabilitative care after hospitalization.

In the present study, there was no significant difference in APACHE II score between sepsis group and non sepsis group. In total SIRS patients APACHE II score was significantly higher in non survivors more than survivors (p<0.001). Also, in both sepsis and non sepsis groups APACHE II score was significantly higher in non survivors more than survivors. APACHE II score was positively correlated with mortality in total SIRS patients, sepsis group, non sepsis group. APACHE II score was positively correlated with hospital stay in total SIRS patients and sepsis group while it was not correlated with hospital stay in non sepsis group. The APACHE II prognostic scoring system is a powerful predictor of hospital mortality in the ICU patient population [8]. Ma, et al. reported that, APACHE II score used as marker of severity and predictor of outcome [9].
Effect of Serum Cytokine Levels on outcome: TNF-α:

In the present study, TNF-α serum levels were significantly increased in the total SIRS patients versus healthy control (p<0.001). Similar to the present study Tadahiko, et al. [10] found that TNF-α was significantly elevated in trauma patients. It was significantly higher in sepsis group versus non sepsis group (p<0.014). In addition Majetschak and his associates [11] found that patients who developed severe posttraumatic sepsis presented with an increased TNF-α cytokine production capacity immediately after trauma when compared with patients without post traumatic complication. On the other hand Mokart and his coworkers [12] found that TNF-α serum levels were not correlated with surgical trauma or the occurrence of septic complication.

In the current study, TNF-α serum levels were found positively correlated with mortality in total SIRS patients. Moreover, serum TNF-α levels were significantly higher in the non survivors in total SIRS patients (p<0.001) and in both sepsis group and non sepsis group (p<0.001 in sepsis, and p<0.001 in non sepsis). In harmony with the present work Tang, et al. [13] reported higher serum TNF-α levels in patients with septic shock who did not survive compared with those who survived (p<0.05). TNF-α serum cytokine levels was found to play a major role in the pathogenesis of sepsis and its complication after burn trauma. In the harmony with the previous study, Gando and his colleagues [14] studied SIRS patients and found that TNF-α serum level was significant elevated in non survivors more than survivors. Furthermore, Bown, et al. [15] found that high TNF-α serum levels were associated with increased mortality.

In the present study, TNF-α cytokine was positively correlated with IL-1β cytokine in total SIRS patients, sepsis group, and non sepsis group. Furthermore, TNF-α cytokine is positively correlated with IL-1ra cytokine in total SIRS patients, sepsis group and in non sepsis group. Similarly, Cavaillon and his associate [16] studied cytokine cascade in sepsis and reported that most pro-and-anti-inflammatory cytokines were positively correlated to each other.

From the previous data we can conclude that, TNF-α is the key of endogenous mediator, It acts on variety of cells and stimulate the production of other cytokines involved in sepsis.

IL-1β:

In our study IL-1β serum levels were significantly higher in the total SIRS patients than control (p value was <0.001). In the current study IL-1β serum levels were found positively correlated with mortality and APACHE II score in total SIRS patients and both sepsis and non sepsis groups. Serum IL-1β levels were positively correlated with TNF-α and IL-1ra cytokines in total SIRS patients and sepsis group while it was positively correlated to TNF-α cytokine only in non sepsis group. Serum IL-1β levels were significantly higher in non survivor patients in total SIRS patients (p < 0.001) and in both groups (p<0.001 in sepsis and, p<0.001 in non sepsis). In accordance with, Tadahiko and his colleagues (2005) [17] reported that IL-1β was significantly elevated in trauma patients.

IL-1ra:

IL-1ra cytokine serum levels increased significantly from in total SIRS patients compared to control (p value <0.001 ). When IL-1ra was compared between SIRS with sepsis and SIRS without sepsis, there was no significant difference (p value 0.506). IL-1ra cytokine serum levels were significantly higher in non survivors versus survivors in total SIRS patients and in sepsis patients (p value <0.001). Also, IL-1ra cytokine serum levels were significantly higher in non survivors versus survivors in sepsis patients (p value 0.005). In the harmony with the present study Slotwinski, et al. [18] found that IL-1ra was significantly increased after surgical injury. They also found, IL-1ra serum concentration significantly higher after surgical injury with complication (like, infection) compared with patients after surgical injury without complication.

