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Association of some adipokines and oxidative stress biomarkers with septic patients

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Abstract

The incidence of sepsis has increased considerably since the late few decades. Sepsis became one of the major causes of death in developed countries. We became in desperate need of updated models for assessing severity of sepsis with no limitations. Therefore, the objective of this study was to determine the diagnostic role of some adipokines and oxidative stress biomarkers in assessing severity and prediction of early outcomes among septic patients. we conducted a cross-sectional analysis of individuals developing sepsis. Consecutive series of all eligible patients had a double venous blood sample drawn one on hospital admission and one after 48 hour of admission for assaying some adipokines such as lipocalin-2 (LCN 2) & resistin and some oxidative stress biomarkers such as malondialdehyde (MDA) & glutathione peroxidase activity (GPX). Admitted patients were followed up to assess early outcomes (length of hospital stay and mortality). The results identified 30 septic cases of them 4 (13%) have SIRS, 5 (17%) have sever sepsis and 21 (70%) with septic shock. We found that lipocalin-2 exhibited elevated levels after 48 hour of admission as compared with on admission measurements (102.9 vs. 85.7 pg/mL, p=<0.001).Resistin showed elevated levels on admission as compared with 48 hour of admission measurements. Malondialdehyde exhibited elevated levels after 48 hour of admission as compared with on admission measurements.Glutathione peroxidase showed significant decrease in its levels after 48 hour of admission as compared with on admission measurements.Also, we found that serum levels of lipocalin-2,resistin and malondialdehyde were significantly higher in non-survivors but glutathione peroxidase were significantly lower in non-survivors than survivors at 28 days -follow-up for mortality. In addition, our markers were positively correlated with APACHE II and SOFA scores respectively. It is concluded from this work that lipocalin-2, resistin, malondialdehyde and glutathione peroxidase are valuable for the risk stratification, early diagnosis and prognostication of sepsis in the ICU.

Keywords. sepsis; lipocalin-2; resistin; malondialdehyde; glutathione peroxidase

1 Introduction

In the developed world, sepsis is dramatically increasing by an annual rate of between 8-13 % over the last decade, and now claims more lives than bowel and breast cancer combined. Reasons are diverse, but include the aging population, increasing use of high-risk interventions in all age groups, and the development of drug-resistant and more virulent varieties of infections. In the developing world malnutrition, poverty, lack of access to vaccines and timely treatment all contribute to death (Peake, et al, 2014). In the developing world sepsis accounts for 60-80% of lost lives per year, affecting more than 6 million newborns and children annually and over 100,000 women contract sepsis in the course of pregnancy and childbirth (Hall, et al, 2011). Sepsis biomarker development represents an important and ongoing area of research within the global sepsis field. Many biomarkers have been proposed over the years. But there is little consensus on which is best and the exact role of individual markers remains uncertain (Marshall, et al, 2003). When sepsis is associated with shock, which is refractory to fluid resuscitation, the patient is considered to be in septic shock. This is associated with an increased production of both pro- and anti-inflammatory cytokines. Cytokines are low-molecular- weight polypeptides or glycoproteins that play an important role in regulating host response to infection, immune responses, inflammation, and trauma. Many of this cytokines are not only synthesized by blood cells, but also by adipose tissue. In contrast with classical views, adipose tissue not only provides a depot for fat storage but has been increasingly recognized as an important endocrine organ, manufacturing

adipokines, and bioactive molecules (Libby, et al, 2010). The inflammatory response to critical illness due to sepsis related syndromes involves the activation of leukocytes and other inflammatory cells that lead to a massive production of reactive oxygen species (ROS). ROS mediated oxidative stress has been implicated in apoptotic cell death and in turn, can be harmful to the patient when the endogenous antioxidant defense mechanisms are overwhelmed. It is now well documented that ROS is involved in the pathogenesis of multiple organ failure following sepsis, often leading to death **(**Preiser, et al, 2000). In this preliminary study, we evaluate the association between adipokines and oxidative stress biomarkers in septic patients.

2 Patients and Methods

Study design:

A cross-sectional analytical study design was used to determine the diagnostic accuracy of serum lipocalin-2, resistin, malondialdehyde and glutathione peroxidase assays for severity of sepsis and prediction of early outcomes among septic patients. The study was conducted at the Critical care Department - Cairo university Hospitals.

