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Assessment of Oxidative Stress in Pediatric Sepsis

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Abstract: Background: Sepsis is at present one of the leading causes of morbidity and mortality in the children population. We performed this study to evaluate the levels of certain antioxidants in the blood of children with sepsis. ***Methods*:** Study group had 48 children with sepsis (0.3 ±11 years) and 20 age -matched healthy controls admitted to Pediatric intensive care unit (pICU) at Mansoura University Children Hospital (MUCH), Mansoura, Egypt. Interleukin 6 (IL6), Malondialdehyde (MDA), Catalase (CAT), superoxide dismutase (SOD) and Glutathione (GSH) by standard techniques. ***Results:*** There was significant elevation in the level of IL6 and MDA in sepsis and correlated with the severity of sepsis when compared to controls. However, GSH level was significantly reduced in septic patients compared to healthy control cases. While, no significant changes in the activity of SOD and CAT in patient compared to healthy cases. Catalase was significantly and positively correlated to glutathione and superoxide dismutase. MDA was significantly but negatively correlated to serum catalase, SOD and GSH levels in the studied septic children. Sepsis severity was significantly but negatively correlated to serum glutathione level. ***Conclusion:*** Our observations show that the toxicity of free radical induced by sepsis affected on pediatric patients by sepsis probably adapt to. These results may be due to the adaptive response of the body to compensate the oxidative stress and active phagocytosis

keywords: Interleukin 6, Superoxide dismutase, Catalase, Glutathione, Lipid peroxidation , Paediatric Sepsis , Mortality.

1.Introduction

Sepsis is a serious clinical syndrome that occurs due to the body's excessive response to stimulation of microorganism products and is characterized by an acute onset and high fatality rate. Sepsis often leads to serious pathophysiological alterations in the vital organs **[1]**. Some factors correlated with sepsis in developing countries were low socio-economic status, poor-hygienic environment and other concomitants diseases, such as diabetes mellitus, immunosuppression, malnutrition, and cancer **[2]** and also impact on high annual economic burden **[3]**. Sepsis affects millions of people worldwide annually and has a 30–50% mortality rate **[4].** Sepsis as an acute response to a pathogen or its toxin in the early stage promotes the elevation of pro-inflammatory cytokine levels, also known as cytokine storm, leading to high fever, tachycardia, and tachypnea **[5].**

Sepsis can be divided into severe sepsis and septic shock which is an important cause of death. Sepsis is a significant health problem since it is one of the frequent causes of mortality in the non-coronary intensive care unit (ICU) **[6].** The mortality of patients with sepsis-induced brain injury combined with sepsis shock and multiple organ dysfunctions have been reported to be as high as 80-90% worldwide **[7].** The mortality rate depends on the level of disease severity.

The activation of leukocytes and some inflammatory cells due to response to critical illness involves leading to a huge production of reactive oxygen species (ROS). ROS mediated

oxidative stress has been implicated in apoptotic cell death and as a result it can be harmful to the person when the endogenous antioxidant defense mechanisms are overwhelmed **[8].** Alterations in the apoptotic mechanism may underlie the dysregulation of the inflammatory action that occurs during sepsis. It is well known that ROS is involved in the pathogenesis of multiple organ failure after infection with sepsis, often leading to death **[9].** So, our context aimed to assess free radical toxicity in pediatric septic ICU children in comparison to healthy controls and studying if their levels are related to sepsis severity and outcome

2. SUBJECT and METHODS

This study was performed in the Pediatric intensive care unit (pICU) at Mansoura University Children Hospital (MUCH), Mansoura, Egypt between 2019- 2020 and was approved by the Ethical Committee of the University Hospital and performed only after parental consent. Sepsis was diagnosed primarily according to the presence of both clinical evidences of infection and systemic inflammatory response (SIRS) that is characterized by specific physiological alterations, including aberrations in heart rate (HR) respiratory rate (RR), temperature and total Leukocyte count (TLC).

