ASSESSMENT OF THE ROLE OF SERUM ISCHEMIA MODIFIED ALBUMIN IN THE EARLY DIAGNOSIS OF NEONATAL SEPSIS

Sawsan H.A. Azzab*, Nasser I.A Abdelrahman and Abdelrahman A. Abdelnaim*

Departments of Pediatrics* and Clinical Pathology**,
Faculty of Medicine, Zagazig University

ABSTRACT

Background: Neonatal sepsis is one of the most challenging problems despite the ongoing progress in diagnosis and treatment. Neonatal sepsis is the single most important cause of neonatal deaths in the community, accounting or over half of them. Ischemia modified albumin (IMA), a Food and Drug Administration-approved serum biomarker of cardiac ischemia and a risk stratification tool for suspected acute coronary syndrome is produced during an ischemic condition or attack and is present in the blood in early and easily detectable levels. Growing evidence suggests that IMA is also increased in diseases associated with oxidative stress such as diabetes mellitus, obesity and hypercholesterolemia. Also, it has been reported that circulating IMA was associated with inflammation markers, because inflammation reduces the capacity of albumin to bind to cobalt. Serum IMA has recently gained interest as marker for neonatal sepsis.

Objectives: The aim of this study is to evaluate the role of serum ischemia modified albumin as a marker for early diagnosis of neonatal sepsis.

Patients and Methods: The present comprised 30 neonates with sepsis, age 35.4 $\pm$ 2.62 weeks and 30 healthy control neonates age 36.50 $\pm$ 1.85 weeks. It was carried out as a case control study. An informed written consent was obtained from parents of all neonates. Patients and controls were subjected detailed history taking, thorough physical examination, routine laboratory investigations (CBC, CRP and blood cultures) and assessment of serum IMA.

Results: Analysis of our results showed statistically significant difference between sepsis group and control group in serum IMA levels. We found that no statistically significant difference was found between full term and preterm neonates with sepsis as regards serum IMA levels.

Conclusion: Serum IMA levels is a useful marker in early diagnosis of neonatal sepsis and further studies are needed to confirm our results in larger groups of patients**.**

Key Words: ischemia modified albumin, neonatal sepsis.

INTRODUCTION

Neonatal sepsis, sepsis neonatorum and neonatal septicemia are terms used to describe the systematic response to infection in the newborn. Neonatal infections account for over one million neonatal death worldwide every year[(1)].

Neonatal sepsis is one of the most challenging problems despite the ongoing progress in diagnosis and treatment. Neonatal sepsis is the single most important cause of neonatal death in the community, accounting for over half of them. Early-onset sepsis is associated with acquisition of microorganisms from the mother. Transplacental infection or an ascending infection from the cervix may be caused by organisms that colonize the mother‘s genitourinary (GU) tract; the neonate acquires the microorganisms as it passes through the colonized birth canal at delivery[(2)].

Laboratory indicators, such as complete blood-cell count, ratio of immature to total neutrophil and C- reactive protein (CRP) do not have high sensitivity especially if measured early in the course of sepsis. Thus, early identification of infants with bacterial sepsis has been recognized to be a major diagnostic challenge[(5)].

Ischemia Modified Albumin (IMA) has been released for clinical use. IMA is a good discriminator between ischemic and non-ischemic patients. Changes in IMA concentration have shown to occur during coronary angioplasty-induced ischemia[(3)].

The detection of ischemia prior to infarction is a challenging concept. It would be very helpful to be able to identify quickly and accurately which patients really have myocardial ischemia and may be in need for either treatment or intervention to prevent subsequent events. IMA has performed reasonably well in clinical trials focusing on its capability for early measurement to characterize ACS patients[(4)].

Ischemia modified albumin (IMA) is a novel marker of tissue ischemia[(6)]. The diagnostic albumin cobalt-binding test (ACB) was based for IMA on the observation that the affinity of serum albumin for cobalt is reduced after N-terminus modifications[(7)]. Recently commercial quantitative IMA detection by ELISA is available and used in this study. Some factors such as hypoxia, acidosis, superoxide-radical injury and energy-dependent membrane disruption could result in IMA formation[(4)].

