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STUDY THE LEVELS OF NITRIC OXIDE, MALONDIALDEHYDE AND TOTAL ANTIOXIDANT CAPACITY AMONG TERM NEONATES WITH SEPSIS

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ABSTRACT

Neonatal sepsis with its high mortality rate still remains a diagnostic and treatment challenge for neonatal health care providers. Sepsis represents an oxidative stress condition when occurs in neonates due to rapid changes in tissue oxygen concentrations with immature antioxidant mechanisms. Our study aimed to study serum levels of oxidants and antioxidants levels among term neonates with sepsis. This case-control study included 30 full term newborns with sepsis in addition to 30 healthy newborns as controls, recruited from NICU of the pediatric department at Al-Alawiya pediatric hospital. Full history, thorough clinical examination, complete blood count (CBC), Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and blood culture were performed to all included patients. Serum levels of nitric oxide (NO), malondialdehyde (MDA) and total antioxidant capacity (TAO) were assayed to all included neonates using colorimetric methods. The overall results showed statistically significant higher serum levels of MDA and NO with lower TAO levels among septic neonates when compared with the controls (P value<0.01). Additionally, all the previously measured biochemical oxidative stress markers didn't show significant differences between early and late onset septic groups. It can be concluded from this study that there is a strong evidence of involvement of oxidative stress in neonatal sepsis, mainly NO, MDA and TAO, and could be helpful in its diagnosis. Also, targeting of therapeutic strategies towards the pro-oxidant pathways may be beneficial in neonatal sepsis.

KEYWORDS: Nitric oxide; Malondialdehyde; Total antioxidant capacity; Neonatal sepsis.

INTRODUCTION

Sepsis is one of the common leading factors of neonatal morbidity and mortality. Oxidative stress together with inflammation is involved in neonatal sepsis with subsequent impairment of organ functions and death.[1] Early onset neonatal sepsis (EOS) is defined as a positive culture of (blood, urine obtained by suprapubic tab or in-and- outcatheterization or cerebrospinal fluid) occurring on 1st, 2nd or 3rd days of life, while late onset sepsis (LOS) is defined as a positive culture occurring between 4th and 120th days of life.[2] The oxidative stress (OS) was previously defined as an imbalance between reactive oxygen species (ROS) and endogenous antioxidant power with subsequent lipid peroxidation, DNA damage and protein oxidation,[3] but recently OS refers to the imbalance between the oxidants and antioxidants in favor of oxidants that results in molecular damage or redox signaling arrest.[4] Oxidative damage from pathologic conditions such as sepsis have serious effects in pediatric patients more than in older people due to the lower functional reserve and the requirement for subsequent growth of tissue to follow normal development.[5] The profile of oxidative stress and antioxidant defense during neonatal sepsis is less extensively studied when compared to adult patients.[1] Thus, the current study aimed to assess the serum levels of malondialdehyde (MDA) and nitric oxide (NO) as oxidative stress markers in addition to total antioxidant capacity (TAO) as antioxidant defense marker in full term neonates with sepsis to investigate the possible involvement of oxidative stress and its possible diagnostic value in neonatal sepsis.

PATIENTS AND METHODS

The present case-control, hospital-based study was conducted on 30 full term newborns with sepsis in addition to 30 healthy newborns as controls, recruited from NICU of pediatric department at Al-Alawiya pediatric hospital in Baghdad/Iraq during the period from September 2017 to August 2018.