Eighty four children (27 males and 21 females) with sepsis diagnosed within the previous 12 h were investigated along with twenty healthy children. The age of these patients 1.0 year with mean 0.3±11.0 years. The healthy group included 12males and 8 females, The age of these healthy children 1.0 year with mean 0.3±11.0 years. Patients were categorized as (10 sepsis, 30 severe sepsis (infection was severe enough to cause organ failure (heart, brain or kidney) and 8 with septic shock (sepsis accompanied by very low arterial blood pressure and requiring vasopressors to maintain mean arterial pressure).

Sample collection and preparation: Blood (5 ml) was collected from all septic and healthy pediatrics at the time of confirmed diagnosis before starting treatment on admission to the hospital. Collected blood samples were collected in dry tubes and lifted 30 minutes to clot and then centrifuged 3000 rpm for 15 minutes and obtained serum was divided into parts and stored at -20°C until performing the analyses of biochemical markers. IL6, MDA, CAT, SOD and GSH were estimated (Abbexa Ltd.) using Human ELISA Kit

Statistical Analysis:

The collected data is analyzed using Statistical package for Social Science (IBM Corp. Released 2017, IBM SPSS Statistics for Windows, version 25.0. Armonk, NY: IBM Corp.). Mann Whitney Test (U test) was used to assess the statistical significance of the difference of a non-parametric variable between two study groups. Correlation analysis was used to assess the strength of association between two quantitative variables. The correlation coefficient defines the strength and direction of the linear relationship between two variables. Logistic regression analysis is used for prediction of risk factors, using generalized linear models. p value is considered significant if <0.05 at confidence interval 95%.

3. RESULTS

1. Demographic and biochemical data among studied groups

Table (1) represents demographic data of the present study, which conducted on 48 sepsis cases [their median age was 1.0 year, ranged from 0.3 to 11 years]. Both males and females were included, they were 27 males (56.3%) and 21 females (43.8%)] and 20 healthy control children [their median age was 1.0 year, ranged from 0.3 to 11 years. Both males and females were included, they were 12 males (60%) and 8 females (40%)]

Also, Table (1) shows biochemical markers levels [inflammatory marker interleukin-6 (IL-6), oxidative stress lipid peroxidation marker (MDA), and the serum antioxidants (Catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH)] of healthy control and sepsis cases. The levels of IL-6 and MDA showed significantly elevation in the septic children compared to healthy children. On the other hand, GSH level showed significant decrease in septic group when compared to healthy group. Moreover, the activities of CAT and SOD showed no significant change between sepsis and healthy cases as shown in Figures (1- 5)

Table (1): Comparison of demographic and biochemical data among studied groups

		Control		Septic Cases		*p*
		N=20		**N=48**		
		median	**range**	**median**	**range**	
Age		1	0.3-11	1	0.3-11	0.584
Gender	Males	12	60%	27	56.3%	0.776
	Females	8	40%	21	43.8%	
IL6 (ng/L)		7.5	3.6-15.3	24.9	3.2-642	0.001
MDA (nmol/mL)		4.1	3.6-4.6	9.4	0.6-35	<0.001
CAT (ng/mL)		5.5	3.2-7.8	4.9	0.3-11.7	0.696
SOD (U/mL)		140	119-161	124	24-235	0.140
GSH (ng/mL)		146	140-153	116	13-230	0.001

Values are shown as median (inter quartile range)