It has been proposed that reactive oxygen species (ROS) generated during ischemia results in IMA formation. Thus, IMA is accepted as a marker of oxidative stress[7]. Growing evidence suggests that IMA is also increased in diseases associated with oxidative stress such as diabetes mellitus, obesity and hypercholesterolemia[8].

Also, it has been reported that circulating IMA was associated with inflammation markers because, inflammation reduces the capacity of albumin to bind cobalt. Serum IMA has recently gained interest as marker for neonatal sepsis and to the best our knowledge, only one published research has investigated this diagnostic role of serum IMA worldwide[9].

The aim of the study is to evaluate the role of serum ischemia modified albumin (IMA) as a marker for early diagnosis of neonatal sepsis.

PATIENTS AND METHODS

The present study was carried out in the Neonatal Intensive Care Unit (NICU) of Zagazig University Hospitals during the period from March 2014 and August 2014. It comprised 30 neonates with sepsis, age 35.4 $\pm$ 2.62 weeks and 30 healthy control neonates age 36.50 $\pm$ 1.85 weeks.

They were divided into 2 groups :

Group I:

Comprised 30 neonates with proven neonatal sepsis based on clinical and laboratory data with positive blood culture results.

Group II:

Comprised 30 healthy neonates matched for both age and sex.

Exclusion criteria:

Neonates with other diseases that cause oxidative stress[9] were excluded from the study, such diseases include :

1. Acute renal injury or dialysis
2. Suspected congenital metabolic disease
3. Cyanotic congenital heart disease.
4. Serious congenital malformation
5. Perinatal asphyxia
6. Neonates with maternal diabetes.
7. Intracranial hemorrhage
8. Suspected and diagnosed necrotizing enterocolitis
9. Neonates treated with indomethacin, ibuprofen and amphotericin B
10. History of or a requirement for surgical intervention
11. Patent ductus arteriosus (PDA)

The approach:

This study was carried out as a case control study.

Methods of the study:

An informed written consent obtained from parents of all neonates.

All of the studied patients were subjected to the following:

- Complete history taking.
- Complete clinical examination including weight, length, skull circumference, vital signs and neonatal reflexes.
- Laboratory investigations including CBC using sysmex XS500i 5 part differential, CRP by high sensitive turbidimetric assay, blood culture (oxoid signal blood culture system medium) and measurement of IMA levels using sandwich ELISA technique.

Statistical analysis:

All the statistical calculations were analyzed with the Statistical Package for Social Science (SPSS) computer program, version 20 Microsoft Window, Statistical analysis all measurements were performed in duplicate. The results of each group are expressed as the mean value ± Standard deviation of the mean. Differences between groups were assessed by compare means two paired samples t-test unpaired t-test and independent samples t-test. Correlations were determined by Pearson's coefficient (r). $p<0.01$ was considered to indicate.

RESULTS

Our results were tabulated as follows:

Table (1): Demographic characteristics of the studied neonates in both groups

Variable	Sepsis N=30				Control N=30				t	X	p
Gestational age (weeks)	Mean		SD		Mean		SD		1.87	-	0.06
	35.40		2.62		36.50		1.85				
Sex	Male		Female		Male		Female		-	0.16	0.9
	n	%	n	%	n	%	n	%			
	15	50%	15	50%	14	46.6%	16	53.3%			
Maturity	Preterm		Full term		Preterm		Full term		-	0.01	0.7
	N	%	n	%	n	%	n	%			
	22	73.3%	8	26.7%	20	66.7%	10	33.3%			

This table shows no statistically significant difference between the two groups as regard gestational age, sex and maturity.

