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Diagnostic Value of Biomarkers in Neonatal Sepsis at Neonatal Intensive Care Unit at Minia University Hospitals

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ABSTRACT

Background: Due to the non-specific clinical manifestations, neonatal sepsis (NS), one of the major concerns with considerable morbidity and mortality in newborn intensive care units (NICUs), poses a significant challenge for clinicians and the laboratory. Blood culture, non-specific biomarkers, and clinical presentation are currently used to diagnose newborn sepsis. Traditional biomarkers with low sensitivity and positive predictive value include total leucocytic count (TLC) and C-reactive protein (CRP). The aim of the current study is to evaluate the value of apelin, procalcitonin, and proadrenomedullin as biomarkers in the diagnosis of neonatal sepsis.

Patients and methods: This study was conducted, over a period of one year, at NICU in Minia University Hospitals on 60 neonates diagnosed as sepsis representing *Group-I* who were further sub-grouped into *Group I-a* (Early Onset sepsis) including 36 neonates and *Group I-b* (Late Onset Sepsis) including 24 neonates. A total of 30 apparently healthy neonates represented *Group-II* (control group) with no manifestations or laboratory findings of sepsis. Samples were collected from each neonate for CBC, CRP, blood culture and evaluation of serum procalcitonin, apelin and proadrenomedullin by ELISA. **Results**: Significant correlations were found between serum procalcitonin, apelin and proadrenomedullin with the routine investigations done (TLC, platelets count and CRP). Higher procalcitonin, proadrenomedullin and apelin levels were observed in septic group (early onset sepsis and late onset sepsis) with positive blood culture results. Staphylococcal infection was the most frequent type of infection.

Conclusion: Measuring procalcitonin, apelin and proadrenomedullin levels are valid and can aid in the diagnosis of NS, but alone cannot be dependable for accurate diagnosis.

Keywords: NICU, Apelin, Procalcitonin, Proadrenomedullin, Neonatal sepsis, Total leucocytic count.

INTRODUCTION

Neonatal sepsis (NS) still represents a major cause of neonatal death, affecting more than 2% of live births and about 16% of neonatal mortality at NICUs [1]. NS is challenging to diagnose because its manifestations are non-specific (e.g. respiratory distress, hypotension, and apnea) which could be presented in non-infectious conditions. Furthermore, the time to administration of antibiotics affects its outcome; therefore, there are both clinical and submissive encourages for identifying and treating neonates with sepsis rapidly [2,3]. After the above facts, clinicians ordinarily use serum biomarkers to evaluate inflammation and infection and to assess the risk of sepsis.

In contrast to late-onset sepsis (LOS), which refers to infection diagnosed from day 4 onward, where the source is either community-acquired or from the hospital environment, early-onset sepsis (EOS) refers to getting an infection in the first 72 hours of life with pathogens most likely acquired through the birth canal [4]. As vital as ever, precise biomarkers are required to aid in the quick and correct diagnosis of NS. The ideal biomarker should reveal a consistent and foreseeable trend in both antibiotic response and diagnosis. Additionally, it must be able to provide predictive information, be quick and simple to measure, and take a small amount of blood [5].

The conventional and accurate way for diagnosing NS is the removal of the causative bacteria from blood. The length of time it takes to arrive at a diagnosis is the biggest barrier to culture-based diagnosis. Recently, diagnostic procedures as acute phase reactants were employed. These procedures included the use of cell surface markers, granulocyte colony-stimulating factor, cytokines, molecular genetics, and molecular cell proteomics [6].

Acute phase reactants, such as haptoglobin, CRP, fibronectin, lactoferrin, and procalcitonin, are collections of endogenous peptides made by the liver in reaction to an infection or tissue injury [7].

Human monocytes and hepatocytes produce the hormone procalcitonin (PCT). After being exposed to bacterial endotoxin for 4 hours, it starts to climb, peaks after 6–8 hours, and stays elevated for at least 24 hours with a half-life of 25–30 hours. Procalcitonin cut-off (2.3 ng/ml) has a poor sensitivity of around (48%) for NS diagnosis but good specificity and PPV of (97% and 96%, respectively). According to a report, even in cases when blood cultures come back negative, antibiotic therapy should be prolonged because a procalcitonin level of more than 2.3 ng/ml suggests a significant likelihood of newborn sepsis. In most circumstances, it is not a simple tool to obtain for assay, albeit [8].

