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Evaluation of Polymorphnuclear Leucocyte Elastase as Diagnostic Tool in Neonatal Sepsis

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ABSTRACT

Objectives: Neonatal sepsis is clinical syndrome of bacteremia with systemic signs and symptoms. Neonatal sepsis is still a leading cause of mortality in neonatal intensive care units all over the world. Early diagnosis and treatment of the newborn infant with suspected sepsis are essential to prevent severe and life threatening complications.

Objective: The aim of this study was to evaluate polymorph nuclear (PMN) leucocyte elastase as a diagnostic tool in neonatal sepsis.

Subjects and Methods: This study was conducted on 45 full term and preterm neonates suspected as neonatal sepsis compared to 45 apparently healthy neonate with matched age and sex, as a control group.

Results: 55.6% of our patients group were full-term patients and 44.4% were preterm. All the patients were subjected to full history taking, full clinical examination, lab investigation including CBC, CRP, blood culture and sensitivity, and measurement of serum polymorph nuclear leucocyte elastase enzyme.

Conclusion: Significant elevation of serum polymorph nuclear leucocyte elastase level in neonatal sepsis with high specificity.

Keywords: Polymorphonuclear leucocyte - Escherichia coli - Early onset sepsis - Late onset sepsis

INTRODUCTION

Neonatal sepsis is the cause of substantial morbidity and mortality. The clinical manifestation range from subclinical infection to severe manifestation of focal or systemic disease. The source of pathogen might be attributed to an utero-infection, acquisition from maternal flora, or postnatal acquisition from the hospital or community. The timing of exposure, the immune status, and virulence of the causative agent influence the clinical expression of neonatal sepsis [(1)].

The neonate immune response to sepsis is driven by increased expression of neutrophil and monocytes. By contrast there is net suppression of the adaptive immune response as characterized by a decrease in expression level by T and B cells [(2)].

Polymorphonuclear leucocyte elastase is major serine proteinase in man, which is secreted by neutrophils and macrophages during inflammation. It plays a role in degenerative and inflammatory disease by its proteolysis of collagen IV and elastin of the extracellular matrix [(3)].

Early diagnosis before obvious clinical signs of neonatal sepsis is an important goal, as delay in commencing antibiotics will result in serious consequences. On the other hand over treatment of neonates with antibiotics based on false positive result will promote the presence of multiresistant bacteria in neonatal intensive care unit [(3)].

AIM OF THE WORK

The aim of the work is the evaluation of the blood polymorphonuclear elastase as diagnostic tool in neonatal sepsis.

SUBJECTS AND METHODS

This cross sectional comparative study was conduced on ninety (90) neonates of both sexes from neonatal intensive care units, and from the well-baby department in Al–Zahraa University Hospital after approval of ethical committee of Al-Zahraa Hospital University for girls from June 2017 till December 2017.

Group I: Included forty five (45) full term and preterm neonates who were selected from the neonatal intensive care units, they were (15) females and (30) males, with early and late onset neonatal sepsis.

Group II: Included forty five (45) apparently healthy neonates, they were (23) females and (22) males as control group.

Inclusion criteria: Age: neonates; preterm or full-term from 0-28 days of both sexes. Neonates with early and late onset suspected sepsis. The clinical signs for diagnosis of neonatal sepsis include any of the following signs: respiratory rate > 60 breath/min., grunting, lethargic or unconscious, tachycardia, and convulsions (1).

Exclusion criteria: Neonates with major congenital anomalies or associated syndromes. All cases with different clinical presentation resembling neonatal sepsis (hypoxic ischemic encephalopathy necrotizing enterocolitis).

Methods: The study groups were subjected to the following: Full history taking, prenatal, natal and postnatal includes: Intrapartum fever > 38°C, premature rupture of membrane, mode of delivery, and invasive procedures as mechanical ventilation. Full clinical examination includes: Determination of gestational age using Ballard score (4), assessment of body weight, length and head circumference, clinical evidence of neonatal sepsis as: Lethargy, temperature

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instability, respiratory distress–apnea, tachycardia, bradycardia, and poor perfusion, gastrointestinal manifestation (vomiting, diarrhea, abdominal distension, hepatosplenomegaly), colors (Jaundice – Cyanosis – Pallor), bleeding tendency, convulsions, hypotonia, umbilical sepsis, and mottling. Newborn were diagnosed as suspected clinical sepsis based on their (hematological score ≥ 3 and Tollner score ≥ 10) (5).

Laboratory investigations including:

1- **Complete blood count:** Complete blood counts with differential was measured at (0) hour (time of sepsis evaluation) was performed on counter T890 (coulter counter, harpenden, UK).

2- **C-reactive protein:** Principle of CRP test: Serum CRP was determined using latex agglutination test.

