Study of Serum Presepsin and Amyloid-A as Biomarkers Predictor in Newborn Suspected to Neonatal Sepsis

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Abstract

Background: Infections are chief reasons of morbidity and mortality in neonates and known contributor for morbidity and mortality. One of most common and widely spread infection, is neonatal sepsis as it is the main reasons for hospitalization of newborns and responsible for 30- 50% of annual neonatal deaths in developing countries. Early diagnosis of neonatal septicemia is essential to initiate accurate antimicrobial therapy and currently available diagnostic tools are inadequate.

Aim: To investigate the role of presepsin and SAA as simple applicable tests for early prediction of newborn suspected to sepsis against sepsis scoring system.

Method: This is a case control study comprising 90 neonates with gestational age ≥34 weeks; 60 neonates and 30 controls. Presepsin, SAA, Check list of infant's presenting symptoms or signs and laboratory data were evaluated and recorded on first and third days of admission for neonates while were measured once on admission for controls.

Results: Serum presepsin and SAA levels significantly higher in patients than controls and were detected earlier than both clinical and laboratory data.

Conclusion: This study revealed that serum levels of Presepsin and SAA were accurate and highly sensitive and specific markers for the prediction and diagnosis of early onset sepsis in comparison to clinical and laboratory data. Recommendation: Presepsin and SAA are innovative combined biomarkers that have a highly predictive value in the diagnosis of early neonatal sepsis.

Keywords: Neonatal sepsis, Presepsin, Serum Amyloid A, sepsis scoring system.

برسبين وأميلويد A فى مصل الدم كمؤشرات حيوية مقابل التنبؤ السريري في تشخيص تسمم الدم فى حديثى الولادة

الخلفية: يعتبر تسمم الدم فى حديثى الولادة من أخطر الحالات المرضية والتى تهدد الحياة محدثة معدلات ملحوظه فى كلا من الإعتلال والوفيات. ولذلك يعد سرعة ودقة كلا من التشخيص وبداية والعلاج أمر أساسي لتحسين النتائج المرجوه. برغم أن انخفاض معدل الوفيات هو التحدي الرئيسي في طب الطوارئ إلا أن كلا من تحاليل الدم التى تستخدم فى تشخيص تسمم الدم والعلامات السريرية كلاهما غير دقيق. لذا كان لازاما من وجود مؤشر حيوى دقيق يساعد على التشخيص فى أسرع وقت ممكن. والبرسبين يعدوا أكثر المؤشرات الحيويه الواعدة فى التشخيص المبكر لتسمم الدم.

الهدف: مقارنة كلا من التحاليل الطبية مع العلامات السريرية لمعرفة أيهما أكثر دقة ويمكن استخدامه للتنبؤ بتسمم الدم فى حديثى الولادة.

الحالات وطرق البحث: هذة الدراسة هى دراسة مقطعية مقارنة، أجريت بالتعاون بين كلية الدراسات العليا، جامعة عين شمس، المركز القومي للبحوث. لقد أجريت هذه الدراسة على عدد ٩٠ حديثى ولادة فى الفترة من ٢٠١٨- ٢٠١٩ فى وحدات الرعاية المركزة للمبتسرين (الحضانات) فى مستشفى الوراق المركزى (مستشفى حكومى) وقد خضعت كلتا المجوعتين إلى التاريخ المرضى يجب مراجعة تاريخ الأسرة والأمومة والحمل والولادة وبعد الولادة والتاريخ الاجتماعي وأيضا وزن الولادة ونوع الولادة (طبيعى أم قيصرى). أحداث الولادة والأمراض النسائية مثل: تمزق الأغشية المحيطه بالجنين لفترات طويلة وحمى الأمهات المحيطة بالولادة أو العدوى المستمرة. الإنعاش عند الولادة بحقنية أو أنبوب أمبو، تعرض الجنين لأى مضاعفات أثناء الولادة.

الفحص الإكلينيكى: فحص إكلينيكى شامل لأستبعاد العيوب الخلقية، وفحوصات معملية تشمل نظام تسجيل للمعين للدم HSS، وفحوصات معمليه أخرى (صورة دم- البروتين التفاعلى- SAA- برسبين.

