Resistin as A Marker of Severe Sepsis

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Abstract

Objective: To measure the level of serum resistin in patients with severe sepsis and septic shock and assess its diagnostic and prognostic implications.

Design: A prospective, randomized, controlled single center study.

Patients: The study included 14 (70%) patients with severe sepsis and 6 (30%) with septic shock. Sixty percent of the patients were males. The overall mortality rate was 70% ranging from 20% in the severe sepsis group to 50% among those with septic shock. Sera taken from 10 healthy laboratory and hospital employees, were used as a baseline control for circulating resistin.

Measurements: Resistin was analyzed by ELISA with a range of detection from 0.1ng/ml to 50ng/ml.

Results: This study showed that resistin level was significantly increased in patients with severe sepsis or septic shock with four to eight fold higher median levels compared with 10 healthy controls ($p<0.001$). Also serum levels of resistin were significant elevated in patients with septic shock compared with patients with severe sepsis at 0hrs ($p<0.01$) and bordering on significantly elevated at 24 hrs ($p: 0.06$). This study showed that serum resistin levels correlated to the patient's initial APACHE II. Also resistin levels were higher in non survivors compared with survivors in patients with severe sepsis and septic shock but this was statistically insignificant.

Conclusion: This study showed the role of resistin as an acute phase protein in sepsis and septic shock with a clear association with the severity of the disease as measured by APACHE II scores, this protein can be used as a diagnostic and prognostic tool in infectious and perhaps inflammatory diseases leading to severe sepsis or even septic shock.

Key Words: Sepsis – Severe shock – Resistin – ICU.

Introduction

OBESITY is a growing health care problem worldwide. The prevalence of obesity has increased so rapidly that the obese population in developed countries has more than doubled over the last decade [1].

Obesity and the associated metabolic pathologies are the most common and detrimental metabolic diseases, affecting over 50% of the adult population. These conditions are associated with a chronic inflammatory response characterized by abnormal cytokine production, increased acute-phase reactants, and activation of inflammatory signaling pathways [2].

A new hormone has been identified that links obesity to type 2 diabetes. It has been called resistin (for “resistance to insulin”). Resistin is expressed in white adipose tissue and is induced during adipogenesis. It is one amongst a family of three proteins, known as resistin-like molecules (RELMs), which have a conserved pattern of 1 1 cysteine residues at the C-terminal end of the structure [3].

Sepsis is one of the most frequent complications in the surgical patient and one of the leading causes of mortality in intensive care units [4]. The mortality of severe sepsis (infection-induced organ dysfunction or hypoperfusion abnormalities) and septic shock (hypotension not reversed with fluid resuscitation and associated with organ dysfunction or hypoperfusion abnormalities) in most centers remains unacceptably high [5].

Serum level of resistin is increased dramatically by endotoxemia in humans, and correlate with a marker of inflammation in patients with type 2 diabetes. Thus, systemic inflammation leads to increased resistin production and circulating levels in humans. The increased level of resistin in humans with obesity is likely an indirect result of elevated levels of inflammatory cytokines characteristic of states of increased adiposity. Hence, obesity and acute inflammation are both hyper-reistinemic states associated with insulin resistance [6].
Resistin has recently been recognized to act as a proinflammatory cytokine in humans. Patients with severe sepsis or septic shock had significantly elevated systemic levels of resistin, which correlated with severity of disease. Serum resistin levels were significantly elevated in patients with Gram-positive, as compared with Gram-negative, septic shock \((p=0.004)\). Analyses of tissue biopsies revealed that resistin was highly expressed at the local site of infection.

The systemic and local hyper-resistinemia noted is likely to contribute to the pathogenesis of acute invasive bacterial infections [7].

The theory that white adipose tissue (WAT) could be an active contributor to whole-body homeostasis rather than just a fat depot became tangible with the discovery of leptin in 1994 [8]. WAT has since been found to produce more than 50 cytokines and other molecules. These adipokines engage, through endocrine, paracrine, autocrine or juxtacrine mechanisms of action, in a wide variety of physiological or pathological processes, including immunity and inflammation [9].

WAT is now regarded as a pro-inflammatory state, and several markers of inflammation have been found to be elevated in obese subjects. Adipokines include a variety of pro-inflammatory peptides including tumor necrosis factor [TNF], the secretion of which by adipocytes was observed even before the discovery of leptin [10].