Table (2): laboratory parameters of the studied neonates in both groups

Variable		Sepsis N=30	Control N=30	T value	P value
TLC $\times 10^3$ /mm^3	Mean	19.1	8.8	6.1	0.01**
	standard deviation	8.7	2.3		
CRP (mg/ L)	Mean	83.9	3.3	10.7	0.01**
	standard deviation	40.9	1.5		
IT Ratio	Mean	0.4	0.13	12.5	0.01**
	standard deviation	0.10	0.04		
PLT $\times 10^3$ /mm^3	Mean	95.60	241.20	11.2	0.01**
	standard deviation	68.5	13.7		

** Extremely statistically significant

This table shows that there is high statistically significant difference between sepsis and control groups regarding TLC, CRP, IT ratio and platelet

Table (3): blood culture result in patients group

Result	Number of patients	Percentage
E coli	12	40%
Staph aureus	8	26.6%
Klebsiella	6	20%
Pseudomonas	4	13.3%

This table shows the results of blood culture in patients group where E coli is 40% and Staph aureus 26.6%, and Klebsiella 20%, and pseudomonas 13.3% of cases.

Table (4): Ischemia modified albumin (IMA) among the studied neonates in both groups

Variable		Sepsis N=30	Control N = 30	T value	P value
IMA (ng/ml)	Mean	82.7	24.4	15.1	0.01**
	standard deviation	20.5	4.4		

** Extremely statistically significant

This table shows that IMA level is highly significant among sepsis group compared to control group (82.7 versus 24.4 ng/ml respectively).

Table (5): Ischemia modified albumin (IMA) among the studied neonates with sepsis according to prematurity

Variable		Preterm N=22	Full N=8	T value	P value
IMA ng/ml	Mean	88.2	74.3	1.89	0.068
	standard deviation	16	24.3		

This table shows that there was no statistically significant difference between full term and preterm neonates with sepsis in IMA level. Preterm neonates with sepsis have mean (IMA) of 88.2 ng/ml while full term neonates have IMA of 74.3 ng/ml

Table (6): Correlation between IMA and other sepsis markers among the studied neonates

Correlations					
		IMA	TLC	CRP	IT
IMA	Pearson Correlation	1	0.683**	0.595**	0.373*
	Sig. (2-tailed)		0.000	0.001	0.042
	N	30	30	30	30
TLC	Pearson Correlation	0.683**	1	0.493**	0.363**
	Sig. (2-tailed)	0.000		0.006	0.048
	N	30	30	30	30
CRP	Pearson Correlation	0.595**	0.493**	1	0.711**
	Sig. (2-tailed)	0.001	0.006		0.000
	N	30	30	30	30
IT	Pearson Correlation	0.373*	0.363**	0.711**	1
	Sig. (2-tailed)	0.042	0.048	0.000	
	N	30	30	30	30

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

This table shows that IMA level is significantly correlated with TLC, CRP and I/T ratio the correlation coefficient between IMA, and **TLC** is .683**, and the correlation coefficient between IMA, and **CRP** is .595** and the correlation coefficient between IMA, and **IT** is .373*.

Table (7): Ischemia modified albumin (IMA) among the studied neonates with sepsis in male and female

Variable		Male N=15	female N=15	T Value	P value
IMA	Mean	79.4	86	-.875	0.389
	standard deviation	18.4	22.6		

This table shows that there is no statistically significant difference between male and female neonates regarding serum IMA level. Male neonates with sepsis have mean IMA 79.4 ng/ml while female neonates have IMA of 86 ng/ml.

Table (8): Ischemia modified Albumin in septic neonates as regards outcome

Variable		Living N=26	Died N=4	T value	P value
		86.6%	13.3%		
IMA Ng/ml	Mean	82.3	85.2	-.262	0.795
	Standard deviation	20.7	22.3		

This table Shows that there is no statistically significant difference between livings and died septic neonates as regards serum IMA level. Living neonates with sepsis have mean IMA 82.3 ng/ml while died septic neonates has mean IMA 85.2 ng/ml.

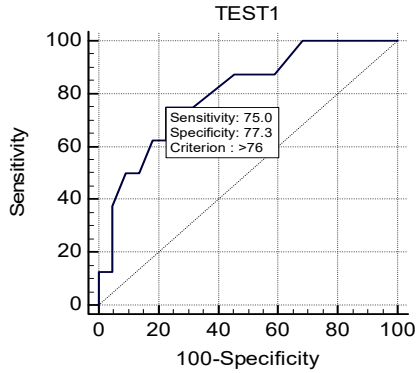

Figure (1): ROC curve analysis for best cutoff value of IMA for detection of neonatal sepsis

This figure shows that the best cutoff value of IMA level for detection of neonatal sepsis is >30 ng/ml.

