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REVIEW ARTICLE

Assessment of The Role of Interleukin 6, C-Reactive Protein and high sensitive C-Reactive Protein in the Diagnosis of Early Onset Neonatal Sepsis (A clinical and Laboratory Approach.

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ABSTRACT

Background: Bacterial sepsis is a life threatening crisis with high mortality and morbidity in neonates, Reliable marker is needed for the diagnosis of neonatal sepsis. C-reactive protein (CRP), IL-6 can be used as Reliable markers for the diagnosis of neonatal sepsis so that early treatment could be achieved.

Aim of the work: to detect the levels of IL-6, CRP and HS-CRP in clinically suspected cases of neonatal sepsis and evaluation and analysis of these measurments as early markers of neonatal sepsis.

Patients and methods: A prospective cohort study was carried on 60 neonates at neonatal intensive care unit of pediatric department and clinical pathology department at Zagazig university hospitals.

Results: IL-6 at a cut off value of 187.5Pg/ml had sensitivity of 87.5% and specificity of 63.6% with a negative predictive value of 93.3%. HS-CRP at a cut off value of 3.3 Mg/L had a higher sensitivity but a lower specificity at 100% and 47.7% repectively. CRP showed higher specificity than HS-CRP and IL-6 with 61.7% with a negative predictive value of 90%. A combination of IL-6 and CRP showed improvement of sensitivity to 100% and a negative predictive value of 100% and is more cost effective than the combination of IL-6 and HS-CRP which showed similar sensitivity and negative predictive value but showed less specificity

Conclusion: a combination of IL-6 and HS-CRP would be a sensitive test for early diagnosis of neonatal sepsis at a sensitivity of 100% and a similar NPV, on the other hand a combination of IL-6 and CRP is less sensitive but more specific.

Key words: neonatal sepsis, CRP, HS-CRP, IL-6, NPV. Early diagnosis.

INTRODUCTION

Neonatal Sepsis is a significant cause of morbidity and mortality in the newborn, particularly in preterm, low birth weight infants, despite advances in neonatal care, infections remain common and sometimes life-threatening in neonates admitted to the neonatal intensive care unit (NICU) [1].

Neonatal sepsis is a significant cause of morbidity and mortality. It has non-specific signs and symptoms, hence makeing the diagnosis difficult. The routinely used laboratory tests are not effective methods of analysis, as they are extremely nonspecific and often cause mal-practice regarding use of antibiotics. Sepsis is an infectious state associated with a systemic inflammatory response with production and release of a wide range of inflammatory mediators [2].

Cytokines are inflammatory mediators with strong action and their levels are elevated during infections, so changes from other inflammatory effector molecules may occur. Although proinflammatory and anti-inflammatory cytokines have been identified as probable markers of neonatal infection in order to characterize the inflammatory response [3].

Bacterial sepsis is a life threatening crisis with high mortality and morbidity in neonates. Diagnosis of sepsis is still difficult due to non-specific clinical presentation. blood culture is the way for reaching diagnosis but it is time consuming. Reliable marker is needed for the diagnosis of neonatal sepsis so that early treatment can be initiated. Various cytokines, chemokines, acute phase reactants, cell surface markers and interferons have been evaluated to find out the effective marker for early diagnosis of neonatal sepsis [4].

According to time presentation, Neonatal sepsis may be categorized as early-onset or late-onset. In early-onset sepsis, 85% present within 24 hours, 5% present at 24-48 hours, and a smaller percentage present within 48-72 hours. Onset is most rapid in premature and preterm neonates [5].

C-reactive protein (CRP) is an acute phase reactant and is released from the liver after stimulation predominantly of IL-6 and other cytokines. During infection, CRP has both pro-inflammatory and anti-inflammatory effects as it mediates elimination of pathogens but also inhibits interaction between endothelial cells and leukocytes. Secretion is started 4 to 6 h after stimulation and reaches peak at 36 h [6].

A lot of studies discussed the role of Interleukin-6 (IL-6) as a cytokine in neonatal population. In infection, IL-6 precedes the increase in C-reactive protein and followed by TNF-α release. It is synthesized by mononuclear phagocytes, endothelial cells, fibroblasts, and the decidua, chorion, amnion, and trophoblast cells soon after stimulation by microbial products [7].

