

RESEARCH ARTICLE

Diagnostic value of resistin and visfatin, in comparison with C-reactive protein, procalcitonin and interleukin-6 in neonatal sepsis

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ABSTRACT. *Aim.* The aim of this study was to evaluate the predictive value of resistin and visfatin in neonatal sepsis, and to compare these adipocytokines with C-reactive protein (CRP), procalcitonin and interleukin 6 (IL-6). *Donors and methods.* A total of 62 term or near term infants with sepsis proven by positivity of blood culture, and 43 healthy infants were included in this study. *Results.* There were no statistically significant differences between the two groups as regards birthweight and gestational age. White blood cell count ($p=0.039$), CRP levels ($p=0.01$), procalcitonin levels ($p=0.01$), IL-6 levels ($p=0.01$), visfatin levels ($p=0.01$) and resistin levels ($p=0.01$) were significantly higher in septic infants. There was a positive correlation between visfatin, resistin and other markers (WBC, CRP, procalcitonin and IL-6). A cut-off value of 10 ng/mL for visfatin, showed 92% sensitivity and 94% specificity, and a cut-off value of 8 ng/mL for resistin showed 93% sensitivity and 95% specificity for neonatal sepsis. *Conclusion.* In the light of these results, visfatin and resistin can be used as a diagnostic marker similar to CRP, procalcitonin and IL-6 in neonatal sepsis. Further studies are needed to better understand the role and predictive value of these molecules in neonatal sepsis.

Key words: visfatin, resistin, neonatal sepsis, CRP, procalcitonin, IL-6

Recent studies indicate an important role of adipose tissue hormones or “adipokines” in obesity-associated complications. Some of these have proinflammatory features and are involved in many inflammatory processes in the human body. Visfatin, an adipokine isolated by Fukuhara *et al.*, corresponds to a protein identified previously as pre-B cell colony-enhancing factor (PBEF), a 52 kDa cytokine that is expressed and secreted by lymphocytes. Although visfatin is an adipocyte-specific protein, it also has biological roles such as glucose-lowering and insulin-mimicking/-sensitizing effects [1]. Expression of the visfatin gene was originally found in human peripheral blood lymphocytes, and was termed pre-B cell colony-enhancing factor (PBEF) as it increased the effect of IL-7 and stem-cell factor on pre-B-cell colony formation [2]. Visfatin appears to be an important mediator of inflammation because it has been shown that recombinant visfatin induced dose-dependent production of both pro-inflammatory cytokines (IL-1, TNF- α and IL-6) and anti-inflammatory cytokines (IL-10 and IL-1 receptor antagonist) in human monocytes [3].

Resistin, also called FIZZ3 (found in inflammatory zone) or ADSF (adipocyte-specific secretory factor) was identified as a 12.5 kDa polypeptide, and is expressed and secreted by white adipose tissue. Resistin may be involved in sensing nutritional status as its mRNA level decreases during fasting and increases after food consumption [4]. Recent studies have shown that resistin might also play a role in inflammation and autoimmunity [5]. Although resistin is predominantly expressed in adipocytes from rodents, analysis of resistin gene expression across a wide array of human tissues has revealed that peripheral blood mononuclear cells (PBMCs), macrophages and bone marrow cells are a major source of human resistin [6]. Therefore, resistin may rather be involved in the inflammatory processes than in the modulation of adiposity and glucose homeostasis in human.

To our knowledge, there is no study which has evaluated the diagnostic value of resistin and visfatin in neonatal sepsis. Therefore, the aim of this study was to evaluate the efficacy of visfatin and resistin in neonatal sepsis and compare this with C-reactive protein (CRP), procalcitonin (PCT) and interleukin-6 (IL-6).

Table 1
Criteria employed for determining the sepsis score.

Groups	Criteria
Group 1 (highly probable sepsis)	At least three sepsis-related clinical signs ^a CRP >1 mg per 100 m. At least two other altered serum parameters in addition to CRP ^b Blood culture; negative or positive
Group 2 (probable sepsis)	Fewer sepsis-related clinical signs ^a CRP >1 mg per 100 mL At least two other altered serum parameters in addition to CRP Blood culture; negative
Group 3 (possible sepsis)	Fewer sepsis-related clinical signs CRP <1 mg per 100 mL Fewer other altered serum parameters Blood culture; negative
Group 4 (no sepsis)	No sepsis-related clinical signs ^a CRP <1 mg per 100 mL No altered serum parameters

^a Sepsis -related clinical signs: temperature instability, apnea, need for supplemented oxygen, need for ventilation, tachycardia/bradycardia, hypotension, feeding intolerance, abdominal distention, necrotizing enterocolitis.

