MEASUREMENT OF SERUM MALONDIALDEHYDE (MDA) LEVELS AS A MARKER OF LIPID PEROXIDATION IN NEONATAL SEPSIS.

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ABSTRACT:

Neonatal sepsis remains a major problem in neonates admitted to the neonatal intensive care units, with high morbidity and mortality rates despite advances in antimicrobial therapy and supportive cares, especially in developing countries. The lipid peroxidation, as a result of reactive oxygen species (ROS) production, play a significant role in pathogenesis of multiple organ failure and septic shock associated with neonatal sepsis which contribute to high morbidity and mortality of neonatal sepsis. This a prospective study carried out to measure the serum malondialdehyde (MDA) levels as a marker of lipid peroxidation in neonates with sepsis who were admitted to the neonatal care unit at Bint-Al-Huda Maternity and Children Teaching Hospital, Thi-Qar governorate, Iraq from first of April 2010 till the end of August 2011. One-hundred eight septic newborns and sixty matched healthy neonates (thirty were full term and thirty were preterm) as control group were studied. Sepsis was confirmed by clinical manifestations and blood culture. Fifty two (48%) of septic newborns were full term and fifty six (52%) were preterm. The most common microorganism isolated from septic newborns was gram negative bacteria especially *Klebsiella pp*. The MDA levels were extremely higher in both full term and preterm neonates with documented sepsis than that in their corresponding controls (P-value < 0.001). These results suggest that newborn infants have insufficient defense mechanisms against free radicals. Both enzymatic and non-enzymatic antioxidant mechanisms in neonates with sepsis and usage of antioxidants drugs and vitamins in the management of neonatal sepsis need further evaluation.

INTRODUCTION:

Neonatal sepsis are a major cause of death worldwide[(1)], with an incidence of 1 to 10 cases per 1000 live births in developed countries and 10 to 50 cases per 1000 live births in developing countries, with even higher rates in low-birth-weight neonates [(2, 3)]. Hospital acquired infections in neonatal intensive care units may also occur as frequently as 30 infections per 100 patients. [(4)] Despite advances in antimicrobial therapy and supportive cares, septicemia continues to be a major cause of morbidity and mortality in the neonatal period. In developing countries, many of the more than 14 million deaths of children under five years of age occur during the neonatal period, with sepsis accounting for up to 70% of total mortality for this age group. [(5, 6)] There are several reports that suggest that reactive oxygen species (ROS) play a significant role in the pathogenesis of neonatal sepsis and its complications. [(7, 8)] The inflammatory response to critical illness, including sepsis, involves the activation of leukocytes and other inflammatory cells leading to a massive

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production of ROS. ROS mediated oxidative stress has been implicated in apoptotic cell death and in turn can be harmful to the patient when the endogenous antioxidant defense mechanisms are overwhelmed. [9] It is now well documented that ROS is involved in the pathogenesis of multiple organ failure following sepsis, often leading to death. [10] Newborns have less protection against oxidation [11]. In comparison with healthy adults, lower levels of plasma antioxidants such as vitamin E, *B*-carotene, and sulfhydryl groups, lower levels of plasma metal binding proteins such as ceruloplasmin and transferrin, and reduced activity of erythrocyte superoxide dismutase are typical of newborn infants. Furthermore, infants frequently have higher plasma levels of nontransferrin-bound iron and higher erythrocyte free iron than adults. Also the neonates have very low levels of melatonin, which is a highly effective antioxidant and free radical scavenger, compare to the adults. [12, 13]

This study was carried out on neonates with documented sepsis, to measure the plasma malondialdehyde (MDA) as a marker of lipid peroxidation in neonatal sepsis.

Patients and methods:

This is a prospective study was carried out at the neonatal care unit of the Bint-Al-Had Maternity and Children Teaching Hospital, Thi-Qar governorate, Iraq, from the first of April 2010 till the end of August 2011. A special questionnaire was designed for the purpose of the study. A total of One-hundred eight neonates (fifty two full term and fifty six preterm), their ages ranged from 1-28 days with documented sepsis and a sixty matched healthy neonates (thirty full term and thirty preterm) as control group were included in the study after exclusion those with prior antibiotic therapy, neonates with obvious congenital anomalies, those with history of birth asphyxia, respiratory distress syndrome (RDS), transient tachypnea of newborn, indirect hyperbilirubinemia, and meconium aspiration syndrome.