IL-6 acts as a signal in the activation of T cells, and it activates the secretion of antibodies by B cells and the differentiation of cytotoxic T cells. other cytokinesn are also stimulated, particularly TNF-α and IL-1β. IL-6 is an early marker in the diagnosis of neonatal sepsis, increasing several hours before the increase in C-reactive protein. The sensitivity of these tests when combined can reach values close to 100%, hence the clinical importance of these markers [8].

Highly sensitive CRP (hs-CRP) is more sensitive than the conventional CRP, hs-CRP assays measures the CRP levels lower than that measured by the conventional CRP assays. CRP can be used as a diagnostic marker of neonatal infection When measured with a high sensitivity analytic method. This is because newborns cannot produce sufficient amount of acute-phase proteins and so they respond to infection with a smaller increase in CRP compared to adults. It has also been demonstrated that hs CRP level below 1 mg/l provides increased sensitivity for neonatal infection [9].

SUBJECTS AND METHODS

This study is a prospective cohort study was conducted at neonatal intensive care unit of pediatric department and clinical pathology department at Zagazig university hospitals. Preterm and term neonates (less than 28 days of age) including males and females presented with both early and late onset sepsis were included in the study.

Infants of diabetic mother, intra utrine growth restriction babies, dysmorphic neonates with chromosomal anomalies, and neonates with history suggestive of perinatal asphyxia were excluded from the study.

60 neonates were enrolled in our study .they were studied for suspecting signs of sepsis, among these 60 neonates 40 showed signs of neonatal sepsis and 20 were considered as our control group, the suspected cases were studied by blood culture and further divided into diagnosed sepsis group with positive blood cultures ,and probable sepsis group which include neonates with positive signs of sepsis but had negative blood cultures. We then designed our study upon three groups.

- **Group A;** control group with no signs of sepsis (control)
- **Group B;** probable sepsis with +ve signs but –ve blood culture
- **Group C;** sure sepsis with +ve signs and blood culture

All included neonates in the syudy underwent full clinical examination, Routine laboratory investigations, and Specific laboratory tests which includes: Quantification of IL-6, Blood culture, Quantification of HS-CRP, and Quantification of CRP.

Written informed consent was obtained from all participants' parents. The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Administrative design:

Approval was taken from the insititutional review board, faculty of medicin, Zagazig university.

Statistical analysis

Data analysis was performed using the software SPSS(statistical package for the social science)version 20.qusantitative variables were described using their means and standard deviations.categorical variation were described using their absolute frequencies and to compare the proportion of categorical data, chi square test and fisher exact test were used when appropriate .

Kolmogrov-smirnov (distribution type) and Levene (homogeneity of variances) tests were used to verify assumptions for use in parametric test. To

compare means of two groups , independent sample t test was used when appropriate .nonparametric test(mann whitney) was used to compare means when data was not normally distributed and to compare medians in categorical data. To compare means of more than two groups , one way ANOVA was used for normally distributed data and kruskal wallis test was used for data which was not normally distributed . ROC curve analysis was used to asses the best cut off of studied parameters . the level statistical significance was set at 5% (*p<0.05)*. highly significant difference was present if $p \leq 0.001$.

RESULTS

Regaeding demographic characteristics of studied groups, of the 60 neonates included in the study of 40 were clinically suspected cases of sepsis admitted to NICU who met the inclusion criteria and were considered cases of which 31 were males and 9 were females with age range of 1 to 28 days and the other 20 neonates were normal and healthy that were considered controls of which 14 were males and 6 females with age range 1 to 28 days.

There is significant difference between studied cases and control regarding weight and personal order (fourth baby significantly has sepsis while the second less liable to develop sepsis). There are non-significant difference between them regarding gestational age, gender or consanguinity (table. 1).

There are statistically non-significant differences between case and control groups regarding history of traumatic delivery, UTI, chorioamnionitis, PROM, type of delivery, APGAR score, history of resuscitation, ETT, central line introduction, and prematurity.

Regarding Clinical picture of studied groups according to diagnosis of sepsis. There are statistically significant differences between case and control groups regarding presence of lethargy and pustules, hypothermia, hypoglycemia, pallor, metabolic acidosis and feeding intolerance. While there are no significant differences between them regarding other symptoms or signs (table. 2).

