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Diagnostic Value Of Resistin And Hepcidin In Neonatal Sepsis

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Abstract: *Diagnosis of sepsis-attributed morbidity and mortality in the neonates still constitute a great challenge. Several attempts have been described to evaluate new diagnostic markers. Resistin and hepcidin are relatively new biomarkers that have been investigated and suggested as possible markers of infection in adult. The aim of this study was to evaluate hepcidin and resistin as new biomarkers of neonatal sepsis (NS) in comparison to the established diagnostic methods as C-reactive protein (CRP), blood culture and detection of bacterial 16S rDNA by PCR. The study included 150 neonates with suspected NS who were subjected to sepsis screen scoring. Complete blood count, CRP and automated blood culture were performed. Broad range bacterial 16S rDNA was detected by PCR without prior enrichment. Resistin and hepcidin serum levels were measured by enzyme immunoassay. Klebsiella was the most common isolated organism (9 isolates) followed by coagulase negative Staphylococci (8 isolates). PCR results for 16S rDNA were positive in 40 cases. Resistin level was significantly elevated in 84 cases; 80 of the sepsis groups and 4 in the control group. Hepcidin level was found to be elevated in 62 cases; 59 of the sepsis groups and 3 cases of the control group. Both resistin and hepcidin levels were significantly elevated in neonates with sepsis and were positively correlated with CRP and band cells count. However, resistin had greater diagnostic value compared to hepcidin. Both of these markers had an efficacy comparable to that of CRP in the diagnosis of NS.*

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I. Introduction

Neonatal sepsis (NS) remains a major cause of newborn morbidity and mortality [1,2]. Moreover, it increases the hospital stay and healthcare cost [1]. In Neonatal Intensive Care Units (NICUs), the incidence NS in full term ranges between 7-24.5% while, the incidence increases up to 40% in low birth weight (less than 1000 g) or preterm newborns [1]. Several attempts have been described to achieve early and reliable diagnosis of NS aiming to justify and rationalize the administration of antibiotics [2]. Although a large number of infants are admitted to NICU present with signs of sepsis and are treated with antibiotics, the pathogens are identified in only a minor proportion of those patients [3]. The clinical outcome of NS is directly correlated with the early diagnosis and management. The diagnosis of NS is problematic due to its vague clinical manifestations and the overlap with other pathological conditions [4]. Recently, there has been a great interest regarding the diagnostic values of a range of biochemical markers, hematological indices and scoring systems [4]. The studies in adults and children have demonstrated the important role of interleukin-6 (IL-6), C-reactive protein (CRP) and procalcitonin (PCT). Also, blood culture remains the gold standard in the diagnosis of NS [3,5,6]. However, obtaining sufficient amount of blood for culture is often difficult. Moreover, it requires long turn-around time and maternal antibiotic administration may constitute an additional barrier in isolating the responsible pathogens [3]. Detection of universal sequences of bacterial DNA is currently suggested by some authors to be a rapid and sensitive supplement to blood culture in diagnosis NS [7].

Resistin is a polypeptide (12.5 kDa) which is predominantly secreted by mononuclear cells and macrophages. Resistin belongs to a new adipokine family called cysteine-rich secretory proteins. Normally, the serum resistin level ranges from 7 to 22 ng/mL [8]. Recent studies suggested that human resistin may have a role in the inflammatory processes rather than in the adiposity and glucose metabolism as thought before [8,9]. Studies in adult patients with sepsis revealed that serum resistin level was elevated in response to acute bacterial

infection [10]. Moreover, it has been considered as an indicator of the severity of infection and an important mediator of the prolonged inflammatory status. However, limited data are available about resistin levels in neonatal sepsis [9].

Hepcidin, a 25-amino acid peptide molecule, is mainly secreted by the liver and mononuclear cells. It plays an essential role in inflammation and iron homeostasis [11,12]. It was found to be expressed by macrophages and neutrophils in patients with bacterial infections in response to IL-6. It is also considered an acute-phase reactant that participates in host defense through pathogens iron deprivation and its direct antimicrobial activity on bacteria and viruses [13]. This association between the hepcidin and infection may suggest it as an early biological marker for NS [14].

Therefore, the aim of this study was to assess hepcidin and resistin as new biomarkers of NS in comparison to CRP and confirmatory diagnostic methods as blood culture and molecular detection of broad-range bacterial 16S rDNA in peripheral blood samples by PCR.