النتائج: ارتفاع HSS بشكل ملحوظ فى مجموعة المرضى عن المجموعة الضابطة وكذلك في مجموعة المرضى بعد ٧٢ ساعة من احتجازهم فى الحضانات (المحضن)، وكان كل من بريسيبين وSAA مفيدا فى تشخيص تسمم الدم كما هو مبين فى الفروق ذات دلالة إحصائية بين الحالات والمجموعة الضابطة. العلامات السريريه كانت ذات دلالة إحصائية فى مجموعة المرضى بعد ٧٢ ساعة من احتجازهم فى الحضانات (المحضن) ولكنها لم تكن ذات قيمة فى بداية التسمم حيث فقدت نسبة من الأطفال المصابة.

الخلاصة: بريسيبين وSAA أكثر دقة وكفاءة فى التنبوء بتسمم الدم فى حديثى الولادة عن العلامات السريرية.

الكلمات المفتاحية: بريسيبين- SAA- الألتهاب- تسمم الدم.

Introduction:

Sepsis, has been considered as a worldwide public health hazard and its defined as a life- threatening organ dysfunction resulting from the host reaction to infection, Neonatal sepsis has several other definitions most common one is, it a systemic inflammatory response that mainly results from bacterial infection in the first month of life (Singer et.al., 2016). In 2014 in Eastern Mediterranean region (EMR) aimed to compare between countries with consideration of the similarities in the geographical location, tradition and culture, Data that were qualified to neonatal mortality (NM) were collected and categorized into social, economic, demographic and perinatal health care (WHO; World Health Statistics., 2015). That's confirmed what said that Egypt, incidence of neonatal sepsis in at risk neonates was 59% in the study of Elwan and Zarouk (2009). Simply neonatal sepsis diagnosis can be divided into clinical and laboratory diagnosis and because the initial diagnosis of sepsis is, by necessity, a clinical one, it is crucial to begin treatment before the results of cultures are available. Clinical signs and symptoms of sepsis are nonspecific, and the differential diagnosis is broad. (Shane et.al., 2017). Early diagnosis of neonatal sepsis gets better prognosis by discovering novel biomarkers become essential (Oeser et.al., 2020). Therefore, a single biomarker is not adequately dependable for diagnosis of neonatal sepsis; so it is necessary to combine different biomarkers to reach conclusions. Two of the most recently discovered biomarkers are Serum Amyloid A (SAA) and Presepsin (Ahmadizar et.al., 2017). Serum amyloid A (SAA) group of polymorphic apolipoproteins, also an acute phase reactant which mainly produced by the liver, have been proposed as a new diagnostic marker of bacterial infection. SAA was shown as a helpful biomarker for the diagnosis and treatment of acute diseases (bacterial, viral, traumatic…) and neonatal sepsis (Özkan et.al., 2019). Presepsin or soluble CD14 subtype, is a trunked portion of soluble CD14, which is released by shedding from the surface of various immune cell lines, such as macrophages, monocytes, and neutrophils, after its stimulation by pathogens, presepsin has recently been demonstrated to be a reliable diagnostic and prognostic marker of sepsis, distinguishing it from non-infectious diseases and the arrangement into severity degrees (Pizzolato et.al., 2014).

Aim:

To investigate the role of presepsin and SAA as simple applicable tests for early prediction of newborn suspected to sepsis against sepsis scoring system.

Ethical Consideration:

The current study was approved by ethical committee of both faculty of Postgraduate Childhood Studies, Ain Shams University and National Research Center, then informed written consent was obtained from the parents after explanation of the aim of the study and its possible benefits for early diagnosis by new markers instead of classic laboratory method.

Subjects& Methods:

In collaboration with faculty of Postgraduate Childhood Studies, This case control study was carried out at the neonatal intensive care unit (NICU) in both 6 October Insurance Hospital and El- Warrak Central Hospital (governmental), Ain Shams University from June 2017 to May 2018. The study was conducted on 90 neonates with gestational age ≥34 weeks. From which, 60 newborns with suspicion of early onset sepsis either clinically or laboratory with Griffin Neonatal Sepsis Score, considered as patient group and 30 apparently healthy newborns were chosen as controls.

- Inclusion Criteria: The maternal criteria included intrapartum fever, urinary tract infection, premature rupture of membrane (PROM) (>8 h). Sepsis screen was done in neonates with presence of more than or equal to two risk factors regardless mode of delivery, number or sex. Sepsis was diagnosed by Griffin Neonatal Sepsis Score or either clinically by Tollner clinical sepsis score (Tollner, 1982) or labrotrary by Hematological sepsis score (HSS) (Rodwell et.al., 1988) in which Total WBC: ≤5000/ mm³, Total PMN count: ≤7800- 14500 cells/mm³Immature PMN count: ≥1440 at first 60h, Immature/ Mature Polymorph: ≥0.3 Immature/ Total WBC: ≥0.2 Degenerative changes of WBC: vacuolization, toxic granulation& Dohle bodiesm Platelet count: <150.000/mm³. Total score is 7, Score ≥3 suggestive of sepsis. While clinical scoring System Total score 7, Score > 2 suggestive of sepsis. (Tollner, 1982).