The following information was taken:

Name, age, sex, date of admission to the neonatal care unit, mode and place of delivery, gestational age (was assessed by Dubowitz criteria) [3], age of onset of symptoms, and any history of previous hospitalization. Data regarding maternal history of prolonged rapture of membrane and its duration, fever, antibiotics used and urinary tract infections are also recorded. The patients are carefully assessed for signs and symptoms of sepsis like poor feeding, lethargy, coffee-ground vomiting, diarrhea, oliguria, convulsions, temperature instability, pallor, jaundice, cyanosis, tachypnea, apnea, respiratory distress, signs of dehydration, signs of intrauterine growth retardation, mottled skin, sclerma, omphalitis, hepatosplenomegally, abdominal distention and delayed capillary refilling (equal or more than 3 seconds). The blood samples from peripheral veins are taken from all neonates with suspected sepsis before initiation of any treatment for blood culture and measurement of plasma malondialdehyde (MDA) levels. The only patients with documented sepsis by a positive blood cultures are included in this study. Also a peripheral vein blood samples are taken from control neonatal group for measurement of plasma MDA levels.

Blood culture:

Blood samples of at least 2 ml were taken from peripheral veins from two separated sites using aseptic techniques. The skin was disinfected by applying tincture of

iodine solution that was left to evaporate, and then wipped off with 70% alcohol solution, beginning at the center and scrubbing in a circular motion out word. The samples were inoculated into a blood culture medium and sent directly to the laboratory where they cultured aerobically and anaerobically under the supervision of an expert microbiologist.

Measurement of lipid peroxidation:

MDA has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give red species absorbing at 535 nm. [14]

Procedure:

1. One ml. of patient or control serum was combined with 2 ml of Trichloroacetic acid (TCA) - Thiobarbituric acid (TBA) – Hydrochloric acid (HCL) solution and mixed thoroughly, when heated for 15 minutes in boiling water bath.
2. After cooling, the precipitate was removed by centrifugation at 3000 rpm for 10 minutes.
3. The absorbency was determined at 535 nm against reagent blank, which was containing all the reagent minus the serum.

MDA (M mol / L) = $\Delta A/1.56 \times 10$

Statistical analysis:

Statistical analysis was done by SPSS-version 18 software. Data were expressed in as mean ± SD. P- value of less than 0.05 was considered as statistically significant, P- value < 0.01 as highly significant, and P- value < 0.001 as extremely significant.

RESULTS:

A total one-hundred eight neonates with documented sepsis by blood culture aged 1–28 days and sixty matched healthy neonates (thirty full term and thirty preterm) as control group were included in the study. Fifty two (48%) of septic neonates was full term and fifty six (52%) was preterm.

Each group of septic neonates was subdivided in to ***early sepsis*** (those with onset of clinical manifestations of sepsis within the first seven days of life) and ***late sepsis*** (those with onset of clinical manifestations of sepsis between 8-28 days of life), and the results was as shown in table-1.

The full term neonates with early and late sepsis and their controls did not differ from each other with respect to gestational age or body weight (P- value not significant) but they are differ in their age at time of admission to the neonatal care unit (P-value < 0.001) and this is due to the fact that early neonatal sepsis presented within the first seven days of life while late neonatal sepsis presented between day eight and day twenty-eight of life. Same results are found among preterm neonates as shown in table-2 and table-3.

The most common causative bacteria of neonatal sepsis among full term and preterm neonates was *Klebsiella pp* in both early and late sepsis, as shown in table-4 and table-5.

The lipid peroxidation, as reflected by serum MDA levels, was extremely significantly higher in early and late neonatal sepsis in both full term and preterm neonates as compared to the serum MDA levels in their corresponding controls (P-value < 0.001), as shown in table-6 and table-7.

DISCUSSION:

Sepsis is a common event in newborns admitted to neonatal intensive care units and it is associated with high rate of morbidity and mortality [2].

There are several reports suggest that reactive oxygen species (ROS) are produced in sepsis eventuating cellular or organ injury [15, 16] which associated with higher morbidity and mortality of sepsis. ROS cause injury to membrane lipids, sulfdryl bands of proteins and nucleotides of DNA.