Pro/anti inflammatory cytokine ratio:

Many authors found that increased proinflammatory levels (like TNF-α) was associated with poor outcome [19]. Other authors reported that increased anti-inflammatory levels (like IL-1ra) were associated with poor outcome [20]. If any imbalance occurs by marked increase in the pro-inflammatory response or marked increase in the anti-inflammatory responses, it leads to poor outcome [21].

In the present study, the ratio between pro and anti inflammatory cytokines was utilized to study the balance between the pro and anti inflammatory cytokines effect. The ratio was more in the total SIRS patients than the control. This means that, although, both pro and anti inflammatory response
were augmented in total SIRS patients, the balance was in favor of more increase the pro-inflammatory response over anti-inflammatory response. Moreover, patients with poor prognosis (non survivors) had even more higher ratio than those with better prognosis (survivors), poor outcome was associated with more increase in the pro-inflammatory response than in the anti-inflammatory response. On dissecting the total SIRS patients into a sepsis and non sepsis group, it was found that the ratio of pro/anti inflammatory cytokines was significantly higher in poor prognosis patients only in the non septic group, while in the septic group the difference was not statistical significant. From this it might be inferred that a balance with more augmentation of anti-inflammatory cytokines may be protective in non septic patients, while it may have no effect on mortality in septic patients.

Ashare, et al. [21] reported that, in sepsis group the balance of tissue pro-to anti inflammatory cytokines directly correlated with severity of infection and mortality. In their study, mice were treated with IL-1ra, and this resulted in decreased pro-inflammatory cytokines, earlier bacterial load, and increased mortality. In the same study also, the authors reported that the initial tissue pro-inflammatory responses to sepsis was followed later by an anti-inflammatory response. The timing and magnitude of the anti-inflammatory response predicted severity of infection and mortality.

Relation between cytokines polymorphisms and cytokines serum levels:

In the present study, there were significant higher TNF-α serum levels in subjects with TNF-α allele 2 versus those with TNF-α allele 1 in total SIRS patients, sepsis group and non sepsis group. Similar to the present study Holmes, et al. [22] found that, TNF-α allele 2 is associated with increased secretion of TNF-α cytokine in vitro and elevated TNF-α cytokine serum levels in vivo.

In the current study, there was no statistical significant difference between IL-1β serum cytokine levels between IL-1β allele 1 patients and IL-1β allele 2 patients in total SIRS patients, sepsis group and non sepsis group.

In the present study, IL-1ra cytokine was higher in IL-1ra allele A2 patients more than IL-1ra allele A1 patients without statistically significant difference in SIRS with sepsis group. In the literature, there was a controversy regarding the relation between IL-1ra gene polymorphism and the cytokine levels. Holmes, et al. [22] found that, the IL-1ra allele A2 is associated with increased IL-1ra cytokine production.

Prognostic value of cytokine gene polymorphisms:

Gene isolation provides the best hope for understanding human diseases at its most fundamental level. Knowledge about genetic control of cellular function will underpin future strategies to prevent or treat disease phenotypes.

Prognostic value of TNF-α gene polymorphisms:

In the present study, there was no statistical difference in frequency of TNF-α alleles between healthy control, sepsis group, and non sepsis group. In the harmony with the previous studies Majestschak [23] found no difference in TNF-α genotypes between SIRS without sepsis (trauma) versus SIRS with sepsis.