^b Serum parameters other than CRP: white blood cell count, absolute neutrophil count, platelet count.

CRP: C-reactive protein.

DONORS AND METHODS

Subjects

Newborn infants born in the GATA Medical Faculty at Haydarpaşa, Istanbul between April 2008 and March 2009 by normal, spontaneous, vaginal delivery were enrolled in the study. Gestational age was assessed by maternal menstrual dates, obstetrical ultrasonography and was confirmed using the Dubowitz scoring system. Infants born to mothers with infection, diabetes mellitus, parathyroid, bone, renal, and gastrointestinal disorders were excluded. Exclusion criteria also included administration of antibiotic treatment at admission and refusal of parental consent. Women with singleton term and near-term (>34 weeks) pregnancies were recruited consecutively from the delivery suite. A total of 178 babies were included in the study and all of them were term or near-term, and appropriate for gestational age (AGA) according to Fenton's intrauterine growth curves [7]. There were 135 late (>5 days) and highly probable septic infants, and 43 healthy infants in our study.

The infants were classified into four groups according to the criteria defined by Gitto *et al.* [8]. Group 1 (highly probable sepsis), group 2 (probable sepsis), group 3 (possible sepsis) and group 4 (no sepsis) (*table 1*). We only collected blood culture positive infants and we had to exclude 73 infants with highly probable sepsis.

GATA Medical Faculty Ethic Committee Approval (April 2008 and 195 session number) was received, and informed consent was obtained before the blood samples were taken. The patients' outcomes were recorded and the same antibiotic protocols were applied to all infants. In our NICU, first line antibiotics for neonatal sepsis is a combination of ampicillin and aminoglycoside. After culture positivity, we change the antibiotic regimen according to the antibiogram.

Blood samples

Blood was collected from infants with late-onset, highly probable, and blood culture-positive, septic infants before antibiotics were started. At the time that sepsis was suspected and/or when sepsis was diagnosed, blood samples were taken from the infants in the sepsis group. The blood samples of the control group were obtained at the time of hospital admission. After clotting, the serum was separated and immediately analysed. A white blood cell count was performed using an automatic counter (Cell Dyn 3700, Abbott Diagnostics Division, USA). Determination of visfatin and resistin levels was performed using enzyme immunoassay (visfatin C-terminal [human] EIA; Phoenix Pharmaceuticals, Belmont, CA, USA). CRP determination was performed using a Roche-Hitachi[®] 912 analyser (Roche Diagnostics, Mannheim, Germany). PCT was measured by monoclonal immunoluminometric assay (Lumitest PCT, Brahms Diagnostica[®] GMBH, Berlin, Germany). The samples for IL-6 were studied using an enzyme-linked immunosorbent test (Bender Medsystems[®] Vienna, Austria), according to the manufacturer's instructions.

RESULTS

There were 43 infants who did not have sepsis (control group), and 62 infants with sepsis (study group). *Table 2* shows the clinical demographics, outcome and laboratory findings of both the study and control groups. There were no statistically significant differences between two groups

Table 2
Characteristics and laboratory results of the infants in the control and study groups

	Control Group n=43	Study Group n=62	p
Birthweight (gr), mean (±SD)	2,660±430	2,520 ± 477	0.174
Gestational age, weeks, mean (±SD)	36 ± 2.1	36.1 ± 2.5	0.299
White blood cell count/mm ³	16,160±5,980	19,840 ± 7,055	0.039
CRP (mg/dL)	1.1±1.7	12.9±	0.01
Procalcitonin (ng/mL)	1.07± 1.7	8.9±4.4	0.01
IL-6 (pg/mL)	5.6±2.9	45 ±18.8	0.01
Visfatin (ng/mL)	5.1±4.3	14.6±4.5	0.01
Resistin(ng/mL)	3.7±3.4	10.7±3.7	0.01

Table 3
Pearson correlation and r values for visfatin, resistin and other markers (WBC, CRP, procalcitonin and IL-6).