Date Of Examination	Score	D1	D2	D3	D4	D4	D5	D6
Apnea, retraction, grunting, cyanosis	1							
Brady, tachycardia, hypotension, poor perfusion	1							
Seizures	1							
Abd- distension, prefed- residual	1							
Irritability, lethargy, poor fed	1							
Hepatomegly, Splenomegly	1							
Hyperthermia Or Hypothermia	1							
Total Score	7							

- Exclusion Criteria: Neonates with traumatic tissue injury, laboratory findings suggestive of inborn errors of metabolism and congenital anomalies, history of perinatal and postnatal asphyxia.

Statistical analysis:

All statistical analysis was performed using statistical software SPSS (Statistical Package for Social Science) statistical program (version 16.0). Graphs were done using SPSS statistical program (version 16.0) and Microsoft Excel program (version 2016).

Results:

Table (1) Comparison between patients and controls as regards clinical and laboratory data (on admission or D1).

		Groups		P- Value
		Patients N= 60	Controls N= 30	
Clinical Score	<2	41 (68.30%)	30 (100%)	0.001
	>2	19.00 (31.70%)	0 (0.00100%)	
HSS	<3	47 (78.3%)	30 (100.0%)	0.015 *
	>3	13 (21.7%)	0 (0.0%)	
PRES	86.26 ± 32.74	36.83 ± 9.86	0.001 **	
SAA	213.38 ± 55.89	117.91 ± 24.14	0.001 **	

- Clinical Scoring System after 72 hours: Comparison between patients

and controls as regard clinical manifestation revealed that was high statistically significant difference between admission and after 72 hours.

Table (2) Comparison between patients as regards clinical data at admission and after 72 hours.

	N	Min.	Max.	Mean± S.D.	Mean Difference	95% Ci	P Value
Clinical Scoring (At Admission)	60	1	5	2.70 ± 1.50	1.52	0.88-2.16	0.001
Clinical Scoring (After 72 hrs)	60	1	6	4.22 ± 1.63			

Table (3) Comparison between patients and controls as regard clinical data:

		On Admission		Control		P- Value
		N	%	N	%	
RD		28	100.0%	0	0.0%	-
Lethargy		22	100.0%	0	0.0%	-
Poor Moro Reflex		30	100.0%	0	0.0%	-
Food Tolence	Interbrated	28	100.0%	0	-	-
	Tolerated	32	76.2%	10	23.8%	0.001 **
Abd. Distention		22	100.0%	0	0.0%	
Hypothermia		40	100.0%	0	0.0%	-
Fever		20	100.0%	0	0.0%	-
Apnea		16	100.0%	0	0.0%	-
Tachycardia		22	100.0%	0	0.0%	-
Bradycardia		10	100.0%	0	0.0%	-
PROM		40	80.0%	0	0.0%	-
Maternal Fever		37	88.1%	5	11.9%	0.001 **
Ambo Bag		42	100.0%	0	0.0%	-

While comparing clinical data between patients revealed that other clinical data were as important as respiratory and food tolerance while were no statistically significant differences in the abdominal distention, tachycardia, bradycardia and hypothermia between patients at admission and after 72hr, they were arranged as below: Fever represented the first clinical presentation of neonatal sepsis after 72hr by (72%) while on admission it only represents (27.8%). Lethargy represented the second clinical presentation after 72hr by (69%) while on admission it only represents to (30.6%). In contrast, tolerated food was highest on admission (72.7%) and lowest after 72hr by (27.3%). Apnea was in the third rank as first clinical presentation after 72hr by (66.0%) while on admission it only represents (34.0%). Poor moro reflex and RD came in the fourth rank by (62.5%) and (62.2%) respectively, after 72hr by (72%) while on admission it only represents (37.5%) and (37.8%) respectively.

Table (4) Comparison between clinical finding among septic group at admission and after 72 hours.