II. Material And Methods

I- Subjects: This study included 150 neonates who were admitted to King Abdul Aziz specialist hospital at Taif, Saudi Arabia, from June 2015 to January 2017. The project was approved by ethical committee of Taif University. An informed parent's consent was obtained for each neonate before involvement in the study. The neonate was considered eligible for the study if showed any signs of neonatal sepsis as described before [15]. Neonates with any congenital malformations and/or laboratory confirmed TORCH infections were excluded from the study. Also, those immediate post-natal hemoglobin level <10 g/dl were excluded because of the role of hepcidin in anemia [13].

II- laboratory methods: Every neonate was assessed initially by complete blood count (CBC), C-reactive protein (Dade Behring, Marburg, Germany) and automated blood culture (Becton Dickinson, NJ, USA) followed by subculture and complete bacteriological standard microbiological identification methods for positive cases [1]. Broad range bacterial 16S rDNA was detected by polymerase chain reaction (PCR) without prior enrichment. DNA was extracted from whole peripheral blood according to the Manufacturer's instructions (Promega, Madison, USA). PCR primers and master mix were used (Macrogen, Seoul, korea) and reactions were set up as described before [15]. PCR products of 500 base pairs were separated by electrophoresis on 1.2% agarose stained by 0.5 µg/ml of ethidium bromide [2]. Resistin and hepcidin serum levels were measured by enzyme immunoassay kits (LINCO Research, Missouri, USA).

III-Statistical analysis:

Normality of distribution was computed (SPSS-20) by Shapiro–Wilk normality test. Quantitative variables were expressed in means ± SDs. Qualitative variables were expressed in frequencies and percentages. The comparison of quantitative data was performed by Kruskal Wallis test for more than 2 groups of data. Comparisons of qualitative variables were done using X^2 test. Spearman correlation coefficients were calculated for association between numeric variables. Sensitivity, specificity, positive and negative predictive values were calculated for different screening tests and ROC curves were drawn for them.

III. Result

According to the laboratory and clinical data the studied neonates were divided into 3 groups as follows: Group (1) proven NS, group (2) probable NS and group (3) control or non-sepsis. Group (1) included 42 neonates with positive either blood culture or PCR results for bacterial 16S rDNA. Group (2) included 59 neonates with sepsis-related clinical signs and positive sepsis screen based on band cells > 10%, polymorphocytosis, elevated CRP >2.5 mg/dl and other parameters according to Malik *et al.*,[15]. Group (3) included 49 neonates that were suspected to have sepsis but they had negative blood culture and PCR results in addition to negative sepsis screen (table 1). Blood cultures were found positive in 36 cases, 2 of them were found to be negative for 16S rDNA. The most common isolated organism was *Klebsiella spp.* followed by coagulase negative Staphylococci (CoNS), then *E. coli* (table 2), PCR results for 16S rDNA were positive in 40 cases, 6 of them showed negative blood culture. The CRP level was significantly elevated (cut-off value = 2.5mg/L [2]) in 87 cases; 40 cases of group (1), 45 of group (2) and only 2 cases of group (3). Resistin level was significantly increased (cut-off value = 60 pg/ml [2]) in 84 cases; 38 of group (1), 42 of group (2) and 4 in group (3). Hepcidin level was found to be elevated (cut-off value = 7 ng/ml [2]) in 62 cases; 30 of group (1), 29 in

group (2) and 3 cases of group (3). Sensitivity and specificity for each test are shown in table (3) and figure (1) while correlation of resistin and hepcidin with other testes were presented in table (4).

Table (1): Blood culture, PCR, C-reactive protein (CRP), resistin and hepcidin, and hematological parameters among the studied groups.

The studied parameter	Group (1) (n=42)	Group (2) (n=59)	Group (3) (n=49)	P value
Gestational age (weeks) (M±D)	25.3±2.7	28.1±1.2	26.2±2.3	0.113 NS
Birth weight (g) (M±SD)	1876±561	1929±346	2016±432	0.933 NS
WBC's ($X10^9$/L) (M±SD)	8.8±3.8	9±1.4	9.7±2.6	0.350 NS
Neutrophils (%) (M±SD)				
- Total	53.5±3.1	52.3±4.1	53.7±2.7	0.348 NS
- Band cells	12.7±2	11±1.4	4±1.4	< 0.001HS
Platelets ($X10^9$/L) (M±SD)	158.4 ±12.1	140.1±7.9	200.5±21.7	< 0.01S
Blood culture				
- Positive	36 (85.7%)	0	0	< 0.001HS
- Negative	6 (14.3%)	59	49	
PCR				
- Positive	40 (95.2%)	0	0	< 0.001HS
- Negative	2(4.8%)	59	49	
CRP (M±SD)	13.2±4.8	3.8±3.2	0.5±0.6	< 0.001HS
Resistin (M±SD)	149.9±71.3	132.4±58.2	55.5±12.7	< 0.001HS
Hepcidin (M±SD)	33.2±23.8	31.6±29.6	5.7 ±3.4	< 0.001HS

HS: Highly significant S: Significant NS: Not significant.