	Visfatin (ng/mL)	Resistin(ng/mL)
White blood cell count/mm ³	0.138	0.242*
CRP (mg/dL)	0.361**	0.388**
Procalcitonin (ng/mL)	0.457**	0.459**
IL-6 (pg/mL)	0.415**	0.555**
Resistin(ng/mL)	0.342**	1

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

with respect to birthweight (control group, 2,660±430 g, study group, 2,520±477, $p=0.174$) and gestational age (control group, 36±2.1 weeks and study group, 36.1±2.5 weeks, $p=0.299$), respectively. There were significant differences between groups for WBC (control group, 16,160±5,980 mm³, study group, 19,840±7,055 mm³, $p=0.039$), for CRP levels (1.1±1.7 mg/dL and 12.9±8 mg/dL, $p=0.01$), for PCT levels (1.07±1.7 ng/mL and 8.9±4.4 ng/mL, $p=0.01$), for IL-6 levels (5.6±2.9 pg/mL and 45±18.8 pg/mL, $p=0.01$), for visfatin levels (5.1±4.3 ng/mL and 14.6±4.5 ng/mL, $p=0.01$), and for resistin levels (3.7±3.4 ng/mL and 10.7±3.7 ng/mL, $p=0.01$), respectively. Correlations between visfatin, resistin and other markers (WBC, CRP, procalcitonin and IL-6) are shown in Table 3 *table 3*.

A cut-off point of 21500 for WBC showed 78% sensitivity and 69% specificity; for CRP, a cut-off value of 0.82 mg/dl had 82% sensitivity and 79% specificity; for procalcitonin, a cut-off value of 2.8 showed 86% sensitivity and 81% specificity; for IL-6, a cut-off value of 15 pg/ml showed 94% sensitivity and 96% specificity; for visfatin, a cut-off value of 10 ng/ml showed 92% sensitivity and 94% specificity; for resistin, a cut-off value of 8 ng/ml showed 93% sensitivity and 95% specificity for sepsis. The area under the curve (AUC) values for WBC, CRP, procalcitonin, IL-6, visfatin and resistin were 0.675, 0.845, 0.898, 0.938, 0.917, 0.912 respectively, and the ROC curve of all markers are shown in *figure 1*.

DISCUSSION

To our knowledge, this is the first study which evaluated the diagnostic value of two adipokines, resistin and visfatin, in neonatal sepsis. This study showed that both resistin and visfatin could be used in the diagnosis of neonatal sepsis with a similar efficacy as other markers CRP, PCT and IL-6.

Despite major advances in neonatal intensive care, neonatal sepsis continues to be an important cause of morbidity and mortality. Although the initial clinical signs and symptoms are subtle, the clinical course can rapidly progress and worsen, and may lead to death within hours. Also, noninfected infants such as those with transient tachypnea of the newborn, meconium aspiration syndrome, and respiratory distress syndrome, are often clinically indistinguishable from septic infants. Therefore, it is important to diagnose neonatal sepsis early and accurately. However, to date, although infection markers might help in the diagnosis,

no single laboratory test provides rapid and reliable identification of early-infected neonates [9].

CRP, PCT and IL-6 are the most widely used markers for neonatal sepsis.

As discussed earlier, resistin and visfatin have proinflammatory roles, and we found higher resistin and visfatin levels, as we did for CRP, procalcitonin and IL-6 in the septic group than in the control group. Jia *et al.* [10] demonstrated that visfatin was synthesized and released by neutrophils in response to inflammatory stimuli, and they reported that it functioned as an inhibitor of apoptosis resulting from a variety of inflammatory stimuli. It was shown that visfatin was expressed at high levels in neutrophils that were harvested from critically ill, septic patients, and contributed to prolonged neutrophil survival in clinical sepsis. Busso *et al.* [11] showed that there was a link between visfatin and inflammatory cytokine secretion by leukocytes. Rongvaux *et al.* [12] showed that visfatin might confer to cells of the immune system, the ability to survive during stressful situations such as inflammation. Moreover, visfatin enhanced the surface expression of the co-stimulatory molecules important for the activation of T cells, such as CD54 (ICAM-1), CD40 and CD80 in monocytes, and was able to act as a potent chemotactic factor for CD14+ monocytes and CD19+ B cells. *In vivo*, intraperitoneal injection of recombinant murine visfatin significantly increased circulating IL-6 levels and IL-6 mRNA expression in the small intestine in mice [3]. Notably, visfatin upregulation accompanied several inflammatory conditions including acute lung injury, chronic obstructive pulmonary disease, inflammatory bowel disease, psoriasis and rheumatoid arthritis [13-15]. However, the role of visfatin was not evaluated in either neonatal or adult sepsis. In the present study, visfatin was shown to be useful in the diagnosis of neonatal sepsis. Resistin enhanced the secretion of proinflammatory cytokines, such as TNF-alpha and IL-12, in both humans and macrophage culture. Bokarewa *et al.* [16] showed that use of recombinant resistin led to a marked up-regulation of the genes for TNF-alpha, IL-6, and IL-1. Resistin probably plays an important role in chronic inflammatory and autoimmune diseases. In inflammatory bowel disease, it was shown that circulating resistin levels were elevated and correlated with WBC, CRP levels and disease activity [17]. Increased circulating resistin levels were also observed in patients with chronic pancreatitis, which might suggest its impact on the development of pancreatic fibrosis [18]. Although similarly higher serum resistin levels have been reported in patients with systemic lupus erythematosus (SLE) compared to those in controls, it has also been