*P<0.05 compared to controls

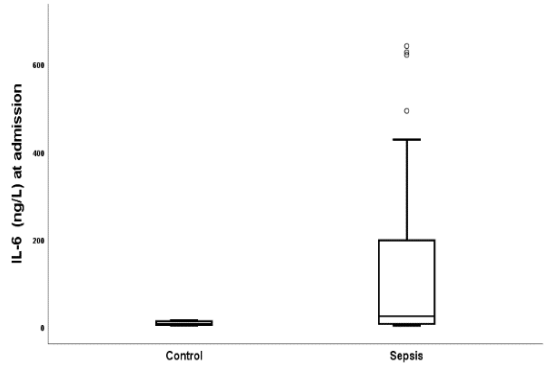


Figure (1): IL-6 level at admission in the septic and heathy control groups

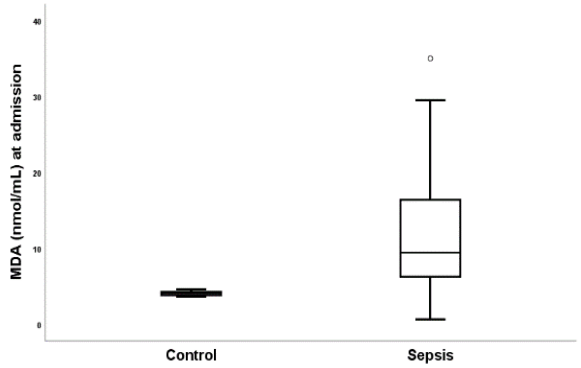


Figure (2): MDA level at admission in the septic and heathy control groups

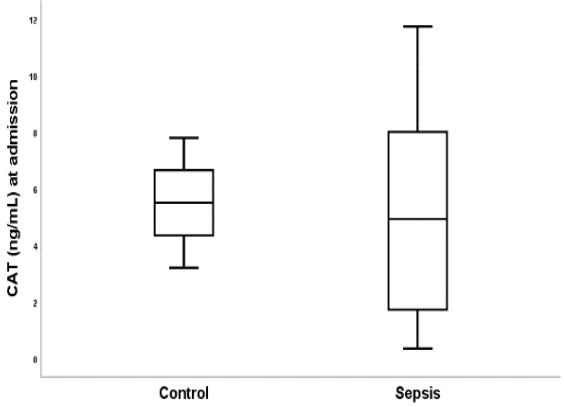


Figure (3): Catalase activity at admission in the septic and heathy control groups

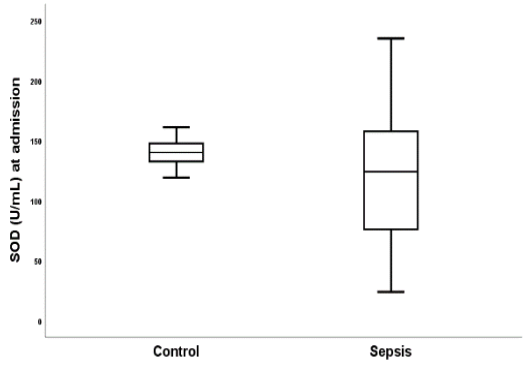


Figure (4): SOD activity at admission in the septic and heathy control groups

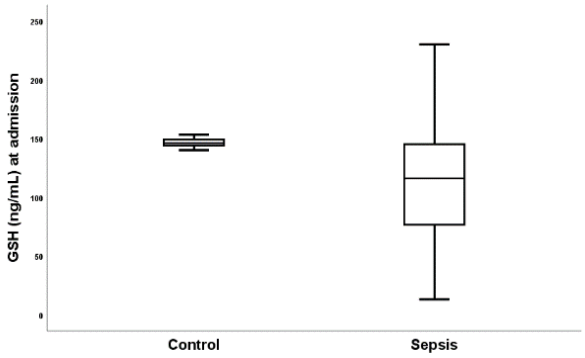


Figure (5): GSH level at admission in the septic and heathy control groups

2. Biochemical data according to severity of sepsis

Table (2) represents a comparison between biochemical markers levels (IL-6, MDA, CAT, SOD, and GSH) and sepsis severity [sepsis (10 cases), severe sepsis (30 cases), and septic shock (8 cases)]. The presented data recorded that IL-6 concentration was increased gradually with severity of sepsis as follow: sepsis (median is 16.9), severe sepsis (median is 24.9), and septic shock (median is 110.8). However, MDA concentration was increased with severe sepsis. While CAT activity and GSH level were

decreased with sever sepsis. Moreover, SOD activity was decreased with septic shock

P1, comparison between all grades

P2, comparison between sepsis and severe sepsis

P3, comparison between sepsis and septic shock

P4, comparison between severe sepsis and septic shock.