In the present study, there was no significant difference in APACHE II score between different genotype of TNF-α gene in total patients, sepsis group, and non sepsis group. TNF-α gene polymorphism was significantly associated with the outcome in total SIRS patients, TNF-α allele 2 was significantly associated with mortality, and mortality was more frequent (50%) in the patient with allele 2 while in the patients with allele 1 it was 20%. In addition, in sepsis group TNF-α allele 2 was significantly associated with non survivors (p=0.045) while in the non sepsis group non survivors were insignificantly more frequent in patients contain TNF-α allele 2 (40%) than patients contain TNF-α allele 1 (15%).

Similar to the present study, Majestschak [23] found that, TNF-α allele 2 polymorphism was associated with mortality in sepsis or septic shock of various origins.

From present study data and the related studies, it is believed that TNF-α gene polymorphism may play an important role in future to identify patients at risk and potentially to design a specific and individualised immune-therapy.

Prognostic value of IL-1β gene polymorphisms:

In the present study, there was no statistical difference in frequency of distribution of IL-1β alleles between healthy control, sepsis group, and non sepsis group. In accordance with the present study Penglin, et al. [24] found that, IL-1β polymorphism did not differ between septic patients and normal control.

In the present study, it was found that there was no association between mortality and any types of IL-1β alleles and no difference in APACHE II...
score between different alleles in total SIRS patients, sepsis group and non sepsis group. In the harmony with the present study Balding, et al. [25] reported no association between meningococcal sepsis outcome and IL-1/β gene polymorphism.

**Prognostic value of IL-1 ra gene polymorphism:**

In the present study there was no statistical difference in the frequency of IL-1ra alleles between healthy control, sepsis group, and non sepsis group. In addition, IL-1ra allele A2 was significantly more frequent in non survivors only in sepsis group while in total SIRS patients and non sepsis group there was no statistically significant difference in the distribution of alleles of IL-1ra gene between the survivor and non survivor subgroups of patients.

APACHE II score was significantly higher in IL-1ra allele A2 in sepsis group While there was no difference in APACHE II score in total SIRS patients, and non sepsis group. Similar to the present study, Penglin, et al. [24] discovered that subject with IL-1ra allele A2 suffered much more from sever sepsis. It was associated with higher mortality rate in septic patients. IL-1ra allele may thus be considered as a high risk genetic marker for sepsis. Also, Yang, et al. [26] found that IL-1ra allele A2 was associated with poor outcome of patients in sepsis, mortality in septic patients with IL-1ra allele A2 was markedly high compared to these with IL-1ra allele A1.

Chen, et al. [27] and Ma, et al. (2002) [9] reported that IL-1ra polymorphism allele A2 was associated with susceptibility to sepsis and authors believed that IL-1ra allele A2 might be important high risk genetic marker for sepsis. Therefore, it has been suggested as a genetic risk factor for sepsis. It was not clear why IL-1ra allele A2 was associated with higher mortality in the sepsis group of patients without having effect on the level of serum IL-1ra cytokine itself. It may be due to hypothesizes that a particular risky allele might have its injurious effect through augmenting the local production of cytokine in certain tissue at the site of reaction without having an impact on the serum level.

In the present work, there were three patients out of 30 patients in sepsis group contained both TNF-α allele 2 and IL-1ra allele A2, these three patients died. One patient in non sepsis group contained both TNF-α allele 2 and IL-1ra allele A2, this one survived. This may suggest potentially interaction of both alleles in sepsis group, correlating with the patients poor outcome. Other study suggested that there was a potentially important interaction of IL-1ra and other gene polymorphism (TNF-β2 allele) as coincidence of TNF-β2 allele and IL-1ra A2 genotypes identified a group with a 100% mortality rate from sepsis [28].

**Conclusion:**

- A balance towards the anti-inflammatory response might be potentially protective in non septic patients but not in septic condition.
- Higher serum cytokine levels is related to mortality.
- TNF-α allele 2 polymorphism is associated with higher levels of TNF-α cytokine and poor outcome.
- Both TNF-α allele 2 and IL-1ra were associated with poor outcome in septic but not in non septic patients. Both TNF-α allele 2 and IL-1ra may be viewed as predictor of poor outcome in sepsis.

**References**