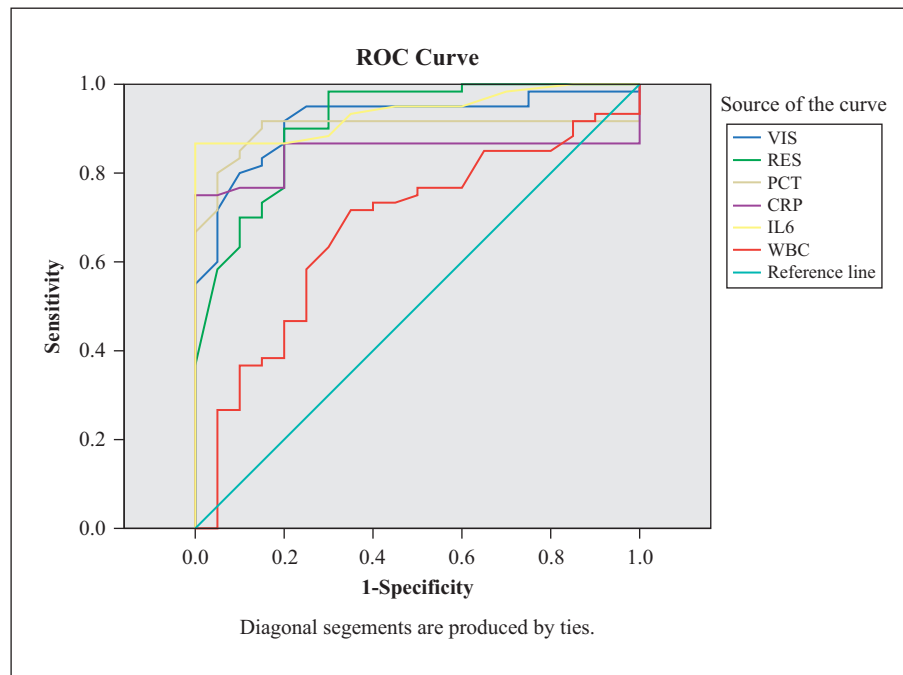


Figure 1

The ROC curve of visfatin, resistin, procalcitonin, CRP, IL-6 and white blood cell count according to the prediction of sepsis in neonates. VIS: visfatin; RES: resistin; PCT: procalcitonin; CRP: C-reactive protein, IL-6: interleukin-6, WBC: white blood cell.

shown that circulating resistin levels are clearly associated with general inflammation, renal disease, treatment with glucocorticoids, and bone loss in SLE patients [19]. Similarly to visfatin, the role of resistin had not been evaluated in neonatal sepsis. This study also showed that resistin could be used in the diagnosis of neonatal sepsis with a similar efficacy of that of CRP, PCT and IL-6. It might be suggested that these markers could play an important role in initiating the inflammatory cascade, leading to the secretion of other markers such as IL-1 and IL-6. These markers might increase earlier and faster than the other, more traditional sepsis markers, and their use might offer an advantage for the early diagnosis of neonatal sepsis.

In conclusion, this is the first study which has shown that resistin and visfatin levels in sepsis correlate positively with CRP, procalcitonin and IL-6 levels (table 2). Both of these markers had an efficacy superior to that of CRP and procalcitonin, but had predictive values similar to that of IL-6 for the diagnosis of neonatal sepsis. In the light of these results, it may be suggested that visfatin and resistin could be used as acute phase reactants similarly to CRP and PCT in the diagnosis of neonatal sepsis. However, further studies are needed to understand better the role and predictive value of these adipokines in both the diagnosis and follow-up of neonatal sepsis.

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