These pro-inflammatory adipokines appear to contribute strikingly to the low-grade inflammatory state of obese subjects, setting up a cluster of metabolic aberrations including cardiovascular complications and autoimmune inflammatory diseases. It is noteworthy that adipokine production by WAT in obesity is strongly influenced by the presence of infiltrating macrophages. Macrophages are an additional source of soluble mediators and might contribute and perpetuate local and systemic inflammation [11].

Recent evidence derived from coculture experiments demonstrates the relationships between adipocytes and macrophages whereby secretory products from adipocytes amplify macrophage-inflammatory cytokine expression in vitro [12].

In view of the role of adipose tissue as an endocrine organ and its huge potential to secrete several pro- and anti-inflammatory mediators such as cytokines, hormones, and peptides, it is likely that under conditions when adipose tissue can contribute between 30 and 50% of total body weight, the increase in morbidity and mortality in septic obese patients could be attributed to this excess adipose tissue. Adipocytes express many of the known Toll-Like Receptors (TLRs). Adipocytes are highly responsive to endotoxin due to the high level expression of TLR-4 [13] and respond by inducing high levels of proinflammatory cytokines at concentrations of endotoxin that reflect a sensitivity to these bacterial cell wall components comparable to or higher than what can be observed in macrophages.

Resistin was originally described as an adipocyte-derived polypeptide that provided the link between obesity and insulin resistance. It was called “resistin” because of the observed insulin resistance in mice injected with resistin [14]. Resistin is expressed at very low levels, if at all, in human adipose cells, whereas high levels are expressed in mononuclear leukocytes, macrophages, spleen, and bone marrow cells.

Inflammation is the first innate immune response to infection or irritation resulting from leukocyte accumulation and their secretion of inflammatory, biogenic chemicals such as histamine and pro-inflammatory cytokines, some like (Kusminski, et al., 2007) [15], believes that resistin participates in this inflammatory response [15].

Recent evidence suggests a role for resistin in human inflammation and the main aim of this study was to investigate whether resistin play a role in severe sepsis and septic shock. In mice, the protein resistin is mainly secreted from adipocytes, but in humans an important source is monocytes, and a number of recent reports suggest that in humans, resistin is likely to be directly involved in inflammation [16].

**Aim of the work:**

To measure the level of serum resistin in patients with septic shock and/or severe sepsis, and assess its diagnostic and prognostic implications.

**Material and Methods**

**The study plan:**

Beginning on the day of inclusion, serum samples were collected from each patient for later analysis and routine biochemical tests at defined time points \((0, 1, 3, \text{ and } 5 \text{ days})\).

Clinical data were registered at all sampling points for each patient. Severity of disease was measured by Acute Physiology and Chronic Health Evaluation (APACHE) II at admittance.
Sera taken from 10 healthy laboratory and hospital employees, ages 22-60 yrs, were used as a baseline control for circulating resistin.

**Resistin analysis:**

Resistin was analyzed by ELISA with a range of detection from 0.1ng/mL to 50ng/mL. All samples were analyzed in duplicate. Samples exceeding the upper limit of detection were reanalyzed at a higher dilution.

**Results**

**Patient characteristics:** The study included 14 (70%) patients with severe sepsis and 6 (30%) with septic shock. Of the patients, 60% were males. The overall mortality rate was 70%, ranging from 20% in the severe sepsis group to 50% among those with septic shock.

Table (1) shows the baseline patient's characteristics and their laboratory findings, divided into two groups the first of 14 patients with severe sepsis and the second of 6 patients with septic shock, resistin level in the six septic patients was markedly elevated in comparison with the 14 patients with severe sepsis.

This study showed that resistin level was significantly increased in patients with severe sepsis or septic shock with four- to eight-fold higher median levels compared with 10 healthy controls ($p<0.001$), Fig. (1).