The protective mechanisms evolved against this injurious process is presence of enzymatic [e.g. superoxide dismutase (SOD) and glutathione peroxidase (GPX)] and non-enzymatic [e.g. TNF-alpha and unconjugated bilirubin] free radical scavengers. These antioxidants protected the cellular integrity against ROS mediated injury [17, 18].

Oxygen free radicals are highly reactive and can initiate chain reactions which form new free radicals. Although the life time of each radical is extremely short, its action may continue by an explosive and proliferative generation of new radicals. Free radicals injure biological membranes by lipid peroxidation [19]. Stable degradation products of such processes e.g. malondialdehyde (MDA) may, therefore, be used as a markers of peroxidation of polyunsaturated fatty acids [20].

In the current study we measured the serum MDA levels as a marker of the extent of lipid peroxidation in neonatal sepsis.

All patient neonates included in this study have documented sepsis by blood culture. Gram negative microorganisms were the most common micro- organisms isolated from these neonates; especially *Klebsiella pp.* while low incidence of *gp. B. hemolytic streptocci* was reported. Similar results were obtained by many studies in Iraq [21, 22, 23], Dubai [24], Saudi Arabia [25, 26] and Mexico. [27]

All septic newborns included in this study (both preterm and full term) have significantly higher levels of serum MDA than their corresponding controls (P-value < 0.001) which indicated that the lipid peroxidation is very high during sepsis process which increase the mortality and morbidity of neonatal sepsis. These results are agree with the results were obtained by Gitto *et al* [28], Cherian *et al* [29] and Kapoor *et al*. [30]

CONCLUSION:

The lipid peroxidation in septic newborn is highly increase as a result of ROS production which is responsible for cellular or organs injury leading to multiple organ failure or septic shock associated with septicemia as listed before. [15, 16]

These results raise the importance of antioxidant agents as a part of management of neonatal sepsis to reduce their mortality and morbidity like melatonin [28], glutamine [31] and Vitamins A, C, and E. [9]

Both enzymatic and non-enzymatic antioxidant capacity of septic newborns need further evaluation.

Table 1: Percentages of early and late sepsis in full term and preterm neonates.

Type of sepsis	Full term neonates	Preterm neonates
Early sepsis	24 (22.1%)	26 (24%)
Late sepsis	28 (25.9%)	30 (28%)
Total	52 (48%)	56 (52%)

Table-2: Characteristics of septic full term newborns and their control.

	Early sepsis N=24 (22.1%)		Late sepsis N=28 (25.9%)		Control N= 30		P-value
	Mean	SD	Mean	SD	Mean	SD	
Age at admission (days)	4.75	1.700	15.93	5.577	10.83	6.341	0.0001
Gestational age (weeks)	38.58	1.316	38.61	1.370	38.83	1.020	NS*
Body weight (kg)	2.825	0.3629	3.0143	0.3978	3.2833	0.4713	NS*

***** Non-significant

Table-3: Characteristics of septic preterm newborns and their control.

	Early sepsis N=26 (24 %)		Late sepsis N=30 (28%)		Control N= 30		P-value
	Mean	SD	Mean	SD	Mean	SD	
Age at admission (days)	4.96	1.455	17.53	5.231	12.23	7.147	0.0001
Gestational age (weeks)	29.08	1.129	29.47	1.570	30.23	2.359	NS*
Body weight (kg)	1.801	0.1780	1.7167	0.3797	1.6967	0.3123	NS*

***** Non-significant

Table-4: causative bacteria in full term neonatal sepsis

Type of bacteria	Full term early sepsis		Full term late sepsis	
	No.	Percentage (%)	No.	Percentage (%)
Klebsiella	6	25%	8	28.6 %
Staphyllococcus albus	5	20.8%	8	28.6%
Str. pneumonae	3	12.5%	0	0.0%
P. aeruginosa	3	12.5%	5	17.9%
E. coli	6	25%	4	14.3%
Str. fecalis	1	4.2%	1	3.6%
Proteus	0	0.0%	1	3.6%
Gp B. hemolytic streptococci	0	0.0%	1	3.6%
Total	24	100%	28	100%

Table-5: causative bacteria in preterm neonatal sepsis

Type of bacteria	preterm early sepsis		preterm late sepsis	
	No.	Percentage (%)	No.	Percentage(%)
Klebsiella	10	38.5%	10	33.3 %
Staphyllococcus albus	6	23.1%	9	30%
Str. pneumonae	0	0.0%	3	10%
P. aeruginosa	4	15.4%	7	23.3%
E. coli	6	23.1%	0	0.0%
Proteus	0	0.0%	1	3.3%
Totall	26	100%	30	100%

Table-6: serum MDA levels in full term septic neonates and their controls group.

