Increased Resistin Levels in Intra-abdominal Sepsis
Correlation with proinflammatory cytokines and Acute Physiology and Chronic Health Evaluation (APACHE) II scores

Abstract: Objectives: Resistin, a hormone secreted from adipocytes and considered to be a likely cause of insulin resistance, has recently been accepted as a proinflammatory cytokine. This study aimed to determine the correlation between resistin levels in patients with intra-abdominal sepsis and mortality. Methods: Of 45 patients with intra-abdominal sepsis, a total of 35 adult patients were included in the study. This study was undertaken from December 2011 to December 2012 and included patients who had no history of diabetes mellitus and who were admitted to the general surgery intensive care units of Gazi University and Bülent Ecevit University School of Medicine, Turkey. Evaluations were performed on 12 patients with sepsis, 10 patients with severe sepsis, 13 patients with septic shock and 15 healthy controls. The patients’ plasma resistin, interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), procalcitonin, lactate and glucose levels and Acute Physiology and Chronic Health Evaluation (APACHE) II scores were studied daily for the first five days after admission. A correlation analysis of serum resistin levels with cytokine levels and APACHE II scores was performed. Results: Serum resistin levels in patients with sepsis were significantly higher than in the healthy controls (P < 0.001). A significant correlation was found between serum resistin levels and APACHE II scores, serum IL-6, IL-1β, TNF-α, procalcitonin, lactate and glucose levels. Furthermore, a significant correlation was found between serum resistin levels and all-cause mortality (P = 0.02). Conclusion: The levels of resistin were significantly positively correlated with the severity of disease and were a possible mediator of a prolonged inflammatory state in patients with intra-abdominal sepsis.

Keywords: Resistin; Systemic Inflammatory Response Syndrome; Sepsis; Shock; Cytokines; APACHE II; Intra-Abdominal Infections.

Advances in Knowledge
- This study provides information regarding the correlation of sepsis with blood resistin levels, using strict inclusion criteria.

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Intra-abdominal infections constitute a considerable portion of general surgery complications and their related mortality and morbidity are an important issue in intensive care (IC). Patients with intra-abdominal infections have a mortality rate of approximately 30%; however, this rate exceeds 50% in cases of sepsis. Although the prognosis of the patient depends on the underlying disease and the patient’s general condition, inflammatory responses can differ and be difficult to measure. With the onset of infection, inflammatory stimuli evoke an inflammatory response that leads to vascular permeability and chemotaxis by several activated cytokines to the inflamed area. The peritoneal surface contains many macrophages and lymphocytes. With the onset of inflammation, the inflammatory response can not only affect the peritoneal cavity, but also lead to sepsis and multi-organ dysfunction. Although the aim of treatment is to resuscitate the patient and eliminate the source of infection, proinflammatory cytokines become the new therapeutic targets. Even after the source of infection has been controlled, ongoing inflammation, or an increased inflammatory response in an individual, can inhibit the effectiveness of the treatment. Therefore, identifying the response of the body to the sepsis would be helpful in determining the prognosis of the disease.

Identifying novel biomarkers for linking the state of inflammation and sepsis is crucial for the further risk evaluation and stratification of patients in IC units (ICUs). Several approaches to risk stratification during ICU admission have been applied, such as measuring inflammatory cytokines and implementing systems that rank illness severity. Procalcitonin, which is a popular marker for infection and risk stratification among IC patients, is related to the severity of disease. Individualised scoring systems are also gaining popularity, as an individual’s inflammatory response depends on several factors, such as their level of obesity and genetic background. Adipose tissue functions not only as a fat deposit, but also as an endocrine organ, manufacturing adiponectin, resistin and proinflammatory cytokines. Although resistin has been produced in adipocytes in animal models, the primary source of it in humans is in the macrophages. Resistin also has proinflammatory properties because of this, it has been used as an indicator of neonatal sepsis. In addition, longer term studies have shown correlations between resistin and inflammatory cytokines during the treatment of sepsis. The ability to predict the severity of an illness at an early stage is important. New markers of illness severity are required to ensure more accurate predictions, of which resistin may be one. As the mechanisms of intra-abdominal sepsis are related to resistin levels, the aim of this study was to determine if patients’ resistin levels were correlated with intra-abdominal sepsis and related mortality. Intra-abdominal infections were targeted to achieve this as they involve large peritoneal surfaces and a variety of infections. Resistin levels were also compared to common mortality prediction models used in ICUs, such as Acute Physiology and Chronic Health Evaluation (APACHE) II scores, blood procalcitonin levels and glucose levels.