Apelin is expressed on the surface of numerous organs, including the endothelium, adipose tissue, GIT, brain, kidneys, and liver. Adipocytes from both mice and humans express and secrete apelin, a pro-inflammatory protein involved in the inflammation of arterial walls [9].

Pre-proadrenomedullin is pre-prohormone of 185 amino acids that is subsequently degraded into proadrenomedullin (ProADM), a 164-amino acid-

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peptide, through cleavage of the signal peptide [10]. ProADM is completely cleaved by peptidase enzyme, into 4 different peptides: Adrenomedullin, amino-terminal peptide of ProADM (PAMP), adrenotensin and mid-regional proadrenomedullin (MR-ProADM) [11].

Therefore, in the current study, we evaluated these biomarkers in NS and determine their value in diagnosis and their correlations with other routine investigations besides evaluation the incidence of the most common organisms associated with NS.

PATIENTS AND METHOD

This comparative study conducted over a period of one year at NICU in Minia University Hospitals on all admitted neonates with clinical signs and symptoms of sepsis at the time of admission.

This work was done, over a period of one year, at NICU in Minia University Hospitals into 60 neonates diagnosed as sepsis representing (*Group I*) who were further sub-grouped into *Group I-a* (Early Onset sepsis) including 36 neonates and *Group I-b* (Late Onset Sepsis) including 24 neonates. 30 apparently healthy neonates representing (*Group II*) as the control group without any manifestations of X or laboratory findings of sepsis.

Inclusion criteria: Any neonate with clinical symptoms and signs or laboratory data of neonatal sepsis as demonstrated in Griffin, Tollner and Hematological sepsis scores. All included neonates subjected to: full history including (antenatal, natal, postnatal history) and full clinical examination.

Routine laboratory investigations: Adequate venous blood samples, under complete aseptic precautions and before starting empirical antibiotics, taken from each neonate for routine complete blood count, CRP and blood culture using (BD™ BACTEC™ FX40 Automated Blood Culture System, Becton Dickinson, Ireland) and subcultures done for positive cases to identify the causative organism [identification and AST done by (VITEK-2, bioMérieux - USA)].

Separated sera were used for CRP by semi-quantitative latex agglutination (TECO DIAGNOSTICS, U.S.A) and the remaining serum was preserved at -80°c until determination of procalcitonin, apelin and proadrenomedullin by ELISA assay kits (Glory Science Co.,Ltd, China).

Ethical consent:

The study was approved by the Academic and Ethical Committee of Minia University. Acceptance of participation in the trial was contingent on the patient providing written informed permission. All procedures involving human subjects in this study have been performed in compliance with the principles outlined in the World Medical Association's Declaration of Helsinki on human research ethics.

Statistical analysis

Data collected and encoded using Microsoft Excel software. Data were then imported into Statistical Package for Social Sciences (SPSS version 20.0) software for analysis. Qualitative variables were presented in the form of frequencies and percentages, quantitative variables were presented in the form of means and standard deviations. Shapiro-Wilk test was used to determine if the data had a normal distribution, The normal distributed quantitative data were compared using Student's t-test. In contrast, the non-parametric data were analyzed by using Mann-Whitney. The Qualitative categorical variables were compared using Chi-square test. In order to assess the inter-relationships among quantitative variables and ranked ones, Spearman rank correlation was used. Partial correlation analysis was used to modify for the time and group effects. Receiver operator characteristic (ROC) curve plots are used to show how the true positive and false positive values of various cut-off points relate to one another. P value <0.05 was considered significant.

RESULTS

In this work, there was a highly significant difference in TLC in *Group I* (Septic Group) when compared with *Group II* (Control Group) (P-value <0.001). Platelets count was highly significant different when compared between both groups (P-value 0.001). As regard CRP results in *Group I* there were 17 (28.3%) neonates with negative CRP and 43 (71.7%) neonates with positive CRP results (**Table 1**). No statistically significant differences were found between *Group I-a* (EOS) and *Group I-b* (LOS) (**Table 2**).