		At Admission		After 72 Hrs		P- Value
		N	%	N	%	
R. D		28	37.8%	46	62.2%	0.036 *
Lethargy		22	30.6%	50	69.4%	0.001 **
Poor Moro Reflex		30	37.5%	50	62.5%	0.025 *
Food Tolerance	Intolerated	28	60.9%	18	39.1%	0.140
	Tolerated	32	72.7%	12	27.3%	0.003 **
Abd Distention		22	47.8%	24	52.2%	0.768
Hypothermia		40	51.3%	38	48.7%	0.821
Fever		20	27.8%	52	72.2%	0.001 **
Apnea		16	34.0%	31	66.0%	0.029 *
Tachy Cardia		22	50%	22	50%	1.000
Brady Cardia		10	50%	10	50%	1.000

Table (5) Correlations of clinical data between patients as regard biomarkers (Presepsin and SAA).

Parameters	Spearman Correlation Coefficient	P Value
Clinical Scoring with SAA (At Admission)	- 0.126	0.336
Clinical Scoring with SAA (After 72 hrs)	0.746**	0.000
Clinical Scoring with PRES (At Admission)	- 0.278	0.132
Clinical Scoring with PRES (After 72 hrs)	0.392**	0.002

**P- value is highly significant at 0.01 level. *P- value is significant at 0.05 level. PRES: presepsin. SAA: Serum amyloid A.

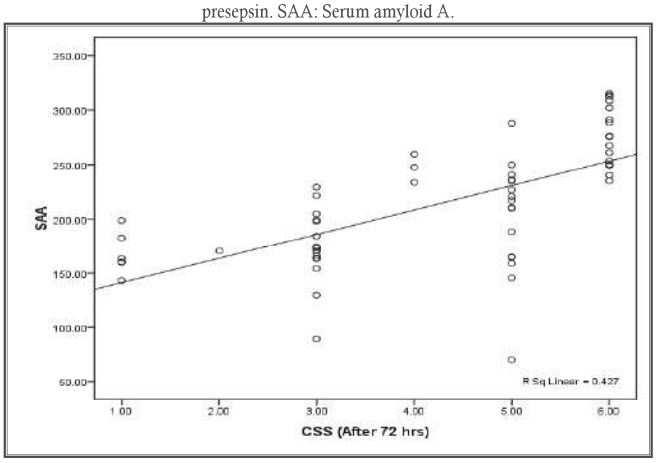

Figure (1) Correlations between Clinical Scoring System and SAA.

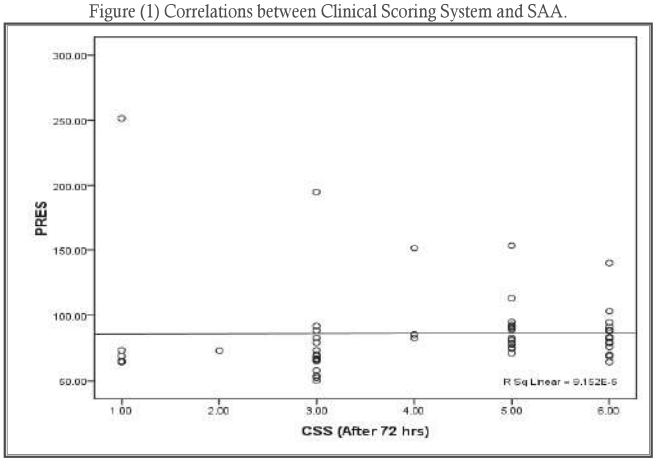

Figure (2) Correlations between Clinical Scoring System and Presepsin.

Discussion:

In current study, clinical assessment of our septic group, there were statistical significant difference on admission and second evaluation as shown in Table (4) The clinical manifestations were ranked as follow: on admission hypothermia was the first clinical presentation by 51.3% while in second position both (Respiratory Distress) RD and poor Moro reflex with minute difference by 37.8%, 37.5% respectively, in the third rank apnea by 34.0% finally lethargy with 30.6%. This agrees with Chan et.al. (2013) who found that temperature instability (Hypo or hyperthermia), poor feeding, followed by respiratory system manifestations: apnea, dyspnea, tachypnea, retractions, flaring, grunting came before gastrointestinal manifestations: abdominal distension, vomiting, diarrhea and hepatomegaly. This agrees with Lutsar et.al. (2014) who confirmed that hypothermia or fever came in the first place followed by: poor sucking, lethargy and RD present before tachycardia or bradycardia, hypoglycemia. In contrary to Hedegaard et.al. (2015) who discovered that RD as a first presentation of sepsis occurring in the form of apnea, mild tachypnea and severe RD requiring mechanical ventilation (MV) occurs in 90% of infants with sepsis. Before illustrating more similar or dissimilar