Table (2): Results of PCR, C-reactive protein (CRP), resistin and hepcidin in relation to the isolated organisms.

The isolated organisms (n)	PCR (+ve)	CRP (M±SD)	Resistin (M±SD)	Hepcidin (M±SD)
Klebsiellaspp (n=9)	9	14.7±1	148.4±70.6	39.6±16.9
Co-agulase negative *Staphylococci* (n=8)	8	16.5±0.6	133.3±67.2	31.7±13
Escherichia coli (n=7)	7	16.6±1.5	167.3±52.7	43.7±39.8
Group B Streptococci (n=5)	5	13±1.6	159.4±72.4	43.8±7.6
Enterobacterspp (n=3)	3	16.8±1.5	165.3±45	38.5±32.5
Staphylococcus aureus (n=2)	1	10.6±1.9	147±24	35.5±0.7
Serratiamarcescens (n=1)	1	14.4±0	123.0±50.1	37.5±0
Enterococcus (n=1)	1	11.4±0	148.2±0	29.6±0
P value	**0.351 (NS)**	**0.125 (NS)**	**0.978 (NS)**	**0.980(NS)**

NS: Not significant

Table (3): Diagnostic accuracy of C-reactive protein (CRP), resistin and hepcidin in neonatal sepsis.

The parameter	**CRP**	**Resistin**	**Hepcidin**
AUC (95%CI)	0.93	0.79	0.57
P value	<0.0001	<0.0001	0.183
Sensitivity	95%	90%	71%
Specificity	56%	57%	49%
Positive predictive value (PPV)	45%	44%	34%
Negative predictive value (NPV)	97%	94%	82%

Table (4): Spearman correlation between resistin, hepcidin and other parameters

	Hepcidin (ng/mL	Resistin (ng/mL)
Band cells	0.43*	0.47*
C-reactive protein (mg/dL)	0.54*	0.69*
Resistin (ng/mL)	0.84*	1

*Correlation was significant ($p < 0.05$)

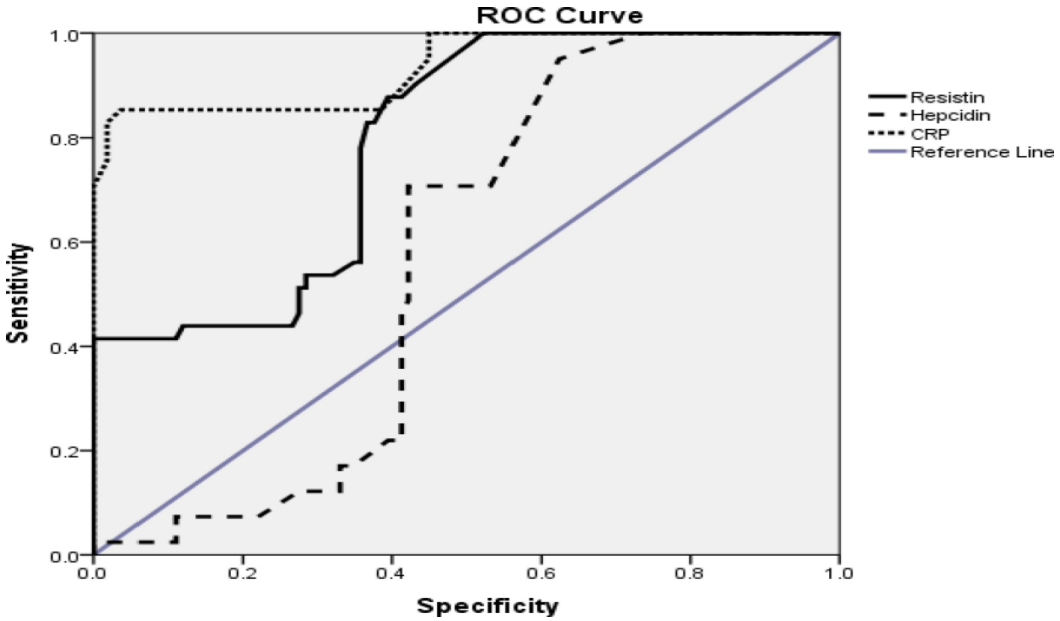

Fig. (1)ROC curve of resistin, hepcidin and C-reactive protein in diagnosis neonatal sepsis.