There is statistically significant difference between case and control groups regarding result of blood culture (table. 3).

Table.1: Demographic characteristics of studied group

	Sepsis group	Control group	P
Weight (KG):			
Mean±SD	2.1 ± .07	2.56 ± 0.85	0.03
Range	0.88 – 3.7	1.2 – 3.8	(S)
Gestational age:			
Mean±SD	35.4 ± 3.03	35.5 ± 3.28	0.908
Range	29 – 40	28 - 38	(NS)
Gender:			
Male	31 (77.5)	14 (70)	0.527
Female	9 (22.5)	6 (30)	(NS)
Personal order:			
First	18 (45)	8 (40)	
Second∞	4 (10)	7 (35)	0.043
Third	11 (27.5)	5 (25)	(S)
Fourth∞	7 (17.5)	0 (0)	
Consanguinity:			
Positive	34 (85)	19 (95)	0.407
Negative	6 (15)	1 (5)	(NS)

Table.2: Clinical picture of studied groups according to diagnosis of sepsis.

	Sepsis group	Control group	P
RD grade:			
0	7 (17.5)	2 (10)	
1	1 (2.5)	0 (0)	0.725
2	10 (25)	7 (35)	(NS)
3	13 (32.5)	8 (40)	
4	9 (22.5)	3 (15)	
Lethargy:			
Absent	8 (20)	18 (90)	<0.001
Present	32 (80)	2 (10)	(HS)
Irritability:			
Negative	34 (85)	15 (75)	0.481

	Sepsis group	Control group	P
Positive	6 (15)	5 (25)	(NS)
Hypotherma:			
Negative	19 (47.5)	18 (90)	0.002
Positive	21 (52.5)	2 (10)	(S)
Fever:			
Negative	36 (90)	18 (90)	1
Positive	4 (10)	2 (10)	(NS)
Seizures :			
Negative	37 (92.5)	20 (100)	0.554
Positive	3 (7.5)	0 (0)	(NS)
Meningitis			
Negative	39 (97.5)	20 (100)	1
Positive	1 (2.5)	0 (0)	(NS)
NEC			
Negative	39 (97.5)	20 (100)	1
Positive	1 (2.5)	0 (0)	(NS)
Feeding intolerance			
Negative	9 (22.5)	15 (75)	<0.001
Positive	31 (77.5)	5 (25)	(HS)
Hypoglycemia			
Negative	27 (67.5)	20 (100)	0.003
Positive	13 (22.5)	0 (0)	(S)
Hyperglycemia			
Negative	40 (100)	20 (100)	
Positive	0 (0)	0 (0)	
Metabolic acidosis			
Negative	14 (35)	17 (85)	<0.001
Positive	26 (65)	3 (15)	(HS)
Jaundice :			
Negative	19 (47.5)	9 (45)	0.855
Positive	21 (52.5)	11 (55)	(NS)
Overt shock:			
Negative	35 (87.5)	20 (100)	0.159
Positive	5 (12.5)	0 (0)	(NS)
Pallor:			
Negative	15 (37.5)	16 (80)	0.003
Positive	25 (62.5)	4 (20)	(S)
Cardiogenic shock:			
Negative	31 (77.5)	15 (75)	1
Positive	9 (22.5)	5 (25)	(NS)
Hepatomegaly:			
Negative	31 (77.5)	18 (90)	0.307
Positive	9 (22.5)	2 (10)	(NS)
Bleeding:			
Negative	35 (87.5)	18 (90)	1
Positive	5 (12.5)	2 (10)	(NS)
DIC:			
Negative	35 (87.5)	20 (100)	0.159
Positive	5 (12.5)	0 (0)	(NS)
Osteomyelitis:			
Negative	40 (100)	20 (100)	
Positive	0 (0)	0 (0)	
Pustules:			
Negative	20 (50)	9 (45)	0.715
Positive	20 (50)	11 (55)	(NS)

	Sepsis group	Control group	P
Renal failure:			
Negative	38 (95)	20 (100)	0.548
Positive	2 (5)	0 (0)	(NS)
Apnea:			
Negative	29 (72.5)	16 (80)	0.753
Positive	11 (27.5)	4 (20)	(NS)

Table.3: Blood culture of studied groups.