Table (1): Routine laboratory investigations of the 2 studied groups:

Variable	Group I Neonatal sepsis (n=60)	Group II Control (n=30)	P-value
TLC (c/mm^3) Mean ± SD	11540 ± 2632.84	4926.66 ± 698.43	**<0.001***
Platelets (c/mm^3) Mean ± SD	196.380 ± 41.230	267.330 ± 53.170	**<0.001***
CRP -Ve +Ve	 17 (28.3%) 43 (71.7%)	 30 (100%) 0 (0%)	**<0.001***

* Significant difference at p value <0.05.

Table (2): Routine laboratory investigations in septic group (*Group I-a* and *Group I-b*):

Variable	Group I-a Early onset sepsis (n=36)	Group I-b late onset sepsis (n=24)	P-value
TLC (c/mm^3) Mean ± SD	10950 ± 2432.24	9866 ± 2312.5	0.154
Platelets (c/mm^3) Range	201.56 ± 48.122	243.150 ± 58.160	0.290
CRP -Ve +Ve	12 (33.3%) 24 (66.6%)	9 (37.5%) 15 (62.5%)	0.290

Regarding blood culture results in *Group I*, there was negative blood culture (No growth) in 28 neonates (46.7%) and positive results were in 32 neonates (53.3%). The common organisms revealed were staphylococcus aureus (11.7%), staphylococcus epidermidis (8.3%), E.coli (8.3%), Enterobacter (6.7%), Klebsiella (5%), streptococcus pyogens (3.3%), non-hemolytic streptococcus (3.3%), staphylococcus saprophyticus (1.7%), pseudomonas (1.7%), proteus (1.7%) and candida (1.7%) [***Table 3 and Figure 1***].

Table (3): Frequency of microorganisms revealed from blood cultures in *Group I*:

Blood culture	Group I (Neonatal sepsis) (n=60)
Blood culture Negative Positive	 28 (46.7%) 32 (53.3%)
Blood culture No growth	 28 (46.7%)
Staphylococcus aureus	7 (11.7%)
Staphylococcus epidermidis	5 (8.3%)
E.coli	5 (8.3%)
Enterobacter	4 (6.7%)
E.coli+ Klebsiella	3 (5%)
Streptococcus pyogens	2 (3.3%)
Non-hemolytic streptococci	2 (3.3%)
Staphylococcus saprophyticus	1 (1.7%)
Pseudomonas	1 (1.7%)
Proteus	1 (1.7%)
Candida	1 (1.7%)