opinions of clinical feature, misfortune most of publication neglect the arrangement of clinical score and discuss the whole score or concentrate on respiratory and gastro- intestinal data but this concentration is very misleading as in this study gastro- intestinal manifestation in form of tolerated food was highest on admission (72.7%) and lowest after 72 hr by (27.3%), while the percentage of abdominal distention was 47.8% on admission and 52.2% after 72 hr. This agree with Izquierdo et.al. (2017) who discovered that abdominal distension was found in 45% of neonatal sepsis cases and progress with progression of the disease. They explained that by NEC or toxic ileus which was reported as a frequent association of neonatal sepsis.

In our study, we should notice that progression of sepsis accompanied by progression of some clinical presentations which were minimal or even not noticeable. Fever represented the first clinical presentation of neonatal sepsis after 72 hr by 72%, while on admission it only represented 27.8%. Lethargy represented the second clinical presentation after 72 hr by 69% while on admission it only represented 30.6%. Apnea was in the third rank after 72 hr of clinical presentation by 66.0% while on admission it only represented 34.0%. Poor Moro reflex and RD came in the fourth rank by 62.5% and 62.2% respectively, after 72 hr by 72% while on admission it only represents 37.5% and37.8% respectively. Tachycardia and bradycardia showed no significant change on admission and after 72 hrs. This agree with Tosif et.al. (2019) who performed a cohort study at three provincial hospitals from 2014- 2016 in Royal Children's Hospital Melbourne, Australia They deduced that if we depend on clinical data this will increase mortality rate by (46.2%) and also agreed with Shane et.al. (2017) who informed that the early symptoms and signs of neonatal sepsis are usually delayed and nonspecific. All those who agreed with us confirmed by data in table (1) as if we depend on clinical data, all that neonates would be missed or exposed to long term complication. This was also proved by (Klingenberg et.al., 2018) who stated that early onset neonatal sepsis generally manifested with respiratory distress, apnea, lethargy or irritability, temperature instability, and feeding difficulties. These symptoms are nonspecific, because many non- infected newborns may show similar symptoms. During the first days of life, different organ systems adapt to extra- uterine life dynamically. A single- point, clinical assessment to diagnose EONS therefore seems impossible.

As regard laboratory diagnosis, blood culture remains the gold standard for diagnosis of neonatal sepsis, despite its low sensitivity which may be due to small volume of blood sample, or empirical antibiotics prior to sampling (Kalathia et.al., 2013). While calculating HSS among cases and control groups showed high significant difference as shown in table (2) This laboratory finding agrees with (Narasimha et.al., 2011) and (Mahallei et.al., 2018). Moreover, current study also found difference among septic groups, which also agreed with the findings of (Mahallei et.al., 2018). This was in disagreement with (Waliullah et.al., 2010) who reported moderate sensitivity and specificity for HSS and stated that HSS could be used as a predictive tool but not always dependable tool. So although hematological parameters were significant as shown in table (1) but the positive predictive values of hematological parameters and inflammatory markers used in the diagnosis of neonatal sepsis are low and serial measurements are required (Benitz et.al., 2015).

Regarding biomarkers, SAA increases rapidly and significantly at the onset of inflammation and promptly returns to baseline levels with the resolution of the inflammation. SAA was established as an accurate and reliable marker for the diagnosis and follow up of neonatal sepsis, SAA was shown to have a protective role during inflammation and stimulate several pro- inflammatory pathways. In a recent study, SAA was found to induce proliferation and inflammation in hepatic stellate cells (Siegmund et.al., 2016). In this study SAA showed statistically being higher between patients and controls and between patients with progression of disease, here SAA takes the same pattern of clinical scoring which was confirmed by table (5).

Presepsin level was significantly higher in the study cases than in controls as shown in table (1) which agreed with Sharma et.al. (2016) and E-Mashad et.al. (2016), in a study carried out on neonates suspected to sepsis either laboratory or clinically to evaluate role of CBC and culture against presepsin.

Conclusion:

Presepsin and SAA are accurate, highly sensitive and specific markers either for prediction or diagnosis of early onset sepsis in contrast to clinical scoring or hematological scoring system (HSS).

Abbreviations:

1. SAA: Serum Amyloid A.
2. HSS: Hematological Scoring System.
3. PRES: Presepsin.

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