	Sepsis group	Control group	P
Blood culture			
Negative	24 (60)	20 (100)	<0.001
Positive	16 (40)	0 (0)	(HS)

Table. 4: Inflammatory markers of studied groups.

	Sepsis group	Control group	MW	P
	Mean ± SD	**Mean ± SD**		
CRP(mg/l)	85.06 ± 77.67	4.81± 8.07	-5.531	<0.001 (HS)
HS_CRP(mg/l)	6.2 ± 1.8	0.59± 0.14	-6.277	<0.001 (HS)
IL 6(Pg/ml)	213.63 ± 34.42	94 ±36.3	-6.191	<0.001 (HS)
Procalcitonin(Ng/ml)	7.24 ± 28.05	0.48 ± 0.15	-5.296	<0.001 (HS)

Table.5: Performance of combined IL 6 and HS-CRP in early detection of neonatal sepsis in studied cases:

IL 6 and HS_CRP		Sepsis		Total
		Present	**Absent**	
	Positive	16	24	40
	Negative	0	20	20
	Total	16	44	60

Table.6: Performance of combined IL 6 and HS-CRP in early detection of neonatal sepsis in studied cases

	Value
Sensitivity	100
Specificity	45.5
PPV	40
NPV	100
+LR	1.83
-LR	0
Accuracy	60

Table.6: Performance of combined IL 6 and CRP in early detection of neonatal sepsis in studied cases

IL 6 and CRP		Sepsis		Total
		Present	**Absent**	
	Positive	16	25	40
	Negative	0	19	20
	Total	16	44	60

	Value
Sensitivity	100
Specificity	43.2
PPV	39
NPV	95
+LR	1.76
-LR	0
Accuracy	58.3

Table.7: Performance of combined IL 6, CRP and HS-CRP in early detection of neonatal sepsis in studied cases.

IL 6 and HS_CRP		Sepsis		Total
		Present	**Absent**	
	Positive	16	26	40
	Negative	0	18	20
	Total	16	44	60

	Value
Sensitivity	100
Specificity	40.9
PPV	38.1
NPV	90
+LR	1.69
-LR	0
Accuracy	56.7

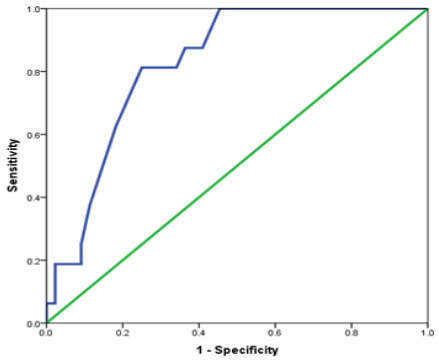

Figure (1): Performance of IL-6 in early detection of neonatal sepsis in studied cases

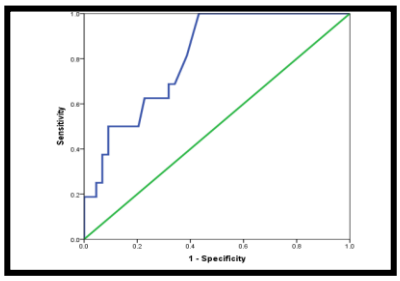

Figure (2): Performance of HS_CRP in early detection of neonatal sepsis in studied cases

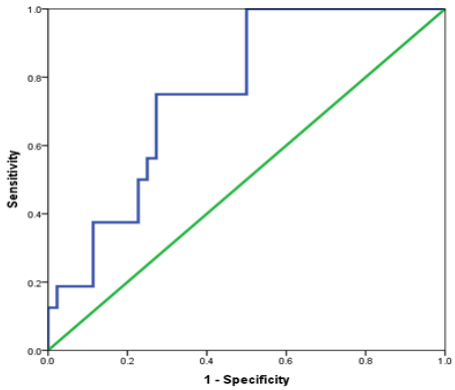

Figure (3): Performance of CRP in early detection of neonatal sepsis in studied cases.