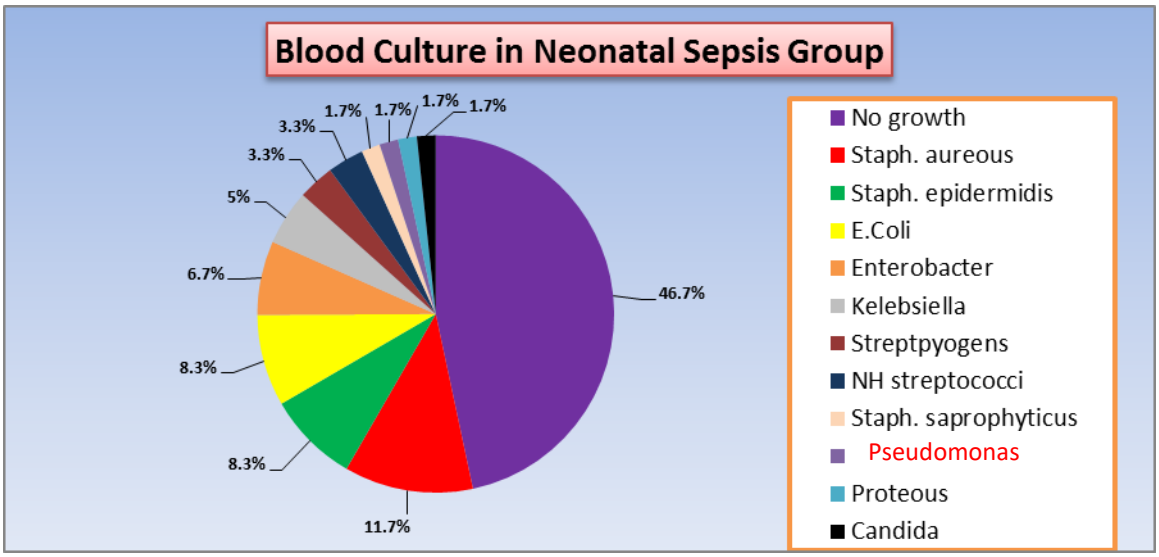

Figure (1): Frequency of revealed organisms from blood culture in *Group I*.

After evaluation of the levels of procalcitonin, apelin and proadrenomedullin in both groups it was shown that they were significantly higher in *Group I* (septic group) when compared with *Group II* (control group) with a P-value <0.001 (Table 4), while there was no significant differences when comparing their results in *Subgroup I-a* (EOS) and *I-b* (LOS) (**Table 5**).

Table (4): Comparison between *Group I* and *Group II* regarding apelin, procalcitonin and proadrenomedullin:

Variable	Group I Neonatal sepsis (n=60)	Group II Control (n=30)	P-value
Apelin (ng/dl) Mean ± SD	1992.1 ± 469.04	1151 ± 112.63	**<0.001***
Procalcitonin (pg/dl) Mean ± SD	157.73 ± 36.22	100.93 ± 4.11	**<0.001***
Proadrenomedullin (ng/L) Mean ± SD	304.4 ± 75.02	40.86 ± 9.66	**<0.001***

*Significant difference at p value <0.05.

Table (5): Apelin, procalcitonin and proadrenomedullin levels in *Group I-a* and *Group I-b*:

Variable	Group I		P-value
	Group I-a Early onset sepsis (n=36)	Group I b Late onset sepsis (n=24)	
Apelin (ng/dl) Mean ± SD	2056.69 ± 469.08	1895.21 ± 461.72	0.194
Procalcitonin (pg/dl) Mean ± SD	157.41 ± 35.39	158.21 ± 34.69	0.943
Proadrenomedullin (ng/L) Mean ± SD	308.27 ± 76.32	298.58 ± 68.18	0.673

*Significant difference at p value <0.05.

Procalcitonin had positive correlation with proadrenomedullin, TLC, platelets and CRP (**Table 6**).

Table (6): Correlation between procalcitonin and other routine parameters in *Group I*:

Variable	Procalcitonin (pg/dl)	
	r	P value
Proadrenomedullin (ng/L)	0.355	0.005*
TLC (c/mm^3)	0.255	0.049*
Platelets (c/mm^3)	-0.304	0.018*
CRP (mg/l)	0.407	0.001*

*Significant difference at p value <0.05.

Apelin had positive correlation with procalcitonin, TLC and platelets count (**Table 7**).

Table (7): Correlation between Apelin and other routine parameters in-group I:

	Apelin (ng/dl)	
	r	P value
Procalcitonin (pg/dl)	0.470	<0.001*
TLC (c/mm^3)	0.294	0.023*
Platelets (c/mm^3)	-0.355	0.005*
CRP (mg/l)	0.218	0.094

*Significant difference at p value <0.05.

Proadrenomedullin had positive correlation with only procalcitonin (**Table 8**).

Table (8): Correlation between Proadrenomedullin and other routine parameters in *Group I*:

Variable	Proadrenomedullin (ng/L)	
	r	P value
Procalcitonin (pg/dl)	0.355	0.005*
TLC (c/mm^3)	0.147	0.261
Platelets (c/mm^3)	-0.041	0.755
CRP (mg/l)	0.219	0.092

*Significant difference at p value <0.05.