Methods

This prospective clinical study was undertaken from December 2011 to December 2012 and included 45 consecutive adult patients with intra-abdominal sepsis in the general surgery ICUs of Gazi University and Bülent Ecevit University School of Medicine in Turkey. A control group was composed of 15 adult patients who had undergone abdominal surgery without any findings indicating a systemic inflammatory response. All patients were over 18 years old, with secondary peritonitis following intra-abdominal conditions, such as pancreatitis or a colon perforation.

The patients were divided into a sepsis group and a septic shock group. Sepsis was defined according to the criteria proposed by the American College of Chest Physicians/Society of Critical Care Medicine. At least three of the following criteria with an identifiable site of infection were accepted to signify sepsis: temperature of >38 or <36 °C; heart rate of >90 beats/minute; respiratory rate of >20 breaths/minute, and a white blood cell count of >12,000 or <4,000/mm³. Patients were considered to have septic shock if they had sepsis and an episode of cardiovascular collapse requiring vasopressor support. Patients who had developed symptoms of sepsis less than 72 hours...
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Table 1: Comparison of the mean resistin, TNF-α, IL-1β, IL-6, procalcitonin, blood glucose and blood insulin levels and APACHE II and SOFA scores among the control, sepsis and septic shock groups

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong> n = 15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistin in μg/mL</td>
<td>3.6 ± 1.2</td>
<td>18.4 ± 1.2</td>
</tr>
<tr>
<td>TNF-α in pg/mL</td>
<td>4.4 ± 1.0</td>
<td>31.0 ± 6.0</td>
</tr>
<tr>
<td>IL-1β in pg/mL</td>
<td>1.7 ± 0.2</td>
<td>7.7 ± 2.1</td>
</tr>
<tr>
<td>IL-6 in pg/mL</td>
<td>92.0 ± 12.0</td>
<td>164.0 ± 28.0</td>
</tr>
<tr>
<td>Procalcitonin in ng/mL</td>
<td>0.42 ± 0.1</td>
<td>1.50 ± 0.1</td>
</tr>
<tr>
<td>APACHE II scores</td>
<td>-</td>
<td>11.1 ± 2.6</td>
</tr>
<tr>
<td>Blood glucose in mg/dL</td>
<td>81.8 ± 9.2</td>
<td>126.2 ± 23.5</td>
</tr>
<tr>
<td>Blood insulin in IU/mL</td>
<td>8.2 ± 2.1</td>
<td>10.2 ± 2.8</td>
</tr>
<tr>
<td>SOFA scores</td>
<td>-</td>
<td>4.6 ± 2.1</td>
</tr>
</tbody>
</table>

**Sepsis** n = 12

| Resistin in μg/mL | 42.1 ± 7.2 | 41.7 ± 7.3 | 43.1 ± 7.3 | 0.82*** |
| TNF-α in pg/mL | 57.8 ± 6.8 | 57.9 ± 6.8 | 58.1 ± 6.8 | 0.99*** |
| IL-1β in pg/mL | 19.6 ± 5.2 | 20.2 ± 5.2 | 20.3 ± 5.2 | 0.78*** |
| IL-6 in pg/mL | 389.2 ± 33.0 | 392.4 ± 33.4 | 394.2 ± 33.6 | 0.74*** |
| Procalcitonin in ng/mL | 3.5 ± 1.2 | 3.6 ± 1.2 | 3.6 ± 1.2 | 0.98*** |
| APACHE II scores | 14.8 ± 2.9 | 14.9 ± 2.9 | 15.0 ± 2.9 | 0.96*** |
| Blood glucose in mg/dL | 151.1 ± 29.0 | 151.2 ± 29.1 | 152.1 ± 29.2 | 0.97*** |
| Blood insulin in IU/mL | 5.9 ± 3.3 | 6.0 ± 3.3 | 6.1 ± 3.4 | 0.95*** |
| SOFA scores | 8.6 ± 2.4 | 8.7 ± 2.4 | 8.8 ± 2.4 | 0.95*** |