Table (1): Baseline patients characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Severe sepsis</th>
<th>Septic shock</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>10</td>
<td>2</td>
<td>12 (60%)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>4</td>
<td>4</td>
<td>8 (40%)</td>
</tr>
<tr>
<td>Age, mean ± SD</td>
<td>58.4±18.8</td>
<td>59.9±14.8</td>
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<tr>
<td>Hemoglobin, g/L, mean ± SD</td>
<td>10.6±17</td>
<td>10.5±17</td>
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<tr>
<td>WBC, 10^9/L, mean ± SD</td>
<td>13.2±7.8</td>
<td>13.8±10.9</td>
<td></td>
</tr>
<tr>
<td>Platelet count, 10^9/L, mean ± SD</td>
<td>190±130</td>
<td>142±113</td>
<td></td>
</tr>
<tr>
<td>Resistin, pg/mL, mean ± SD</td>
<td>44.000±36.000</td>
<td>78.000±66.000</td>
<td></td>
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<tr>
<td>APACHE II, mean ± SD</td>
<td>17±8.2</td>
<td>22.1±9.4</td>
<td></td>
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</tbody>
</table>

APACHE = Acute physiology and chronic health evaluation.

Serum levels of resistin were significantly elevated in patients with septic shock compared with patients with severe sepsis at 0hrs ($p<0.01$) but borderline elevated at 24 hrs ($p=0.06$) (Fig. 2).

Furthermore, statistical analysis of the data using Spearman's rank correlation coefficient showed that serum levels of resistin correlated to the patient's initial APACHE II scores at 24hrs, correlation ($p$) was 0.36, a test of the significance of this coefficient was made using Hotelling-Pabst test showed a $p$-value of (0.0005).

This study included 20 patients, 14 (70%) with severe sepsis and 6 (30%) with septic shock, the overall mortality rate was 70%, ranging from 20% in severe sepsis group, up to 50% among those with septic shock. Interestingly, non survivors had higher level of resistin than survivors throughout the study, but the difference did not reach statistical significance Figs. (3,4).
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Discussion

This study establishes the role of resistin as an acute phase protein in sepsis and septic shock with a clear association with severity of disease, as measured by APACHE II scores.

Also, significantly increased level of resistin were found in patients with septic shock compared with those with severe sepsis and in both groups compared with healthy controls.

Overall levels in these septic patients were considerably elevated, with individuals exhibiting concentrations >200ng/ml and even 300ng/ml compared with 14ng/ml, the highest level measured in a healthy control.

In a recent study done by Sundén-cullberg, et al. [17] showed that the level of resistin in patients with severe sepsis or septic shock was significantly increased and also levels were higher in non survivors of septic shock compared with survivors, but the difference did not reach statistical significance.

In the same study a clear association with the severity of disease, as measured by APACHE II and SOFA scores, as well as with established markers of sepsis including D-dimer and lactate and also with the cytokines IL-6, IL-8, IL-10, and TNF-α, was established [17], although the increased of resistin was delayed compared with other cytokines, but the authors noted that despite the increase of resistin concentrations was slower, its concentrations were then sustained at high levels throughout the studied period, and this persistently elevated levels were demonstrated in 10 of 15 patients sampled on day 14 of the study period, at which point the correlation between resistin levels and disease severity also was remarkably strong.

This persistently elevated expression and the indications of an increasing degree of correlation between resistin and clinical status with passage of time raise the intriguing possibility that hyper-resistinemia is important in maintaining persisting inflammation. Two recently published papers on resistin-induced cytokines both present results showing release of TNF-α and IL-6 from peripheral blood mononuclear [18] and TNF-α and IL-12 from macrophages [19].

Taken together, this available evidence was enough to conclude that resistin is indeed an important propagator of inflammation [11]. Previous study demonstrated that resistin up regulates vascular cell adhesion molecule-1 on endothelial cells, ICAM-1 is an important cell adhesion molecule, aiding the attachment of cells of the immune system to a site of infection, thus the relation between HMGB-1 and resistin and their contribution to a persistent pathologic proinflammatory state requires further study [20].

There is much work left to do in delineating the role of resistin in the inflammatory pathways of sepsis pathogenesis, particularly concerning its role in prolonging inflammation and its complicity in the “diabetes of injury” often found in septic patients. Furthermore, the strong correlation found with severity of disease scores implies that resistin, regardless of its biological functions in sepsis, may be used as a prognostic tool in the fields of infectious and perhaps also inflammatory diseases. Such a marker in critical care patients would be clinically very useful.
References


