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RESEARCH ARTICLE

Resistin as an Early Diagnostic Marker for Identification of Neonatal Sepsis in Comparison With C-reactive Protein

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Abstract

Background: The study was designed to evaluate the diagnostic value of resistin in neonatal sepsis, and to compare these adipocytokine with C-reactive protein (CRP).

Neonates were recruited from neonatal intensive care unit (NICU) of Ain Shams University in the period from January 2012 till September 2012.

This study included 90 neonates, group1 (proved sepsis) 30 neonates were diagnosed to have neonatal sepsis clinically and laboratory [18 males (60%) and 12 females (40%)], Group2 (suspected sepsis) 30 neonates were suspected to have neonatal sepsis clinically but laboratory free [13 males (43.3%) and 17 females (56.7%)] and control group (group3) of 30 health neonates [16 males (53.3%) and 14 females (46.7%)].

Methods: All patients in the study were subjected to history taking, full clinical examination, CBC, CRP, blood culture and serum Resistin assay at the time of diagnosis of sepsis by enzyme linked immunosorbant assay (ELISA).

Results: That neonates with neonatal sepsis had their serum resistin levels significantly higher than those of the control group, as mean serum resistin level was (38.5±7.3 ng/ml), (23.9±8.5 ng/ml), and (7.2±2.6 ng/ml) in group 1, 2 and 3 respectively.

Our study found that serum resistin is of diagnostic value in early onset neonatal sepsis ($p<0.01$) and it had superior efficacy to CRP i.e the best cut off level was 17.5 ng/ml and 12.5 ng/ml for sepsis and suspected sepsis respectively.

Conclusion: Resistin is of diagnostic value in early onset neonatal sepsis as serum resistin values were significantly higher in septic neonates compared to healthy controls with positive correlation with CRP in cases.

Introduction

Background

Neonatal sepsis is a bacterial infection in the blood. It is found in infants during the firstmonth of life. Despite major advances in neonatal intensive care, neonatal sepsis continues to be an important cause of morbidity and mortality. Therefore, it is important to diagnose neonatal sepsis early and accurately [1].

Early recognition and diagnosis of neonatal sepsis are difficult because of the variable and nonspecific clinical presentation of this condition. It is extremely important to make an early diagnosis of sepsis, because prompt institution of antimicrobial therapy improves outcomes [2].

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 like leptin, adiponectin, visfatin, resistin, apelin, and proinflammatory cytokines [3].

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 [1].

Resistin is a hormone secreted by adipose tissue. It is also known as "serine/cysteine rich adipocyte-Specific Secretory Factor" (ADSF). The length of the resistinpre-peptide in human is 108 amino acids (in the mouse and rat it's 114 aa); the molecular weight is ~12.5 kDa. Among the hormones synthesized and released from adipose tissue (adiponectin, angiotensin, estradiol, IL-6, leptin, PAI-1, TNF-α, and resistin; also known as ADSF or FIZZ3 [found in inflammatory zone][4].

Resistin has been shown to increase transcriptional events leading to an increased expression of several pro-inflammatory cytokines including (but not limited to) interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-12 (IL-12), and tumor necrosis factor-α (TNF-α) [5].

Resistin also upregulatesintracellular adhesion molecule-1 (ICAM1) and vascular cell-adhesion molecule-1 (VCAM1), all of which are occupied in chemotactic pathways involved in leukocyte recruitment to sites of infection [6].

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 this marker could play an important role in initiating the inflammatory cascade, leading to the secretion of other markers such as IL-1 and IL-6. This marker might increase earlier and faster than the other, more traditional sepsis markers, and its use might offer an advantage for the early diagnosis of neonatal sepsis [1].

Resistin and visfatin levels in sepsis correlate positively with CRP, procalcitonin and IL-6 levels. 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[1].

The study was designed to evaluate the diagnostic value of resistin in neonatal sepsis, and to compare these adipocytokine with C-reactive protein (CRP).

Subjects and Methods

This case control study was conducted in neonatal intensive care unit, Ain Shams University.

90 neonates admitted to NICU were recruited for the study. They were divided into three groups: Group 1 (sepsis):- It included 30 neonates diagnosed as having proved early onset neonatal sepsis based on hematological sepsis score [7] clinical and laboratory data, Group 2 (suspected sepsis):-It included 30 neonates diagnosed as having early onset suspected neonatal sepsis based on clinical data only and laboratory free and Group 3(no sepsis):-Control group it included 30 healthy term newborns.
Neonates with gestational age > 32 week and body weight > 1.500 kg were included while neonates with major congenital anomalies, surgical problems or congenital heart were excluded from the study.