**Septic shock** n = 23

| Resistin in μg/mL | 43.1 ± 7.3 | 42.4 ± 7.4 | 42.7 ± 7.4 | 0.79*** |
| TNF-α in pg/mL | 58.1 ± 6.8 | 58.3 ± 6.8 | 58.6 ± 6.8 | 0.82*** |
| IL-1β in pg/mL | 20.3 ± 5.2 | 20.4 ± 5.2 | 20.5 ± 5.3 | 0.92*** |
| IL-6 in pg/mL | 394.2 ± 33.6 | 396.4 ± 33.8 | 398.2 ± 33.8 | 0.91*** |
| Procalcitonin in ng/mL | 3.6 ± 1.2 | 3.6 ± 1.2 | 3.6 ± 1.2 | 1.00*** |
| APACHE II scores | 15.0 ± 2.9 | 15.1 ± 2.9 | 15.2 ± 2.9 | 0.99*** |
| Blood glucose in mg/dL | 152.1 ± 29.2 | 153.4 ± 29.3 | 155.1 ± 29.4 | 0.98*** |
| Blood insulin in IU/mL | 6.1 ± 3.4 | 6.2 ± 3.4 | 6.3 ± 3.5 | 0.95*** |
| SOFA scores | 8.8 ± 2.4 | 8.9 ± 2.4 | 9.0 ± 2.4 | 0.96*** |

*Comparison between the control and sepsis groups using analysis of variance (ANOVA)/Scheffé’s post hoc test; **Comparison between the sepsis and septic shock groups using ANOVA; ***Comparison between the sepsis and septic shock groups using a Chi-squared test.

before their admission to the ICU and those who developed symptoms of sepsis during treatment were included in the study.

Patients with diabetes, coronary artery disease, suspected pregnancy, acute or chronic renal failure, severe chronic liver disease or a score of less than 8 on the Glasgow Coma Scale were not included in the study. Patients who had had cardiac resuscitation or a major cardiac operation in the previous five days were also excluded. In addition, patients were excluded if it was found that the initial cause of sepsis was not intra-abdominal. In total, 10 patients were excluded from the study, resulting in a sample of 35 patients.

Intra-abdominal infections were determined by microbiological investigations. Sequential Organ Failure Assessment (SOFA) and APACHE II scores for all patients were calculated daily for five consecutive days. The day following the diagnosis of sepsis, daily fasting venous blood samples were obtained each morning at 6 am from all patients. The blood samples were centrifuged for 10 minutes at 2,000 × g and stored at −80 °C. The patients were then kept under observation for 28 days.

Blood resistin levels were determined by the sandwich enzyme-linked immunosorbent assay (ELISA) method using the RD191016100 Resistin Human ELISA immunoassay (BioVendor Inc., Brno, Czech Republic) according to the manufacturer’s instructions (detection range: 0.1–50 ng/mL). Serum levels of interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumour necrosis factor alpha (TNF-α) were determined using a Fluorokine® Multi-Analyte Profiling Kit (R&D Systems, Inc., Minneapolis, Minnesota, USA) as described by the manufacturer and using a Luminex® platform (Luminex Corp., Austin, Texas, USA). Procalcitonin was assessed with a LIAISON® chemiluminesence analyser (DiaSorin, Salugia, Italy) and plasma insulin levels were determined using a radioimmunoassay technique.

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) Version 12.0 (IBM Corp., Chicago, Illinois, USA). Parametric results were compared with analyses of variance using Scheffé’s post hoc tests. Results from the control group were not included in the correlation tests. Non-parametric data (including the mean SOFA and APACHE II scores, age and body mass index [BMI] of the patients) were compared with Chi-squared tests. A comparison of the mean blood resistin levels in male and female patients was also performed using Chi-squared tests. Correlations between the serum resistin levels and the TNF-α, IL-6, procalcitonin, IL-1β, blood glucose and insulin levels, as well as the APACHE II scores and patient ages, were analysed using bivariate Pearson’s correlation coefficient tests, where P <0.05 was considered statistically significant.

This study was approved by the Gazi University Clinical Research Ethic Committee and written informed consent was obtained from each patient and, where relevant, their spouse.