	Early sepsis N=24 (22.1%)		**Late sepsis N=28 (25.9%)**		**Control N= 30**		**P-value**
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	
S.MDA levels	1.7913	0.1825	1.7454	0.14436	0.5790	0.40886	0.0001

Table-7: serum MDA levels in preterm septic neonates and their controls group.

	Early sepsis N=26 (24 %)		**Late sepsis N=30 (28%)**		**Control N= 30**		**P-value**
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	
S.MDA levels	1.7715	0.2616	1.5447	0.05740	0.5687	0.39913	0.0001

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قياس مستوى المالوندايلدهايد في المصل كعلامة لأكسدة الدهون في الأطفال حديثي الولادة المصابين بالإنتان الدموي

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المقدمة

الانتان الدموي يبقى مشكلة كبيرة بين الاطفال حديثي الولادة الراقدين في وحدات رعاية حديثي الولادة المركزة مع معدلات مراضه و وفاة عالية على الرغم من التطور الحاصل في العلاج عن طريق المضادات الحيوية و العلاجات الساندة الاخرى خصوصا في البلدان النامية. أكسدة الدهون، كنتيجة لتكون اصناف الأوكسجين التفاعلية ، تلعب دور هام في نشوء و تطور عجز الأعضاء المتعدد و الصدمة الانتانية المصاحبة للإنتان الدموي في الاطفال حديثي الولادة.

هذه الدراسة تمت لقياس مستوى المالوندايلدهايد في المصل كعلامة لأكسدة الدهون في الاطفال حديثي الولادة المصابين بالإنتان الدموي الذين رقدوا في وحدة رعاية حديثي الولادة في مستشفى بنت الهدى للولادة و الاطفال التعليمي في محافظة ذي قار، العراق، خلال الفترة الممتدة من اول شهر نيسان ٢٠١٠ حتى نهاية شهر اب ٢٠١١.

مائة وثمانية اطفال حديثي الولادة مصابين بالإنتان الدموي و ستون طفل حديثي الولادة طبيعيين من دون اي مرض (ثلاثون منهم ولدوا بعد ٣٧ اسبوع من الحمل و ثلاثين ولدوا قبل ٣٧ اسبوع من الحمل)، كمجموعة مقارنة، شملوا في هذه الدراسة. اثبات الانتان الدموي تم عن طريق الاعراض و العلامات السريرية بالإضافة الى مزرعة الدم.

اثنان و خمسون (٤٨%) من الاطفال حديثي الولادة المصابين بالإنتان الدموي والمشمولين بهذه الدراسة كانوا مولودين بعد ٣٧ اسبوع من الحمل بينما ستة وخمسون(٥٢%) منهم مولودين قبل ٣٧ اسبوع من الحمل.
البكتريا المسببة للإنتان الدموي الاكثر شيوعا في حديثي الولادة المصابين بالإنتان في هذه الدراسة كانت البكتريا السالبة لصبغة كرام خصوصا بكتريا الكليبزلا. مستوى المالوندايلدهايد في المصل كان عالي جدا عند حديثي الولادة المصابين بالإنتان الدموي بالمقارنة بأقرانهم الغير مصابين.

هذه النتائج تبين أن الأطفال الحديثي الولادة لديهم تقنيات دفاعية غير كافية ضد الجذيرات الذرية الحرة المسببة لأكسدة الدهون.
التقنيات الانزيمية و اللاانزيمية المضادة للأكسدة في الأطفال حديثي الولادة المصابين بالإنتان الدموي بالإضافة الى استعمال الادوية و الفيتامينات المضادة للأكسدة في علاج الانتان الدموي تحتاج الى دراسات اكثر لتقييمها.

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