Study population:

We identified 30 individuals (20 males and 10 females).Their ages ranged between 18 to 85 years old. After clinical examination they are admitted to the intensive care department, Cairo university hospitals with Sepsis and its related syndromes. The blood samples are drawn once on admission before any vaso - active medications administration to those patients and one time after 48 hour of admission. A 28 day observation study for mortality will be carried out to predict biomarkers related mortality. We excluded patients who had abnormal renal function before admission, chronic heart disease, and Chronic liver disease. We defined sepsis cases as hospitalizations for serious infection with two or more systemic inflammatory response syndrome (SIRS) criteria. SIRS criteria included body temperature > 38°C (100.4°F) or < 36°C (96.8°F), heart rate > 90 beats per minute, respiratory rate > 20 breaths per minute or arterial carbon dioxide tension (PaCO2) < 32 mm Hg, and abnormal white blood cell count (>12,000/μL or < 4,000/μL or >10% immature band forms). Ethical approval for the study was obtained from the Research Committee at critical care department –Cairo university hospitals.

Study procedure:

From each subject, blood samples (about 8 ml) were collected on admission and after 48 hour of admission in a dry plastic tube with a clot activator. The samples were Left to clot (20 minutes at 37^0 C) and they were centrifuged at 3000X g for 15 minutes. The serum was then divided into two aliquots, one for the investigated parameters and the other was for the routine tests. Commercial biomarker kits used in the study included ELISA kit for Lipocalin-2 and resistin from (R&D Systems, Minneapolis, Minnesota) and direct enzymatic assay from (Cayman chemicals). The laboratories performed all assays in duplicate, using the average value in the analysis. All medical data including patient's history, past admissions, age and sex were collected from the medical records from the archival unit at the department. The vital data that were used for the calculation of APACHE II and SOFA scores were collected completely from the referring clinician.

Statistical analysis:

All the statistical calculations were performed with the SPSS 13 statistical software package (SPSS Inc).Values were expressed as mean ± standard deviation (SD). P-values < 0.05 was considered statistically significant. Spearman rank correlation was used for estimating the correlation between variables. Logistic regression was applied for univariate and multivariate analyses to determine the risk factors regarding mortality. Variables with $p < 0.1$ in univariate analyses were entered into multivariate analyses.

3. Results

A total of 30 patients were recruited into this study. Our cohort study included four patients with SIRS (13.3 %), five patients with severe sepsis (16.7%) and twenty one patients with septic shock (70 %). According to source of sepsis, we had twelve patients with chest infection (40%), ten patients with peritonitis (33.3%) and eight with multiple soft tissue infections (23.3%). In total, 17 participants were survived (56.7%) and 13 participants were died (43.3%) as shown in Tables 1&3.

The plasma levels of serum adipokins and oxidative stress bio markers were measured on patient admission to the

Table 1. Demographic and clinical characteristics

Characteristics	**Value**
Age, median (IQR)	55 (18-85)
Sex n (%)	
Male	20 (66%)
Female	10 (34%)
Source of sepsis	
Chest infection	12 (40%)
Peritonitis	10 (33.3%)
Soft tissue infection	8 (26.7%)
Groups classification	
SIRS	4 (13.3 %)
Severe Sepsis	5 (16.7 %)
Septic shock	21 (70 %)
Final out come	
Survivors	17 (57 %)
Non survivors	13 (43 %)

Table 2. Biomarkers & scoring systems measures on admission & after48 h. from admission

Variant	**On admission**	**After 48 hour of admission**	**P-value**
Biomarkers			
Lipocalin-2 (ng/mL)	85.75 (± 26.0)	102.94 (± 27.2)	<0.001
Resistin (ng/mL)	35.94 (± 9.79)	27.76 (± 7.33)	<0.001
Malondialdehyde (ng/mL)	14.27 (± 3.14)	17.64 (± 3.42)	<0.003
Glutathione peroxidase (U/l)	0.40 (± 0.14)	0.25 (± 0.07)	<0.001
Scoring system			
APACHE II (O/A)	23.21 (± 4.65)	---	<0.001
SOFA (O/A)	9.86 (± 2.17)	---	<0.001
SOFA (48 hours)	---	11.95 (± 2.07)	<0.001