All full term newborns admitted to NICU and presented with clinical suspicion of sepsis and proved by hematological (leucocytosis, neutrophilia with or without thrombocytopenia, CRP>6 mg/dl, ESR>15 mm after the first hour) and/or bacteriological (positive blood culture) laboratory workup were included in the current study, while those with congenital malformation, meconium aspiration, gastro-intestinal obstruction, congenital heart diseases, inborn error of metabolism or respiratory distress syndrome were excluded. The parents of the included patients were subjected to history taking with special concern on prenatal history (maternal diseases, pre-eclampsia, congenital infections, maternal fever, rupture of membranes more than 18 hour), natal history (mode of delivery, Apgar score 1 min and 5 min, resuscitation measures), post-natal history (respiratory distress symptoms, convulsions and other neurological symptoms, or NICU admission of any of their siblings), gestational age assessment using New Ballard Score method and pregnancy date.[6] Complete general examination including general condition, pulse, blood pressure, respiratory rate, oxygen saturation percent, temperature, and color were performed, in addition to neurological examinations including anterior fontanel, muscle tone, grasp, Moro and suckling reflexes. Clinical diagnosis of sepsis included symptoms and signs suggestive of sepsis in the form of poor feeding, temperature instability, vomiting, diarrhea, constipation, apneic spells, cyanosis, persistent tachycardia or bradycardia, prolonged capillary refilling time, hypotension, apathy, irritability, hypertonia, hyperreflexia, seizures, tremors, jitteriness, poor Moro and suckling reflexes, jaundice, petechiae, purpura, sclerema, omphalitis, or signs of meningitis (in the form of high pitched cry, head retraction, bulging anterior fontanel and convulsions). Cranial ultrasound was done and lumbar puncture if meningitis was suspected.

Venous blood samples were drawn from every included neonate at the same day of life from both groups and introduced on plain tubes and centrifuged at 3500 rpm for 15 minutes. Separated serum from each tube was divided into aliquots using 1 ml cryotubes which was stored at -80°C until the performance of biochemical analysis in the form of measuring serum malondialdehyde, nitric oxide and total antioxidant capacity using colorimetric methods (Chem-7, Erba diagnosctics, Germany). MDA, NO, and total antioxidants assays were done using commercially available colorimetric kits according to Nair et al.,[7] Liu et al.,[8] and Valko et al.,[9] respectively and as described in previously published studies.[10-13]

Statistical analysis

Data were analyzed using SPSS 21. Quantitative data were represented as mean ± standard deviation. The Student t-test was used to compare means of the two groups. When the data were not normally distributed, Kruskal Wallis test for comparison of three or more groups and Mann-Whitney test was used to compare between the two groups. Qualitative data was presented as number and percentage and compared using either Chi square test or Fisher exact test.

RESULTS

The current study was conducted on 30 term septic neonates, 15 (50%) were males and 15 (50%) were females, with their mean gestational age was 39 weeks ±1.64 SD and the mean birth weight was 3.09 kg ±0.54 SD. They were compared with 30 healthy neonates, 14 males (46.7%) and 16 (53.3%) females. Their mean gestational age and birth weight were 39 weeks ±1.65 SD, 3.29 kg ±1.21 SD respectively, with non-significant differences with the included cases in respect to age, birth weight and sex matching. Apgar score for cases was significantly lower than that of controls at 1st min (4 ± 3 vs. 7 ± 3) with non-significant differences at 5th min as shown in table (1).

Results showed that 10 (33.3%) of cases and 8 (26.7%) of controls had normal vaginal delivery. The study revealed that the percentage of mothers who had either fever, premature rupture of membranes, or urinary tract infection were higher in cases 10 (33.3%), 8 (26.7%) and 3 (10%) respectively] than controls [6 (20%), 3 (10%) and 1 (3.3%) respectively as seen in table (1).

Table (1): Baseline characteristics of the studied groups.

Demographic characteristics	Term septic neonates (n=30)	Control (n=30)	P-value
Type of birth (number and percentage)			
CS	20 (66.7%)	22 (73.3%)	> 0.05
NVD	10 (33.3%)	8 (26.7%)	
Maternal risk factors (number and percentage)			
Fever	10 (33.3%)	6(20.0%)	> 0.05
PROM	8 (26.7%)	3(10.0%)	> 0.05
Maternal UTI	3(10.0%)	1(3.3%)	> 0.05
Sex (number and percentage)			
Male	15 (50.0%)	14 (46.7%)	>0.05
Female	15 (50.0%)	16 (53.3%)	>0.05
Gestational age (Mean ±SD)			
GA (weeks	39 ± 1.64	39 ± 1.65	>0.05

Apgar score			
At 1st minute	4 ± 3	7 ± 3	< 0.05
At 5th minute	7 ± 5	9 ± 7	>0.05
Birth weight (Mean ±SD)			
Birth weight (kg)	3.09 ± 0.54	3.29 ± 1.21	>0.05
Length (Mean ±SD)			
Length (cm)	47.9 ± 2.1	48.5 ± 3.21	>0.05
Head Circumference (Mean ±SD)			
Head Circumference (cm)	35.1 ± 0.79	35.3 ± 2.11	>0.05
N.S. (>0.05) Non-significant; significant, <0.05. CS: Cesarean Section; NVD: Normal Vaginal Delivery; PROM: Premature Rupture of Membranes; UTI: Urinary Tract Infection; GA: Gestational Age.			