Results

A total of 35 patients were included in the study, of which 12 had sepsis and 23 had septic shock. Among the subjects, 62% were male. The aetiology of intra-abdominal sepsis among the patients included colon perforation (n = 8), infected pancreatic abscess (n = 6), gastric and duodenal perforations (n = 6), postoperative anastomotic leakage (n = 6) and biliary sepsis (n = 3), among others (n = 6). Within the 28-day observation period, the mortality rate for both the sepsis and septic shock groups was 22.8%, with no fatalities occurring during the first five days of the study. The mortality
rate was 16.6% in the sepsis group and 26.0% in the septic shock group; this was significantly different between the two groups ($P = 0.02$). The mean ages in the control, sepsis, and septic shock groups were 58.3 ± 5.6, 60.3 ± 12.3 and 64.7 ± 9.7 years, respectively. The mean BMIs of the patients in the control, sepsis and septic shock groups were 26.6 ± 3.1, 24.5 ± 2.9 and 23.2 ± 4.5 kg/m$^2$, respectively. No significant correlations were found between serum resistin levels and BMI ($P = 0.87$), nor did the resistin levels in the blood correlate with the patients’ ages in the sepsis or septic shock groups. There was also no significant difference in the mean resistin levels between male and female patients ($P = 0.98$).

The mean resistin, TNF-α, IL-6, procalcitonin, IL-1β and glucose levels were significantly elevated among the sepsis and septic shock groups ($P < 0.001$). In addition, the mean resistin, TNF-α, IL-6, procalcitonin, IL-1β and glucose levels were significantly increased in the septic shock group compared to the sepsis group ($P < 0.001$), whereas the mean blood insulin levels were significantly higher in the sepsis group than in the septic shock and control groups. A clinical comparison of the sepsis and septic shock groups using the patients’ SOFA and APACHE II scores ($P = 0.02$ and 0.03, respectively) found that these scores were significantly higher in the septic shock group than in the sepsis group. There were no significant correlations between the mean BMI and resistin levels ($P = 0.92$), or between the patients in the sepsis and septic shock groups with regards to their mean BMI ($P = 0.987$) or age ($P = 0.867$).

There were significant positive correlations between the levels of resistin and TNF-α, IL-1β, IL-6 and procalcitonin [Figures 1A–D], with resistin levels increasing as proinflammatory cytokine levels increased. The correlation between resistin levels and fasting glucose levels was also significantly positive ($R = 0.44; P < 0.001$) [Figure 2]. In contrast, blood insulin levels decreased as the disease worsened and a significant negative correlation was seen between resistin and serum insulin levels ($R = 0.271; P = 0.011$) [Figure 3]. There was a significant positive correlation between APACHE II ($R = 0.51; P < 0.001$) and SOFA ($R = 0.45; P < 0.001$) scores with the blood resistin levels [Figure 4]. The mean resistin levels in the surviving and non-surviving patients were not significantly different, at 33.4 ± 5.8 and 38.1 ± 4.5 µg/mL, respectively ($P = 0.082$).
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Discussion

The findings of this study support the role of resistin as an acute-phase protein with a significant association to disease severity, as demonstrated by the the levels of proinflammatory cytokines and the SOFA and APACHE II scores among the studied population.

As expected, no inflammatory response was seen in the control group. The blood resistin levels were significantly increased in the septic shock group in comparison to the control and sepsis groups. However, the resistin levels in the control group, which had a mean BMI of 26.6 kg/m², were lower than those seen in a similar study from Sweden by Sundén-Cullberg et al. This variation might be related to ethnic differences.

In their study, Sundén-Cullberg et al. found no correlation between BMI and blood resistin levels. As with the current study, Lehrke et al. observed that the blood resistin levels in non-survivors were higher than those of survivors, although this was not significant.

Both Sundén-Culberg et al. and Lehrke et al. confirmed that adiposity appears to play no significant role in the increased levels of resistin induced by acute stress. In this regard, the results of the current study were similar to others in the literature. As mentioned earlier, the primary origin of resistin in humans is in the macrophages, as opposed to the adipose tissue. Therefore, as expected, because human resistin is part of the inflammatory response to infection, resistin levels were not elevated among obese patients.

Lehrke et al. also demonstrated that the inflammatory cascade is sufficient and necessary for the production of resistin synthesis in the macrophages. The peritoneum is an important source of inflammatory cells, particularly macrophages. Gene expression and protein expression of resistin have been shown to increase with inflammation. Lipopolysaccharides also activate several cytokines and inflammatory pathways. After an early and short increase in the primary cytokines, secondary to lipopolysaccharide induction, the production and enhancement of resistin in macrophages is seen.