All neonates were subjected to full history taking, thorough clinical examination to detect clinical signs of sepsis (temperature instability, respiratory dysfunction, circulatory dysfunction, GIT dysfunction and neurological dysfunction), Laboratory investigations [Complete blood count (CBC) using blood samples collected in EDTA tubes by coulter counter T660 with differential leucocytic count using Leishman-stained blood smear by counting at least 200 cells; c-reactive protein (CRP) estimated by latex agglutination assay; Blood culture using a signal blood culture system (oxoid) both CBC1 and CRP1 drawen at admission, CBC2 and CRP2 at diagnosis of sepsis and CBC3 and CRP3 at discharge, Bacterial subculture was done on MacConkey agar plate, blood agar plate and Sabouraud's dextrose agar plate (SDA).

Serum Resistinlevel by ELISA technique (using the Assay Max Human Resistin ELISA kit). Blood samples were collected aseptically by venipuncture when sepsis was suspected blood was left to clot then centrifuged for 10 minutes at 5000 rpm. The sera were separated and stored at -20 ºC until the time of the assay.

The study protocol gained the approval of the local ethics committee of Department of Pediatrics, Children's Hospital, Ain Shams University. An informed consent was taken from the parents.

Statistical methodology:

Analysis of data was done by IBM computer using SPSS (statistical program

for social science version 12). Quantitative variables are described as mean, standard deviation (SD) and range. Qualitative variables expressed as number and percentage. Chi-square test was used to compare qualitative variables between groups. Unpaired t-test used to compare quantitative variables, in parametric data (SD<50% mean). Mann Whitney test used in non parametric data (SD>50%mean). Spearman Correlation co-efficient test used to rank variables versus each other positively or inversely. ROC (receiver operator characteristic curve) was used to find out the best cut off serum resistin for the risk of neonatal sepsis and the associated specificity and sensitivity levels.

Results were considered significant if $P \leq 0.05$, highly significant if $P \leq 0.01$

and insignificant $P > 0.05$.

Results:

This study included 90 neonates, group1 (proved sepsis) 30 neonates were diagnosed to have neonatal sepsis clinically and laboratory [18 males (60%) and 12 females (40%)]with mean gestational age (37±1.6 weeks), mean birth weight (2.4±0.74 Kg).

Group2 (suspected sepsis) 30 neonates were suspected to have neonatal sepsis clinically but laboratory free [13 males (43.3%) and 17 females (56.7%)] with mean gestational age (36.6±1.39 weeks), mean birth weight (2.5±0.67 Kg).

A control group (group3) of 30 health neonates [16 males (53.3%) and 14 females (46.7%)] with mean gestational age (37.1±1.33 weeks), mean birth weight (2.86±0.95 Kg).

The three groups were matched regarding sex (p=.429), gestational age (p=.448) 37±1.6 weeks, 36.6± 1.3 weeks and 37.1±1.3 weeks, birth weight (p=.039) 2.4±.74 kg, 2.5±.67 kg and 2.8±.59kg and length (p=.289) 43.7±5.4 cm, 44.3±2.5 cm and 45.2±2.1 cm, in group1, 2 and 3 respectively.
It was found that APGAR score was significantly lower in group1 compared to group2 (p=.007) and no non-significant difference as regard other demographic data as shown in (table 1)

In the current study it was found that C.R.P1 and C.R.P2 was significantly higher in group1 (23.6 ± 34ng/ml), (39.6 ± 32.9 ng/ml) than group2 (.00 ±.00), (.00 ±.00) with (p=.001) and no significant difference as regard C.R.P3 as shown in (table 1).

Neonates in group 1 had significant lower Plt1 and Plt2 in group1 (235.7 ±152.7 $\mathbf{x10^3/mm^3}$) and (164.4 ± 153.8 $\mathbf{x10^3/mm^3}$) than in group2 (270.5 ± 94.8 $\mathbf{x10^3/mm^3}$) and (254.7 ± 115 $\mathbf{x10^3/mm^3}$) with p=.03, .001 respectively and

show highly significant higher band cells (p=.001) in group1 (8.9 ± 3.6 **%**) than in group 2 (2.6 ±.93 **%**) and no significant difference between group1and group2 as regard other element of C.B.C 1, C.B.C 2 and C.B.C3 as shown in table1.