DISCUSSION

The present study was designed to assess the role of serum ischemia modified albumin in the early diagnosis o neonatal sepsis.

Our results revealed that serum IMA levels were significantly higher among the sepsis group compared with the control group (82.7 versus 24.4 ng/ml respectively). These results were in agreement with **Yerlikaya et al.[9] and Erden et al.[10]** who reported higher levels of serum IMA levels in neonates with sepsis compared to healthy control neonates.

In our study we found no statistically significant difference between serum IMA levels in full term versus preterm infants. Full term septic neonates had a mean serum IMA level of 74.3 ng/ml while preterms had a mean level of 88.2 ng/ml, a difference which was not statistically significant. No other studies have been carried out before our study to compare the serum levels of IMA with the gestational age of the neonate.

In our study, there was no significant differences between serum IMA levels in survivors and died neonates with sepsis (82.3 vs 85.2 ng/ml.

In our study, IMA was significantly correlated with TLC, CRP and I/T ratio the correlation coefficient between IMA, and TLC is .683**, and the correlation coefficient between IMA, and CRP is.595** and the correlation coefficient between IMA, and I/T is 0.373.

The present work showed that there was a positive correlation between (TLC, I/T ratio, IMA). This result came in agreement with that of **Yerlikaya et al.**[9] who showed that there is a positive correlation between IMA, TLC, CRP and PCT), but in our study we did not use procalcitonin (PCT) as a marker or sepsis.

Serum CRP and IMA levels were positively correlated in the sepsis group. Serial CRP estimation has greater value as compared to single CRP estimation in judging the course and outcome of neonatal septicemia. This result came in agreement with that of **Erdem et al.**[10] **and Duarte et al.**[11].

Our study showed that the incidence of neonatal sepsis was more among the premature neonate. 22 premature neonate (73.3%) had sepsis, while 8 full-term (26.7%) have sepsis. This agreed with **Zaakouk et al.**[12] and **Ahmed et al.**[13] who found that the incidence of sepsis is more among the premature neonate.

Also, **Belling**[14] found that the incidence and severity of sepsis is inversely related to the gestational ages of the infant.

Also our study agree with **Politis**[15] who found that the premature neonate in particular have a relatively permeable mucosal surfaces that allow for trans-epithelial passage of bacteria and other pathogens. Also loss of maternal antibodies, as well as non specific alterations in macrophage phagocyte and clearance of invading pathogen, impaired T-cell and B cell responses and

altered production of complement and antibodies.

In our study we found that there was no significant statistical difference between sex of patient and incidence of sepsis .15 male cases (mean IMA =79.4) with sepsis and 15 female cases (mean IMA =86) with sepsis i.e. female: male=1:1. This agreed with **Mohamed et al.**[16] who found that there was no statistically difference between sex of patient and incidence of sepsis.

Shin et al.[17] found that more male than female infants developed both early-onset and late-onset sepsis; gender differences were more pronounced in culture-confirmed cases but the differences by onset were not statistically significant (P=0.14, confirmed-sepsis; P= 0.85, clinical sepsis).

Aslan and Apple[18] found that no statistically significant diffe-rence between male and female. Male neonates with sepsis had mean IMA 79.4 ng/ml while female neonates have IMA of 86 ng/ml.

Blood culture is the gold standard method for isolation of the micro-organisms. Blood culture should be obtained before initiation of antibiotics[16].

In our study, blood culture results revealed that E. coli was the most commonly detected organism (40%), followed by staph aureus (26.6%) then klebsiella (20%) and finally pseudomonas was (13.3%).

Our study was in concordance with **Zaakouk et al.**[12] who found that E-coli was the most commonly detected organism (42.9%).

Our result were in disagreement with the study of **Dzwonek Agnieska**[19] in which nearly half of the positive cultures grew klebsiella pneumonia.