3- **Blood culture and sensitivity testing:** 1 to 5ml of blood was drawn from venipuncture using sterile needle and then blood was injected in the blood culture bottle for bactec microbial detection system (Bactec 9050, Becton-Dickinson company, 1 Becton Drive, Franklin lakes, New Jersey). This is a closed automated system that uses a chemical sensor to detect increases in carbon dioxide production produced by the growth of microorganisms. The sensor is monitored every 10 minutes fro increased fluorescence, which is proportional to the amount carbon dioxide present.

4- **Polymorphonuclear leucocyte** elastase was determined by enzyme linked immunoassay (ELIZA):

Sample collection: A serum separator tube was used and samples were allowed to clot for two hours at room temperature before centrifugation for 15 minutes at 1000xg. Serum was removed and stored at -20°C.

Principles of assay: Principles of the assay employed the quantitative sandwich enzyme immunoassay technique.

Statistical Analysis

The quantitative data were presented as mean, standard deviations and ranges when their distribution found parametric and median with inter-quartile range (IQR) when their distribution found non parametric while qualitative data were presented as number and percentages.

The comparison between two independent groups with qualitative data was done by using Chi-square test and/or Fisher exact test only when the expected count in any cell found less than 5.

The comparison between two independent groups with quantitative data and parametric distribution was done by using Mann-Whitney test.

RESULTS

Table (1): Organisms of the patients group.

	No.	%
Candida ablicans	7	15.6%
E.coli	20	44.4%
Enterobacter	2	4.4%
Klebsiella	4	8.9%
Pseudomonas	3	6.7%
Staph aureus	6	13.3%
Staph epidermis	3	6.7%
Total	45	100%

Table (2): Comparison between control group and patients group regarding laboratory data

		Control group	Patients group	Fisher's exact tests	
		n = 45	n = 45	Chi2	p-value
CRP	Negative	45 (100.0%)	2 (4.4%)	82.340	<0.001
	Positive	0 (0.0%)	43 (95.6%)		

Table (3): Comparison between control group and patients group regarding PMN elastase (ng/ml)

PMN Elastase (ng/ml)	Control group	Patients group	Mann-Whitney test	
	n = 45	n = 45	Z	P-value
Median (IQR)	0.52 (0.17 - 0.74)	0.85 (0.37 - 3.44)	2.527	0.012
Range	0.06 - 2.82	0.08 - 6.4		

Table (4): Comparison between non survival and survival patients regarding PMN elastase (ng/ml).

Outcome	PMN elastase (ng/ml)		Mann-Whitney test	
	Median (IQR)	Range	Z	P-value
Non survival	5.20 (3.27 – 5.50)	3.17 – 6.4	3.492	<0.001
Survival	0.67 (0.26 – 1.54)	0.08 – 5.50		

Table (5): Relation between PMN elastase and CRP among patients group.

		PMN elastase (ng/ml)		Mann-Whitney test	
		Median (IQR)	Range	Z	P-value
CRP	Negative	0.11 (0.09 – 0.13)	0.09 – 0.13	-2.093	0.036
	Positive	0.85 (0.38 – 3.64)	0.08 – 6.4		

Table (6): Comparison between PMNL and type of organism.

Organism	PMN elastase (ng/ml)		Mann-Whitney test	
	Median (IQR)	Range	Z	P-value
Candia ablicans	0.37 (0.16 – 0.52)	0.09 – 5.4	14.221	0.027
E.coli	3.21 (0.9 – 4.36)	0.08 – 5.5		
Enterobacter	1.13 (0.12 – 2.13)	0.12 – 2.13		
Klebsiella	0.24 (0.16 – 0.55)	0.12 – 0.82		
Pseudomonas	0.9 (0.71 – 1.51)	0.71 – 1.51		
Staph aureus	0.65 (0.14 – 3.44)	0.1 – 6.4		
Staph epidermis	0.41 (0.13 – 0.43)	0.13 – 0.43		

Table (7): Comparison between non survival and survival patients regarding PMN elastase (ng/ml).

Outcome	PMN elastase (ng/ml)		Mann-Whitney test	
	Median (IQR)	Range	Z	P-value
Non survival	5.20 (3.27 – 5.50)	3.17 – 6.4	3.492	<0.001
Survival	0.67 (0.26 – 1.54)	0.08 – 5.50		

DISCUSSION

Neonatal sepsis, a clinical disorder developed by blood stream infections, is one of the serious global public health problems that must be addressed. More than one million of the estimated global new born deaths per year are occurred due to severe infections. The clinical diagnosis and treatment are highly complicated. Microbiological surveillance and assessment of anti microbial resistance is a key component decreasing the rate of neonatal sepsis and the associated mortality [(6)].