There were significant higher mean leucocytic and granulocytic count, CRP, and ESR (17.15 (10^9/l) ±4.65, 71.55 (10^3/mm^3) ±7.95, 75.15 (mg/dl) ±56.22, 31 (mm) ±8.17 respectively) among septic neonates when compared with the controls (10.75 ±3.87, 54.9 ±4.95, 6 ±3.59, 11 ±3.29 respectively), with p value <0.05 for all. There were also significant lower mean hemoglobin levels and platelets count in cases (10.91 (g/dl) ±3.45, and 119.95 (10^9/l) ±41.54 respectively) versus the control group (15.1 ±3.12, and 293 ±51.15 respectively) with a P-value <0.05 for both as shown in table (2).

Table (2): Routine laboratory investigations in the form of complete blood count (CBC) parameters, CRP and ESR among septic neonates versus controls.

Variables	Septic neonates (n=30)	Control (n=30	P value
WBC (10^9/l)	17.15 ± 4.65	10.75 ± 3.87	< 0.01
GRA (10^3/mm^3)	71.55 ± 7.95	54.9 ± 4.95	< 0.01
LYM (10^3/mm^3)	25.71 ± 6.64	26.95 ± 2.31	> 0.05
MON (10^3/mm^3)	8.35 ± 4.51	8.75 ± 3.21	> 0.05
RBC (10^6/mm^3)	3.52 ± 1.15	4.05 ± 1.32	> 0.05
HGB (g/dl)	10.91 ± 3.45	15.1 ± 3.12	< 0.01
PLT (10^9/l)	119.95 ± 41.54	293 ± 51.15	< 0.01
HCT (%)	40.95 ± 6.12	46.91 ± 5.45	< 0.01
MCV (µm^3)	101.0 ± 7.67	99.0 ± 8.89	> 0.05
MCH (pg.)	32.46 ± 3.51	32.14 ± 4.36	> 0.05
CRP (mg/dl)	75.15 ± 56.22	6 ± 3.59	< 0.01
ESR (mm)	31 ± 8.17	11 ± 3.29	< 0.01
N.S. (> 0.05): Non-significant, <0.05: S: Significant, <0.01: H.S: highly significant. WBC: White Blood Cells; GRA: Granulocyte; LYM: Lymphocyte; MON: Monocyte; RBC: Red Blood Cells; HGB: Hemoglobin; PLT: Platelet; HCT: Hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; CRP: C-Reactive Protein; ESR: Erythrocyte Sedimentation Rate			

Among the included cases, 20 (66.6%) had early onset sepsis (EOS) with positive blood culture in 8 (40.0%) of them, while the remaining 10 (33.3%) had late onset sepsis (LOS) and blood cultures were positive in 4 (40%). Group B. Streptococci and E. coli were the most frequent causative organisms in EOS group (62.5%, and 20.0% respectively), while, in LOS group, Staphyloccus aureus, Klebsiella and Pseudomonas were the frequently causative organisms (50%, 25% and 25.0% respectively) (Table 3).

Table (3): Blood culture and causative organisms among septic newborns (EOS & LOS).

Blood cultures (No., %)	**Early onset sepsis, (EOS) (n=20, 66.6%)**		**Late onset sepsis, (LOS) (n=10, 33.3%)**	
	Positive blood culture	**Culture Negative blood culture**	**Positive blood culture**	**Negative blood culture**
	8 (40.0%)	**12 (60.0%)**	**4 (40%)**	**6 (60%)**
Causative organisms (No., %	Early onset sepsis, (EOS) (n=8)		Late onset sepsis, (LOS) (n=4)	
	Group B. Streptococci	5 (62.5%)	Staph. aureus	2 (50.0%)
	E. coli	2(20.0%)	Klebsiella	1 (25.0%)
	Listeria monocytogens	1 (17.5%)	Pseudomonas	1 (25.0%)

There were significant higher mean serum levels of NO and MDA (36.21 (µmol/l) ±15.13, and 4.75 (nmol/ml) ±4.64 respectively) with significant lower mean TAO level (0.14 (mm/l) ±0.11) among neonates with sepsis

when compared with the control group, with p value <0.01 for all, while, there were non-significant differences between oxidative stress biomarkers among EOS and LOS groups, with p value >0.05 for all (Table 4).