DISCUSSION

Neonatal sepsis is still considered one of the major causes of mortality in neonates. The blood culture is considered the gold standard of neonatal sepsis but it is time consuming and often negative due to inadequate sampling [10]. In this study the cytokine IL-6 and the most commonly used acute phase reactant CRP as well as HS-CRP were evaluated to find out which of the above markers alone or in combination would be reliable and a better

predictor of neonatal sepsis, especially early onset neonatal sepsis. The markers showed significant difference between the cases and the control groups. Blood culture was positive only in 16 out of 40 neonates in clinically suspected cases of neonatal sepsis. the most common organisms isolated were Klebsiella species (56.3%). A study done by **PrasHant et al** [11]. showed that Klebsiella species was the most common organism. CRP is the most extensively studied and commonly available laboratory test used for the diagnosis of neonatal sepsis ,its delayed response in the beginning of inflammation process accounts for low sensitivity in the beginning of neonatal sepsis but it is a good indicator in serial measurements [11].

In our study, the serum C-reactive protein (CRP) level was significantly higher in the clinically suspected neonatal sepsis groups than the control group. A study done by **Prashant A et al [11].** showed that CRP has high sensitivity (above 90%) which correlates with our study results that also suggest the high sensitivity of C-reactive protein (CRP) level . A study of **Ganesan et al [4].** suggested that at a cut off value of 13.4 CRP holds a sensitivity of 80% which is similar to the results in our study.

A study by **Ganesan et al [4]** stated that The HS-CRP value <0.5mg/l indicates no risk of infection, the value 0.5-1mg/l indicates low risk, the value 1-3 mg/l indicates average risk and the value >3 mg/l indicates high risk of infection in neonates ,this consists with our study in which we conclouded the cut –off value for HS-CRP as 3.3 with sensitivity of 100% and a similar NPV. The serum level of highly sensitive C-reactive protein (hs-CRP) was significantly higher in the clinically suspected sepsis group than in the control group in our study and it's also stated in the study by **abdollahi et al** [13]. .in our study the HS-CRP was also higher in sure sepsis group than in probable sepsis group. HS-CRP is more sensitive than the conventional CRP as it can measure very low levels of CRP. Similar results were observed in a study done by **abdollahi et al** [13].

In our study HS-CRP has very low specificity and positive predictive value than the other markers in the diagnosis of neonatal sepsis. HS-CRP is used mostly to evaluate the cardiovascular risk and it can also be used as the prognostic indicator in patients with acute coronary syndrome. A study by **Rifai et al** [14]. stated the high coeffiecient index of HS CRP in evaluating the heart and coronary risk in seemingly healthy individuals. Also in the study by **Ganesan et al [4]**, it stated the low specificity of HS-CRP in relation to other markers such as IL6 and CRP which correlates with our study.

In our study the serum level of interleukin-6(IL-6) is significantly higher in the clinically suspected cases of neonatal sepsis group than the normal healthy controls. Its also suggested in our study that the levels of IL6 is higher among sure sepsis group than probable sepsis group and both are higher in relation to our control group. A study by by **Messer et al [15].** stated that at a cut off value of 100 pg/dl or greater, IL6 obtained after 12 hours of birth has a high sensitivity and specificity for neonatal sepsis which correlates with our study. in this study by messer et al it was suggested that a combination of IL6 and CRP would be usually useful which we proved in our study.

In the study by **Ganesan et al [4].** the sensitivity of IL6 was 100% which was higher than CRP as we stated at our study, it also had better specificity than other markers as we concluded. But it showed different sensitivity of the marker than our study at a different cut off value .this variation may be due to differences at the age of the neonates at the beginning of sepsis or absence of a clear cut off value for IL6.

In our study the specificity of IL-6 is 62% which correlates with the study done by **Ganesan et al [4].** Our study also shows a negative predictive value of 100% which almost correlates with study done by **Messer et al [15]. and Ganesan et al [4].**

CONCLUSION

The overall accuracy of IL6 is higher than other studied markers with sensitivity of 87.5% ,specificity of 63.6% and a NPV of 93.3%. HS-CRP is more sensitive than CRP but less specific with a similar overall accuracy. Conventional CRP is a sensitive test for neonatal sepsis with average specificity at 61.7 % and a NPV of 90%. a combination of IL-6 and HS-CRC would be a sensitive test for early diagnosis of neonatal sepsis at a sensitivity of 100% and a similar NPV on the other hand a combination of IL-6 and CRP is less sensitive but more specific. In case of neonates with unclear infectious status, the assay these sepsis markers will be very helpfull. These markers can be reliably used only if there is a standardization of inclusion, exclusion criteria, cut-off values and the methods used for quantification of the markers.

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