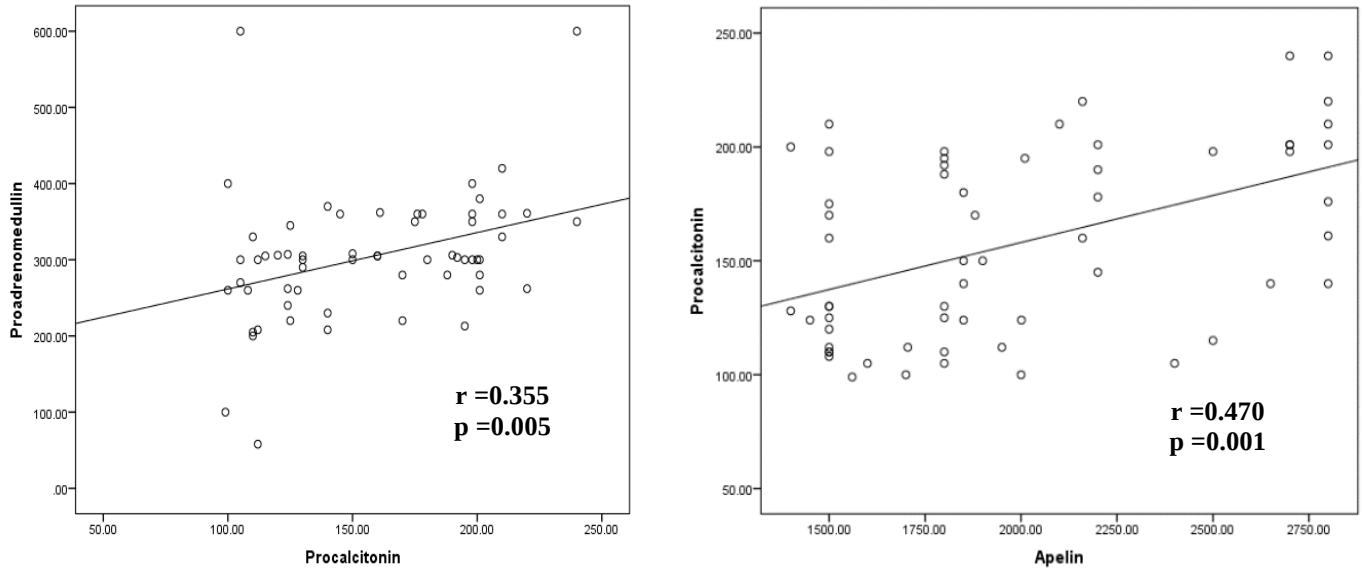

Figure (2): Correlation between procalcitonin and proadrenomedullin and apelin in *Group I*.

At a cut-off value of 1300 ng/dl for apelin, 110 pg/dl for procalcitonin and 70 for proadrenomedullin in *Group I-a*, it was found that apelin had higher sensitivity than procalcitonin and proadrenomedullin (100%, 88.8% and 97.2%) respectively (**Table 9**). Moreover, at the same cut-off value for the three biomarkers in *Group I-b*, it was found both apelin and proadrenomedullin have higher sensitivity than procalcitonin (100%, 100% and 75%, respectively) (**Table 10**).

There were higher levels of procalcitonin, proadrenomedullin and apelin in septic neonates both (EOS and LOS) with positive blood culture when compared with those of negative blood culture.

Table (9): Sensitivity, Specificity, PPV and NPV for procalcitonin, proadrenomedullin and apelin in EOS *Subgroup I-a*:

Variable	Optimal cutoff point	AUC	P value	Sensitivity	Specificity	PPV	NPV	accuracy
Procalcitonin (pg/dl)	>110	0.977	<0.001*	88.89	100	100	88.2	93.94
Proadrenomedullin (ng/L)	>70	0.994	<0.001*	97.22	100	100	96.8	98.48
Apelin (ng/dl)	>1300	1	<0.001*	100	100	100	100	100

Table (10): Sensitivity, Specificity, PPV and NPV for procalcitonin, proadrenomedullin and apelin in LOS *Subgroup I-b*:

Variable	Optimal cutoff point	AUC	P value	Sensitivity	Specificity	PPV	NPV	accuracy
Procalcitonin (pg/dl)	>110	0.936	<0.001*	75	100	100	83.3	88.89
Proadrenomedullin (ng/L)	>70	1	<0.001*	100	100	100	100	100
Apelin (ng/dl)	>1300	1	<0.001*	100	100	100	100	100