IV. Discussion

Definitive diagnosis of NS is difficult because of the variable and non-specific clinical presentation. Therefore, many neonates may be subjected to unnecessary antibiotic therapy with subsequent development of microbial resistance [16,17,18]. Diagnosis in most centers is based on combination of clinical signs and classic laboratory markers as CRP and the percentage of the neutrophils and band cells. Blood culture continues to be the most confirmed method for diagnosis of NS [19]. However its sensitivity in neonates doesn't exceed 35% [20]. Detection of 16S rDNA by PCR which is common for all bacteria represents an excellent alternative method for diagnosis of bacterial sepsis in well-equipped laboratories [18]. In the current study, the statistical analysis of the clinical and laboratory data revealed that birth weight and gestational age showed non-significant differences between the studied groups (P = 0.113 and 0.933 respectively). Also, there were no significant differences regarding white blood cells (WBCs) and total neutrophils count while, band cells were significantly higher in both proven and probable NS groups compared to the controls. Out of the 150 neonates screened for sepsis, 40 cases were 16S rDNA positive and 36 cases were blood culture positive. Compared to blood culture, PCR was revealed to have comparable sensitivity and specificity (94.4 % and 94.8% respectively) however, PCR results were negative in 2 samples obtained from blood culture positive neonates, and it was positive in 6 samples that were negative in blood culture. These results my support many previous studies [2,15] that recommended 16S rDNA PCR assay as a reliable tool in evaluating NS. The causative organisms of NS showed notable regional variation between the developed and developing countries. In the present study, Gram-negative organisms as *klebseilla* (9 isolates) and *E coli* (7 isolates) were the most recovered organisms followed by CoNS (8 isolates) while Group B Streptococcus (GBS) was isolated only from 5 cases. These findings are consistent with many previous studies [18,21,22]. The predominance of Gram-negative organisms and decline of GBS was attributed to the maternal intra-partum antimicrobial prophylaxis. Moreover, the neonates might acquire those Gram-negative rods from the maternal fecal flora during delivery. While, other studies reported that co-agulase negative *staphylococci* (CoNS) was still to be the most common etiology of NS [23].

Resistin was described by Steppan*et al.;* [24] as a pro-inflammatory adipokine and it was initially considered to be a link between obesity and the development of insulin resistance. Recently, it was reported that bacterial infections induce its production by monocytes and macrophages [25]. The present study showed that resistin levels of the neonates with sepsis (either proven or probable NS) were significantly (P < 0.001) higher than controls. Also, resistin level had positive correlation with CRP level and band cells count. These results may recommend it as a good marker of sepsis with considerable sensitivity and specificity (90% and 57% respectively). Cekmez*et al.;* [9] reported similar results indicating that resistin had similar efficacy as that of CRP and procalcitonin in the diagnosis of NS. Moreover, resistin level might increase earlier than the other markers, and its use might offer an advantage for the earlier diagnosis [26].

Hepcidin is produced in response to inflammation so, it is recently considered as an acute-phase biomarker for inflammation and sepsis [27]. Also, it has antimicrobial properties through limitation of the availability of iron to the invading pathogens [28,29,30]. In the current study, hepcidin level was significantly (P

< 0.001) higher among the neonates with sepsis than controls. Also, it was found to be correlated positively with CRP level and band cells count. However, in our results it showed lower sensitivity (71%) and specificity (49%) compared to resistin (90% and 57% respectively). These results are consistent with previous reports [13] that showed that hepcidin concentrations were significantly increased in sepsis. Moreover, after termination of antibiotic therapy, hepcidin serum concentrations were comparable with levels in healthy infants [13]. In addition, a recent prospective study reported the superiority of hepcidin over CRP and the leukocyte count in assessing the severity of sepsis [31]. Also, Kemna*et al.;* [32] mentioned that hepcidin synthesis was induced by bacterial lipopolysaccharide and IL-6, and could be quantitatively detected in the urine which can enable serial periodic evaluations of sepsis [33]. These properties suggest that hepcidin may be a useful biomarker for NS diagnosis in adjunct to blood culture and other traditional markers.

V. Conclusion

Resistin and hepcidin levels in NS were positively correlated with CRP and band cells count. Both of these markers had an efficacy comparable to that of CRP in the diagnosis of NS, however, both the sensitivity and specificity of resistin were superior to that of hepcidin. Our results may suggest that resistin and hepcidin could be used as acute phase reactants in the diagnosis of NS. However, further larger studies are recommended to evaluate their role in NS diagnosis and follow-up.

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