Also in the study done by **De Benedetti et al.**[20], the isolated pathogen included klebsiella pneumonia (47.5%), pseudomonas (20%), E. coli (10%), Candida albicans (10%), staph aureus (7.5%),and Enterococcus (5%).

The authors concluded that variety of microorganisms cause neonatal sepsis, with local variations in organism type. Klebsiella species and S aureus are especially likely to induce severe lung damage, producing micro-abscesses and empyema.

In our study, the area under the curve for IMA was 0.804. This agreed with **Turedi et al.**[21] who found that the area under the curve for IMA was 0.889.

CONCLUSION

- Ischemia modified albumin is a new biochemical marker, which

can be used with high sensitivity and specificity in the early diagnosis of neonatal sepsis.

- According to our results, serum IMA level is a good predictor of morbidity, but not of mortality.

RECOMMENDATION

Further large scale studies are needed to confirm the validity of serum IMA level in early prediction of neonatal sepsis and to assess its usefulness in prediction of early onset and late onset neonatal sepsis and also to assess its significance as a prognostic factor throughout the course of treatment.

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تقييم دور الزلال المتحور بنقص الدم في المصل في التشخيص المبكر لمرض الإنتان الدموي في الأطفال حديثي الولادة

د. سوسن حامد عبد اللطيف – د. ناصر عبد الرحمن

د. عبد الرحمن احمد عبد النعيم*

قسمي طب الأطفال والباثولوجيا الإكلينيكية* – كلية الطب – جامعة الزقازيق

رغم التطورات المستمرة في تشخيص وعلاج مرض الانتان الدموي في حديثي الولادة، إلا أن هذا المرض لا يزال من المشاكل العظمي في وحدات حديثي الولادة بالمستشفيات. ولا يزال هذا المرض هو السبب الأكبر والأهم في وفيات الأطفال حديثي الولادة وخاصة في الدول النامية ودول العالم الثالث، ولا زالت الأبحاث تجرى هنا وهناك لمحاولات الاكتشاف المبكر لهذا المرض مما قد يحسن من نتائج العلاج.

وقد ظهر في السنوات القليلة الماضية أحد الدلالات الكيميائية الحيوية التي قد نساعد في التشخيص المبكر لهذا المرض ألا وهو الزلال المتحور بنقص الدم.

وقد وافقت الهيئة الأمريكية للأغذية والأدوية على اعتبار الزلال المتحور بنقص الدم دلالة كيميائية حيوية هامة في تشخيص حالات قصور الشريان التاجي إلا أن الأبحاث التي أجريت على هذا المركب اثبتت أنه يمكن استخدامه في اكتشاف عدد كبير من الأمراض الأخرى من ضمنها الالتهابات الناتجة عن العدوى البكتيرية.

الهدف من البحث:

تقييم دور الزلال المتحور بنقص الدم في التشخيص المبكر لحالات الانتان الدموي في الأطفال حديثى الولادة.

المرضى وطرق البحث :

أجرى هذا البحث على 30 طفلا حديثي الولادة مصابين بالانتان الدموي، 30 طفلاً حديثي الولادة من الأصحاء كمجموعة ضابطة بعد أخذ موافقة مكتوبة من الأهل على إجراء البحث.

وقد تعرض هؤلاء الاطفال لفحص سريري شامل بالاضافة الى الفحوصات التقليدية، ثم قياس مستوى الزلال المتحور بنقص الدم في المصل.

النتائج :

أثبتت النتائج وجود اختلاف ذي دلالة احصائية بين مستوى الزلال المتحور بنقص الدم في الأطفال حديثي الولادة المصابين بالانتان الدموي مقارنة بالمجموعة الضابطة إلا أن مستوى هذه الدلالة لم يختلف بين الاطفال حديثي الولادة المكتملين والمبتسرين.

الاستنتاج :

يعتبر الزلال المتحور بنقص الدم من الدلائل الحيوية المفيدة في التشخيص المبكر لمرض الانتان الدموي في حديثي الولادة ولا يزال هناك حاجة الى اجراء المزيد من الابحاث على عدد أكبر من الأطفال حديثي الولادة لتأكيد النتائج.