It our study we found highly significant higher resistin level (38.5±7.3 ng/ml) and sepsis score (10.8 ± 1.33) in group1 (p=0.001 and p=.001) than in group2 (23.9±8.5 ng/ml), (6±1) and no significant difference as regard blood culture as shown in table 1.

There was a significant positive correlation between serum resistin level in cases group with CRP (p=.01) as shown in table 2.

Table (1): Comparison between group1 and group2 as regard demographic and laboratory data.

Descriptive data items	Group 1 n =30 (Mean±SD) /%	Group 2 n =30 (Mean±SD) /%	Statistical test Post Hoc / Chi-Square	P value
Gestational age in weeks	37 ± 1.6	36.6 ± 1.39	0.33333	.675
APGAR	7.2 ± 1.09	8.06 ± 1.01	0.866	.007
Birth weight (kg)	2.4 ±.74	2.5 ±.67	0.059	.944
Birth length (cm)	43.7 ± 5.4	44.3 ± 2.5	0.616	.841
Sex Male Female	 60% 40%	 43.3% 56.7%	1.669	0.196
C.R.P1 (ng/ml)	23.6 ± 34	.00 ±.00	-4.004	0.001
C.R.P2 (ng/ml)	39.6 ± 32.9	.00 ±.00	-6.39	0.001
C.R.P3 (ng/ml)	4.8 ± 19.3	3.2 ± 10.4	-.386	0.7
Hb 1 (gm/dl)	13.9 ± 2.8	14.4 ± 2.6	0.523	0.742
T.L.C 1 ($x10^3/mm^3$)	13.7 ± 11.4	10.5 ± 3.4	-0.54	0.58
Plt 1 ($x10^3/mm^3$)	235.7 ±152.7	270.5 ± 94.8	-2.174	0.03
Hb 2 (gm/dl)	12.7 ± 1.88	12.8 ± 2.3	0.053	0.995
T.L.C 2 ($x10^3/mm^3$)	12.7 ± 12.1	9.9 ± 2.6	-0.56	0.56
Plt 2 ($x10^3/mm^3$)	164.4 ± 153.8	254.7 ± 115	-4.28	0.001
Neutrophilies %	37.8 ± 10.9	42.9 ± 10.1	-1.85	0.06
Bands %	8.9 ± 3.6	2.6 ±.93	-6.50	0.001
Hb 3 (gm/dl)	10.8 ± 1.6	11.3 ± 1.4	0.450	0.488
T.L.C 3 ($x10^3/mm^3$)	9.8 ± 2.5	9.4 ± 2	-0.91	0.36

Plt 3 (x10^3/mm^3)	318.1 ± 112.3	316.7 ± 101.3	-0.244	0.8
Blood culture **positive** **negative**	46.7% 53.3%	33.3% 66.7%	1.11	0.29
Resistin (ng/ml)	38.5 ± 7.3	23.9 ± 8.5	14.6	0.001
Sepsis score	10.8 ± 1.33	6 ± 1	4.7	0.001

Hb: Haemoglobin**T.L.C:** Total Leucocytic count **Plt:** Platelet **C.R.P:** C-reactive protine

Table (2): Correlation between Resistin and C.B.C2, C.R.P2 in group1:

Variables	(Mean ± SD)	Pearson correlation coefficient test	P value
Hb 2 (gm/dl)	12.7 ± 1.88	0.14	0.45
T.L.C 2 (x10^3/mm^3)	12.7 ± 12.1	-0.15	0.41
Plt 2 (x10^3/mm^3)	164.4 ± 153.8	-0.044	0.81
Neutrphils %	37.8 ± 10.9	0.12	0.52
Band %	8.9 ± 3.6	-0.11	0.55
C.R.P2 (ng/ml)	39.6 ± 32.9	0.44	0.01

Hb: -Haemoglobin**T.L.C:-** Total Leucocytic count **Plt:** - Platelet
C.R.P:- C-reactive protineCorrelation is significant at the 0.01 level

Figures:

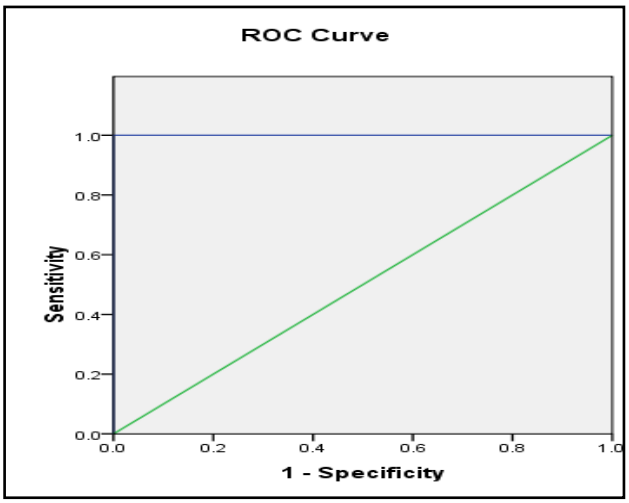

Figure (1): Sensitivity and Specificity of Serum Resistin Level to Detect Sepsis in group1 using receiver operating characteristic (ROC) Curve

Our study revealed that the best cutoff value of serum resistin to detect sepsis in group1 was (17.5 ng/ml) with 100% sensitivity and 100% specificity, and that serum resistin level was reliable to detect sepsis (P <0.01) as shown in figure1.

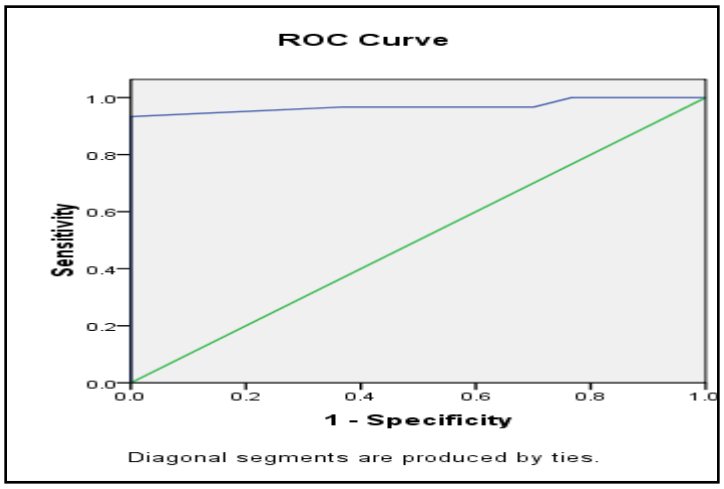

Figure (2): Sensitivity and Specificity of Serum Resistin Level to Detect Sepsis in group2 using ROC Curve.

The ROC Curve defined the best cutoff value of serum resistin to detect sepsis in group2 was (12.5 ng/ml) with 93% sensitivity and 100% specificity, and that serum resistin level was reliable to detect sepsis (P <0.01) as shown in figure 2.

Discussion:

In our study it was found that resistin is higher in case groups than control group as in comparison between cases in group1 (proved sepsis) and control group resistin was (case 38.5 ± 7.3 ng/ml, control 7.2 ± 2.6 ng/ml, p=.001) and between cases in group2 (suspected sepsis) and control (case 23.9 ± 8.5 ng/ml, control 7.2 ± 2.6 ng/ml, p=.001).

This comes in agreement with the study of DidemAliefendioglu et al.,[8]who found that resistin was higher in case group than control group (case 17.5±229.9 ng/ml, control 7.2±176.7 ng/ml, p=.001).

This is due to recent studies had shown that resistin might play a role in inflammation and autoimmunity. 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. Therefore, resistin may rather be involved in the inflammatory processes than in the modulation of adiposity and glucose homeostasis in human [1].

In our study it was found that there was a positive correlation between resistin and CRP.

This comes in agreement with DidemAliefendioglu et al., [8] who found also a positive correlation between resistin and CRP.

In group1 the best cutoff value of serum resistinto detect sepsis was 17.5 ng/ml, yielded sensitivity of 100%, specificity of 100%.

Moreover,in group2 the best cutoff value of serum resistinto detect sepsis was 12.5 ng/ml, yielded sensitivity of 93%, specificity of 100%.

This comes in agreement with study done by Cekmez et al. [1] which show similar results asROC curve for serum resistin level was constructed showing an area under the curve (AUC).912 and cutoff value of serum resistin to detect sepsis was 8 ng/ml, yielded sensitivity of 93%, specificity of 95%.