Table (4): Comparison between serum oxidants (Nitric oxide & Malondialdehyde) and total antioxidant capacity among the study groups.

Variables		**Neonatal sepsis group (n=30)**	**Control group (n=30)**	**P value**
Oxidants	Nitric oxide (µmol/l)	36.21 ± 15.13	25.95 ± 4.64	<0.01
	MDA(nmol/ml)	4.75 ± 4.64	2.13 ± 1.74	<0.01
Total Antioxidant (mm/l)		0.14 ± 0.11	0.45 ± 0.12	<0.01
Variables		Early onset septic neonates (n=20)	Late onset septic neonates (n=10)	P value
Oxidants	Nitric oxide (µmol/l)	35.12 ±13.45	33.15 ±16.57	>0.05
	MDA(nmol/ml)	4.10 ± 0.25	3.95±0.15	>0.05
Total Antioxidant (mm/l)		0.13 ± 0.12	0.12 ± 0.61	>0.05
*N.S. (>0.05): Non-statistically Significant, <0.05: Statistically significant, <0.01: highly statistically significant.				

DISCUSSION

Morbidity and death from bacterial infections represent significant portion among neonates.[14] Neonatal sepsis is prevalent due to the defective infection control.

Measures, insufficient nursing staff and exposure to adult infection with underestimated incidences.[15] in addition to birth condition, pregnancy control or maternal morbidities. The current study revealed a higher portion of the included neonates with sepsis had their mothers with associated co-morbidities in the form of fever, premature rupture of membranes and urinary tract infection. Our findings were in agreement with many investigators.[16-20] In addition, neonates with sepsis exhibited significantly lower Apgar score at first min than the controls which were in line with many studies.[19-22] This could be explained by the fact that neonates with low Apgar score may indicate poor accommodation with the extrauterine life resulting from labour stress and therefore more susceptible to infection as stated by Adatara et al.[19]

Complete blood count (CBC) is frequently used to evaluate the likelihood of infection.[23] In the current study, there were significant higher white blood cell and granulocytes counts, with lower hemoglobin level and platelet counts among septic neonates when compared with the controls. These results agreed with many studies.[24-26] Surapanenik and Vishnu,[26] reported that the membrane of the erythrocytes in the neonates is frequently subjected to oxidative stress due to pro-oxidant predominance. Cellular products of the microorganisms enhance platelet clumping and adherence with subsequent destruction and thrombocytopenia.[27] The significantly higher CRP and ESR noticed among the included septic neonates in our study were in agreement with many studies.[28-30]

The sensitivity of blood culture is low in neonatal sepsis and many factors may affect its accuracy e.g. timing and numbers of cultures taken, organism concentration, culture medium, temperature, technique and blood volume.[31] The current study revealed that 40.0% of neonates with EOS and 40% of the included neonates with LOS have positive blood culture with Group B. Streptococci, Staph. aureus, Klebsiella, E. coli and Pseudomonas among the most frequent causative organisms. These results were in line with many investigations.[32-34]

The immune activation induced by sepsis, will initiate the redox cascade with subsequent production of reactive oxygen and nitrogen species,[35] e.g. nitric oxide (NO). The present study revealed the occurrence of oxidative stress in neonatal sepsis evidenced by significant higher oxidants (NO and NDA) associated with significant lower total antioxidant capacity among the included neonates with sepsis versus the controls, with a non-significant difference in the oxidative stress status regarding the onset of sepsis (EOS and LOS). These results were in accordance with many studies.[36-41] Chuang et al.[42] reported that serum TAO could be used as a marker for clinical severity of sepsis, suggesting that its lower levels in patients with severe sepsis could be explained by the associated excessive pro-inflammatory cytokines and immune activation in such patients.

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