DISCUSSION

An early, sensitive, and specific laboratory test would enable newborn unit clinicians decide whether to start antibiotics in neonatal sepsis [12]. This study was conducted for a period of a year in NICU in Minia University hospitals at 60 neonates diagnosed with NS. Routine laboratory investigations such as TLC, platelets and CRP had been done besides the evaluation of serum apelin, procalcitonin and proadrenomedullin using ELISA. There was a significant difference when comparing TLC and platelet counts in septic group with that of the control group and these results were in agreement with **Marwa *et al.*** [12] and with other studies conducted on neonatal sepsis patients and found that neonates with bacterial sepsis have reduced platelet count and high I/T ratio [13-15]. However, show some differences with other studies as Da Silva *et al.* who found indecision among his results and postulated that the possible sources of heterogeneity were change in gestational age, different methodology used, variant reference values, different cut-off values and different analysis of test results by other investigators [16].

We also showed that newborns with clinically confirmed sepsis had markedly elevated CRP levels. The reliability of CRP as a tool to distinguish between healthy newborns and those with proven or suspected sepsis is proved by the many prior research that showed its higher level in NS [17,18]. This was consistent with those findings.

Blood culture is still the gold standard for the diagnosis of bacterial sepsis; however, its results may take much time, because of the expected low bacteremia, the small-sampled blood volume and early antibiotic therapy prior to blood culture withdrawal. Furthermore, hospital rules and standards recommend that it be collected in the case of a temperature surge to maximize its yield [19].

In the current study, it was found that 28 neonates representing (46.7%) of septic group with no growth blood culture. While 32 neonates representing (53.3%) of septic group with positive blood culture results, this percentage was similar to that revealed in an s study by **Shaw *et al.*** [20] who found that 54.64 % of his cases with show the similar positivity rate. The commonest microorganisms revealed from blood culture results were; staphylococcus aureus (11.7%) similar findings were found with **Sundaram *et al.*** [21] who revealed CoNs in (8.3%) of his cases, E.coli in (8.3%), Enterobacter in (6.7%), Klebsiella in (5%), streptococcus pyogens in (3.3%), Non-hemolytic streptococcus in (3.3%), staphylococcus saprophyticus in (1.7%), Pseudomonas in (1.7%), Proteus in (1.7%) and candida in (1.7%). Staphylococcal infection was the abundant type of infection and this may be due to horizontal transmission from colonized visitors or health care workers, poorly developed immune defenses, the requirement of central venous catheters, a prolonged total parenteral nutrition, and the use of steroids or antimicrobial agents.

In the present study, there were significant correlations between serum procalcitonin, apelin and proadrenomedullin with routine investigations done (TLC, platelets count and CRP). There was a positive correlation between proadrenomedullin and procalcitonin, while there was a negative correlation between proadrenomedullin and TLC, platelets & CRP. In addition, there was a significant correlation between apelin, procalcitonin, TLC and platelet counts in the septic group.

It was found that higher procalcitonin, proadrenomedullin and apelin levels in septic group (EOS and LOS) with positive blood culture results, without any significant difference, when compared with neonates in the septic group with culture negative and these results were similarly found with **ElMeneza *et al.*** [22] who found that serum apelin was significantly higher among culture positive patients than the culture-negative in their study.

This study shown that, when compared to the control group, the serum apelin level in the septic group increased. This was in line with the findings of **Gad *et al.*** [23]**,** who discovered that newborns with sepsis had serum apelin levels that were approximately 8 times higher than those of controls.

Our study also showed that serum proADM levels, were significantly higher in the septic group than in controls and these findings were in agreement with **Fahmey *et al.*** [24] who described serum proADM levels increase in sepsis for a variety of reasons, and the key was excessive proADM synthesis during sepsis.

In conclusion, measuring procalcitonin, apelin and proadrenomedullin levels are valid and can aid in the diagnosis of NS, but alone cannot be dependable for accurate diagnosis.

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