Extensive work is being performed to find the ideal test for early diagnosis of neonatal sepsis. There is still need for further research work to find an ideal test for early diagnosis of neonatal sepsis. Early diagnosis is important to guide the management of at risk neonates and those with suspected sepsis particularly in the face of limited bed space for neonatal care and the high cost of hospital care [(7)].

Polymorphnuclear elastase released from azurophilic granules of polymorphs plays an important physiological function in degrading phagocytosed substance and facilitating diapedesis excessive amounts of free un bound may result in degradation of the essential elements of the interstitium (elastin, collagen, proteoglycan) and decomposition of plasma proteins proteinase inhibitors, blood coagulation factors, immunoglobulins, the basal membrane of renal glomeruli, and the ciliary epithelium of the respiratory tract [(8)].

The aim of this work was to evaluate polymorph nuclear leukocyte elastase level and using it as biomarker for neonatal sepsis.

This study was conducted on 45 neonates suspected as neonatal sepsis 23 females and 22 males, compared to 45 health neonates 15 female and 30 males.

There was no significant difference between both groups as regarding age and this agrees with ***Ferreira et al.*** [(9)] who detected that infant gestational age < 37 with birth weight less than 1500 gm was not statistically significantly different compared to neonates without sepsis, also ***Xia et al.*** [(10)], in their study detected that age was an independent risk in neonatal sepsis.

Regarding the gender, there was no statistically significant difference between the gender distribution of the patient and the control group, which was in agreement with ***Conkar et al.*** [(11)] who detected this fact in their study.

On the contrary ***Moustafa and Ahmed*** [(12)] in their study revealed that males are more prone for septicemia than females due to the probability of sex linked factor in host susceptibility. The synthesis of gamma globulins is possibly regulated by X-linked immunoregulatory gene and as males are having one X chromosome so they are more prone for neonatal septicemia.

In the current study it was found that positive cultures were 100% in the sepsis group, and E.coli was the most affecting organism by 44.4%. This was agreed with ***Tsai et al.*** [(13)] who detected that E.coli is more common in premature infant and sepsis onset on the 1st day of life than non-E.coli, also ***Simonsen et al.*** [(14)]***; Shah and Padbwy*** [(15)]**,** revealed in their study that E.coli is the organism most frequently involved in early onset sepsis. More over ***Liaquat et al.*** [(16)] concluded that gram negative bacteria are the commonest cause of neonatal sepsis, and in their study they showed that E.coli is the most common gram negative bacteria causing neonatal sepsis.

As regard fungal infection in our current study it was (15.6%) and this was in agreement with ***Saha*** [(17)] who detected that C. albicams was by far the predominant species responsible for 60% of all cases of infection in late onset neonatal sepsis, also, ***King et al.*** [(18)] showed in his study that Candida represent the third most common cause of blood stream infections in neonates and children that result in significant mortality and death. Also ***King et al.*** [(18)] in their study detected that preterm and LBW are more vulnerable to invasive Candidiasis, and neonatal candidiasis acquired after 6 days of life, is the most common form of invasive Candidiasis in neonates.

Statistical significant increase was found in the level of CRP in the patients group versus the control group ($p < 0.001$) and this was agreed with ***Saleh et al.*** [(19)] whose study detected that CRP value was high in all cases while it was normal in control group. Also ***Monica et al.*** [(20)] detected in their study that CRP is good diagnostic and therapeutic tool and it has high sensitivity and good protective value. On the other hand ***Markic et al.*** [(21)] concluded that in spite that CRP are used in

the laboratory evaluation of patients with suspected infection or sepsis, however it is still not always possible to accurately predict the presence of bacterial infection on the basic of clinical appearance and those laboratory tests.

In our current study PMN elastase level the patients group was positively significantly different than in the control group (p= 0.012) and this agrees with ***Pessar*** [8] who concluded in her study that raised PMN level was found to be diagnostic and prognostic markers in neonates with sepsis. Also ***Pessar*** [8] detected in her study that PMN elastase is major indicator for early diagnosis of neonatal sepsis.

On the other hand ***Tagami et al.*** [22] showed that selective elastase inhibitor for treating acute respiratory distress syndrome didn't affect significantly 28 days mortality but it may improve the respiratory function.

Lastly in our current study there was positive statistically significant difference between PMN elastase in preterm than full-term patients and this agrees with ***Fang et al.*** [23], who detected that neutrophil elastase is predictor of single birth prematurity.

CONCLUSION

Significant elevation of serum polymorph nuclear leucocyte elastase level in neonatal sepsis. Significant elevation of serum polymorph nuclear leucocyte in relation with CRP. Significant elevation of serum polymorph nuclear leucocyte elastase with non survival patients than survival one. Significant elevation of serum polymorph nuclear elastase with E.coli and staph aureus organism.

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