In contrast to study of DidemAliefendioglu et al., [8] found that AUC for resistin was.74 and cut of value of sermresistin to detect sepsis was 50 ng/ml with sensitivity of 73.3%, specificity of 45.8%. This high sensitivity in our study might be attributed to small number of the studied patients group, yet it suggests that the role of serum resistin in neonatal sepsis should be further investigated.

In our study we showed that resistin could be used in the diagnosis of neonatal sepsis with a similar efficacy of that of CRP as in group1 (proved sepsis) CRP was elevated (39.6±32.9 ng/ml) and resistin also was high (38.5±7.3 ng/ml).

In group2 (suspected sepsis) we found that resistin had superior efficacy to that of CRP as CRP was negative while resistin was elevated (23.9±8.5 ng/ml), also in one of our cases resistin was elevated even before appearance of clinical manifestation of sepsis.

This comes in agreement of Cekmez et al. [1]who showed that resistin could be used in the diagnosis of neonatal sepsis with a similar efficacy of that of CRP, PCT and IL-6 and it had an efficacy superior to that of CRP.

In contrast to us DidemAliefendioglu et al., [8] found that resistin may be used as an indicator of sepsis in preterm babies. However, it has a limited value compared with other inflammatory markers, including CRP, PCT, and IL6. This may be because their study was done on preterm babies only while we did our study on near term and full term babies.

In conclusion that resistin is of significant diagnostic value in early onset neonatal sepsis as serum resistin values were significantly higher in septic neonates compared to healthy controls with positive correlation with CRP in case group and it has superior efficacy to that of CRP with cut off value 17.5ng/ml with 100% sensitivity and 100% specificity.

List of abbreviation:

CRP C-reactive protein

NICU neonatal intensive care unit

ADSF adipocyte-Specific Secretory Factor

CBC complete blood picture

IL interleukin

TNF tumor necrosis factor

ICAM1 intracellular adhesion molecule-1

VCAM1vascular cell-adhesion molecule-1

SDA Sabouraud's dextrose agar plate

SPSS statistical program for social science

GIT gastrointestinal tract

SD standard deviation

ROC receiver operator characteristic curve

PCT procalcitonin

FIZZ3 Found in inflammatory zone

Competing interests:

'The authors declare that they have no competing interests'.

Authors' contributions:

IA had made substantial contributions to conception and design, SA acquisition of data, NR analysis and interpretation of data; NMA had been involved in drafting the manuscript, revising it critically for important intellectual content; and had given final approval of the version to be published; and I A agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

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Endnotes:
Resistin was of significant diagnostic value in early onset neonatal sepsis as serum Resistin values were significantly higher in septic neonates and it had superior efficacy to that of CRP.

References:

1-Cekmez F, Canpolat FE¸ etinkaya M, Aydinöz S, Aydemir G, Karademir F, Ipcioglu OM, Sarici SÜ **: Diagnostic value of resistin and visfatin, in comparison with C- reactive protein, procalcitonin and interleukin-6 in neonatal sepsis Eur.** Cytokine Netw 2011, 22(2): 113-117.

2-Verboon-Maciolek M.A, Krediet T.G, GerardsL.J :**Severe neonatal parechovirus infection and similarity with enterovirus infection.** Pediatr Infect Dis J 2008, 27 (3): 241-245.

3-Libby P, Okamoto Y Rocha VZ : Inflammation in atherosclerosis; transition from theory to practice. Circ J 2010, **74:**213-220.

4-Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA: **The hormone resistin links obesity to diabetes**. Nature 2001, 409:307.

5-Silswal N, Singh AK, Aruna B: **Human resistin stimulates the pro-inflammatory cytokines TNF-α and IL-12 in macrophages by NF-B-dependent pathway.** BiochemBiophys Res Commun 2005, 334:1092–1101.

6-Verma S, Li SH, Wang CH:**Resistin promotes endothelial cell activation: Further evidence of adipokine-endothelial interaction.** Circulation 2003, 108:736–740.

7-Töllner U : **Early diagnosis of septicemia in the newborn.** Clinical studies and sepsis score 1982, 138(4): 331-337.

8-Didem Aliefendioglu, TugbaGursoy, Osman Caglayan, AlevAktas, FahriOvali: **Can Resistin be a New Indicator of Neonatal Sepsis.** Pediatrics and Neonatology 2014**,** 55(1): 53-57.