Table 3. Mortality screen outcomes on admission & after48 h. from admission

Mortality		N	Mean	SD	p-value	Significance
LCN 2 (O/A)	died	13	110.9262	10.00058	0.001	H.S
	survived	17	66.5118	15.78448		
LCN 2 (48 h)	died	13	125.0885	14.19064	0.001	H.S
	survived	17	86.0165	22.20012		
Resist.(O/A)	died	13	45.4708	5.35909	0.008	H.S
	survived	17	28.6624	4.70478		
Resist.(48 h)	died	13	34.9946	4.39619	0.000	H.S
	survived	17	22.2306	2.80939		
MDA (O/A)	died	13	17.3285	1.67876	0.004	H.S
	survived	17	11.9376	1.57207		
MDA (48 h)	died	13	20.8462	1.45814	0.000	H.S
	survived	17	15.2000	2.22332		
GPX 3 (O/A)	died	13	.2885	.04598	0.000	H.S
	survived	17	.5000	.12062		
GPX 3 (48 h)	died	13	.1892	.04092	0.000	H.S
	survived	17	.3006	.04802		
APACHE II	died	13	27.4538	3.08992	0.001	H.S
	survived	17	19.9765	2.52914		
SOFA (O/A)	died	13	11.9200	.91278	0.001	H.S
	survived	17	8.2929	1.36184		
SOFA (48 h)	died	13	13.4423	1.52525	0.009	H.S
	survived	17	10.8247	1.71159		

ICU and also after 48 hours of admission. Our experimental results showed significant high levels of lipocalin 2, resistin & MDA on admission and after 48 hour of admission as compared with control groups, with exception that Resistin was slightly decreased after 48 hour of admission than on admission measurements. By contrast, glutathione oxidase showed decreased level on admission and after 48 hour of admission as compared with control groups as shown in Table 2.

Further correlation analysis was performed with respect to on admission results of LCN 2 was found to be closely positively correlated with resistin & MDA ($r = 0.698$, $p < 0.001$) & ($r = 0.800$, $p < 0.001$) respectively. While LCN 2 with GPX showed negative correlation ($r = -0.54$, $p < 0.002$). Multivariate analysis showed that the significant factors related to survival outcome during a 28 days - observation study for lipocalin-2, resistin, malondialdehyde and glutathione peroxidase were ($P < 0.001$, $P < 0.008$, $P < 0.004$ & $P < 0.001$) respectively which indicates a strong correlation between these biomarkers and mortality (Table 3).

4 Discussion

This prospective study investigated the plasma levels of LCN 2, resistin, MDA and GPX in septic patients on

admission to the ICU & after 48 hours from admission time. The LCN 2, resistin & MDA was found to be elevated on admission & after 48 hours. Our findings agree with a previous study stated that at the time of first sample collection in the ED, gene expression of Interleukin (IL)-10 and Neutrophil Gelatinase Associated Lipocalin (LCN 2) were significantly higher in severe sepsis than uncomplicated sepsis. Serum concentrations of NGAL and Resistin were consistently higher in severe sepsis than uncomplicated sepsis at the time of first sample collection in the emergency department (Macdonald, et al, 2014). A 28 days - follow-up was performed for a group of septic patients. It was found that the median levels of serum LCN 2 and TIMP-1 increased with sepsis severity. Serum LCN 2, MMP-9 and TIMP-1 levels were significantly higher in non-survivors than survivors at 28 days' follow-up. The study agreed with our work as it showed that it is not surprising that circulating LCN 2 is increased in sepsis because of its structure and function, and that LCN 2 level increased according to the clinical severity of sepsis in their study (Miaomiao, et al, 2014). Another study proved that Sepsis symptoms developed within the first 6 h. During sepsis, IL6, IL10, monocyte chemotactic protein-1 (MCP1), interferon-gamma (IFNγ) and TNFα significantly increased. The authors added that plasma LCN 2 was already elevated at 6 h, 24 h and 48 h (Gordon, et al, 2013).

An observational prospective study was conducted on forty-one mechanically ventilated patients diagnosed as having sepsis. Adiponectin, resistin, and cytokines were measured upon sepsis diagnosis and every 3 to 4 days thereafter until day 30. The experimental results in this cohort showed that the patients had higher adiponectin and resistin (Dinitra, etal, 2012).So, these observations agreed with our results of resistin. It is well documented that resistin and cytokine levels and routine biochemistry were measured at three to six defined time points during the first 2 weeks after admission and were correlated to other cytokines. Moreover, Serum resistin was significantly elevated compared with healthy controls and correlated with severity of disease (Jonas, et al, 2007) & (Gokmen, et al, 2013). Serum resistin concentrations were closely correlated to inflammatory parameters such as C-reactive protein, leukocytes, procalcitonin, and cytokines such as IL6 and TNF-α, as well as associated with renal failure and liver synthesis capacity. Serum resistin concentrations are elevated in acute inflammation due to sepsis or systemic inflammatory response syndrome (Alexander, et al, 2009).