It has been hypothesised that hyper-resistinaemia may be an important feature in maintaining persistent inflammation, as interleukins and lipopolysaccharides have been shown to also upregulate resistin levels. In the present study, the procalcitonin levels that increased with infection were significantly correlated with resistin levels. This finding supports the induction effect of lipopolysaccharides on resistin secretion.

In addition to the laboratory findings, the patients’ clinical features, as measured by their APACHE II scores, were also positively correlated with their resistin levels. Therefore, increased resistin levels were associated with organ dysfunction and increased APACHE II scores. The significant correlation between the APACHE II scores and resistin levels demonstrates...
that blood resistin levels can be used as an index in sepsis. One of the most important prognostic scoring systems in the ICU is the SOFA, which is usually an ongoing evaluation that uses continuous measurements.\textsuperscript{7} In contrast, the APACHE II assessment is generally performed during the initial admission of the patient. Both scoring systems were used throughout this study.

Koch\textit{et al.} found no association between resistin levels at admission and the survival of patients in IC, or the disease severity as measured by APACHE II scores.\textsuperscript{18} They stressed that high resistin levels were a predictor for an unfavourable prognosis only in non-sepsis cases and that these levels were independent of survival.\textsuperscript{18} However, the incidence of abdominal sepsis was only 18\% in Koch\textit{et al.}’s study, as opposed to the current study where all of the patients had intra-abdominal sepsis. In addition, the patients’ resistin levels in the current study were measured during the duration of their ICU stay, rather than solely upon admission to the ICU. Although resistin has proven to be a reliable marker of non-septic inflammatory conditions, it has also been shown to act as a marker of septic inflammation.\textsuperscript{19}

Blood glucose control is an essential feature of sepsis treatment.\textsuperscript{20} In recent years, strict diet control, even for those without hypo- or hyperglycaemia, has become the accepted treatment for sepsis patients.\textsuperscript{21} Studies have shown that TNF-α induces insulin resistance via the ceramide accumulation in hepatic tissue.\textsuperscript{20,21} Banerjee\textit{et al.} found that peak cytokine and insulin resistance levels among mice began approximately six hours after the administration of lipopolysaccharides, which is close to the approximate time of resistin induction.\textsuperscript{22} As in the current study, Banerjee\textit{et al.} also demonstrated that increased levels of resistin were correlated with increased blood glucose levels and decreased levels of insulin.\textsuperscript{23} On the other hand, Koch\textit{et al.} observed that blood glucose, insulin and resistin levels were not associated in patients whose blood samples were collected promptly on admission.\textsuperscript{18} Although resistin contributes to acute inflammatory responses, it may also have contributing effects within the inflammatory cascade. As such, with the induction of inflammation, macrophages would infiltrate the inflamed area and increase resistin production.

The number of participants in the current study was limited due to the strict inclusion and exclusion criteria. Although this appears to be a limitation, the sample size was similar to that of previous studies on resistin.\textsuperscript{18,15} The current study differed from others in that resistin levels were determined on five consecutive days in an attempt to gauge early phase changes. This extended period of observation was significant as patient risk stratification is generally only performed during admission or soon thereafter. The measurements were taken on five consecutive days in order to minimise the risk of missing the inflammatory response, thereby achieving more reliable results. The consecutive measurements of resistin level also enabled a strict evaluation of the patients’ clinical deterioration and may have increased the sensitivity of the analysis. Additionally, this study included only patients with intra-abdominal sepsis. This was because it is the most frequently seen form of sepsis in general surgery clinics. Although previous studies have measured resistin levels and correlated them with sepsis, those studies were performed during the treatment period as a time series.\textsuperscript{7,8} This differs to the current study, which measured resistin levels during the early phase of sepsis.

**Conclusion**

In summary, blood resistin levels were significantly positively correlated with the severity of intra-abdominal sepsis among this sample of patients. Resistin may also have affected blood glucose metabolism among patients with sepsis. These results indicate that resistin plays an important role in sepsis. However, resistin levels were not predictive of patient survival and future studies are required to determine the clinical application of resistin levels in guiding specific therapies for intra-abdominal sepsis.

**ACKNOWLEDGEMENTS**

A preliminary version of this study with a different patient population was presented at the 7\textsuperscript{th} International Conference on Complexities in Acute Illness in Cologne, Germany, in 2008. The abstract of this preliminary study was previously published in the Journal of Critical Care in June 2008 (Vol. 23, Iss. 2, P. 268).

**References**

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