A supportive study showed that Oxidative stress has been postulated as a mechanism of organ dysfunction, and thus a potential therapeutic target - in sepsis. **(**Scott,L. & Clifford, 2014**)** reported an increase in serum levels of malondialdehyde, a biomarker of oxidative stress-induced lipid peroxidation, in adults with severe sepsis, particularly in non-survivors. Another experimental results showed that serum levels of MDA were higher in severe septic patients than in healthy controls and non-surviving septic patients had higher MDA values than survivors. Moreover, serum MDA levels were associated with severity markers. Finally, these results showed that elevated MDA serum levels probably represent an unbalanced oxidant state and are related to poor prognosis in patients with severe sepsis **(**Lorente, et al, 2013**).** Another research assumed that the lipid peroxidation, as a result of reactive oxygen species (ROS) production, played a significant role in pathogenesis of multiple organ failure and septic shock associated with neonatal sepsis which contribute to high morbidity and mortality of neonatal sepsis. The MDA levels were extremely higher in both full term and preterm neonates with documented sepsis than that in their corresponding controls (Ameen, et al, 2011). Some reviewers aimed to find correlation between plasma and tissue oxidative stress and the anti-oxidative response, by measuring malondialdehyde (MDA) and glutathione (GSH) in late sepsis induced by cecal ligation and perforation. MDA concentrations were increased in the sepsis groups after 24 h. they concluded finally that plasma MDA and GSH were positively correlated with tissue MDA and GSH in intra-abdominal sepsis in a rat model (Koksal, et al, 2004).

A study mainly was done to confirm the influence of systemic inflammatory response syndrome (SIRS) on selenium (Se) levels and prospectively evaluated the relationship between serum Se concentrations (Se), glutathione peroxidase activity (GPx-3) and injury severity in patients at the time of intensive care unit (ICU) admission. Both (GPx-3) and (Se) were determined by standard methods within the first 48 h of admission to ICU. The experimental results showed that In SIRS and MODS patients, (GPx-3) and (Se) decreased significantly ($P = 0.0001$ and $P = 0.002$, respectively). After ICU admission (GPx-3) and (Se) had a predictive value for SIRS (GPx-3) sensitivity. They stated finally that systemic inflammatory response syndrome and MODS were associated with early decreases in (Se) and (GPx-3).So, low [Se] and (GPx-3) after ICU admission had a predictive value for SIRS (William, et al,2009). Some researchers hypothesized that in septic shock, and similar syndromes such as (SIRS), Sel-P binds massively to endothelium, causing a drop in Sel-P plasma concentration. Plasma Se, Sel-P and albumin concentrations, and glutathione peroxidase (GPx) activity were measured in patients with septic shock and SIRS with organ failure, on ICU admission, plasma Sel-P concentrations were 70% lower than control group (Xaver, et al,2009). In a complementary study, evidences suggested that septic shock is a primary radical induction process that has its origins early in the development of sepsis with the accumulation and generalized dispersal of cytotoxic levels of H_2O_2. This arises secondary to glutathione depletion as a result of a systemic inflammatory mediated hyper metabolic state. Studies have shown that systemic inflammation significantly reduces GSH levels, and GSH deficient animals subjected to shock. So the near universal requirement of glutathione for cellular function and the pathological accumulation of H_2O_2 that ensues when glutathione is deficient can affect every organ in the body (Jay, 2014). Another study stated also that decreased plasma selenium and glutathione peroxidase activity have been shown in patients with sepsis, and two small clinical

studies of selenium repletion have been reported (Macdonald, et al, 2003).

Conclusion

In this study, elevated baseline LCN -2, resistin, MDA and decreased GPX were associated with early prediction of sepsis. These adipokines and oxidative stress biomarkers may play a role in the prediction of sepsis risk. The association between mortality outcomes and these biomarkers may be helpful in decreasing incidence of fatal episodes among septic ICU patients.

Limitations

We studied a narrow selection of biomarkers and included a portion of the full cohort. We did not consider other potentially relevant biomarkers. An ideal study would encompass examination of a broader range of baseline biomarkers involving all subjects in the cohort. However, our preliminary study lays the foundation for a more comprehensive examination of a broader range of biomarkers and mechanisms.

Competing interests

The authors do not report any conflicts of interest.

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