P5, comparison of sepsis versus severe sepsis + septic shock.

P6, comparison of sepsis + severe sepsis versus septic shock.

Table (2): Comparison of biochemical data according to severity of sepsis

		Sepsis	**Severe sepsis**	**Septic shock**	***P1***	***P2***	***P3***	***P4***	***P5***	***P6***
		N=10	**N=30**	**N=8**						
IL6	Median	16.9	24.9	110.8	0.046	0.731	0.014	0.037	0.980	0.028
	range	7-621.9	3.2-642	7.7-391						
MDA	Median	6.5	11	8.2	0.136	0.078	0.689	0.197	0.121	0.383
	range	0.6-19	1.2-35	0.6-25.5						
CAT	Median	5.5	4.7	5.2	0.401	0.195	0.563	0.543	0.223	0.771
	range	0.9-11.7	0.3-9.7	0.7-8.8						
SOD	Median	137.5	121.5	119.5	0.446	0.241	0.265	0.929	0.204	0.782
	range	52-211	24-235	42-177						
GSH	Median	130	108	111.5	0.318	0.018	0.014	0.900	0.034	0.580
	range	43-199	13-230	40-140						
	range	0.2-0.4	0.1-0.5	0.1-0.3						

Table (3): Correlation of biochemical data with length of ICU stay

	1 day		**3 days**		**10 days**		***p***
	N=18		**N=18**		**N=12**		
IL6	31	3.3642	132.4	3.2-628	8.7	3.9-428.1	0.962
MDA	6.5	0.6-19	11	1.2-35	8.2	0.6-25.5	0.448
CAT	5.5	0.9-11.7	4.7	0.3-9.7	5.2	0.78.8	0.569
SOD	137.5	52-211	121.5	24-235	119.5	42-177	0.681
GSH	130	43-199	108	13230	111.5	40-140	0.728

Table (4): Comparison of biochemical data according to survival in all studied case

	Survived		**Not survived**		***P***
	N=22		**N=26**		
	Median	**range**	**Median**	**range**	
IL6	17.9	3.2-628	31.01	3.3-642	0.042
MDA	7.9	1-27	11.7	1-35	0.048
CAT	5.9	0.6-8.4	4	0.3-11.7	0.385
SOD	135	40-179	121.5	24-235	0.521
GSH	126.5	38-160	98.5	13-230	0.043

p value is significant <0.05.

Table (5): Correlation of studied markers with each other

	MDA		**CAT**		**SOD**		**GSH**	
	rs	***p***	***rs***	***p***	***rs***	***p***	***rs***	***p***
IL-6	0.234	0.110	0.171	0.246	0.212	0.148	0.156	0.289
MDA		.	0.863	<0.001	0.873	<0.001	0.872	<0.001
CAT				.	0.944	<0.001	0.913	<0.001
SOD						.	0.936	<0.001
GSH								.

rs, correlation coefficient; p value is significant <0.05.

3. Correlation of Biochemical data of septic children with length of ICU stay

As shown in Table (3), no significant association between the studied biochemical markers levels in the septic children such as IL-6, MDA, CAT, SOD, and GSH with length of ICU stay through 1day, 3 day, and 10 days).

4. Biochemical data according to survival in septic cases

Table (4) represents a comparison of studied biochemical markers (IL-6, MDA, CAT, SOD, and GSH) according to survival in septic cases. The data showed that significant elevation in the levels of IL6 (median: 31.01) and MDA (median: 11.7) in died cases and associated with mortality, while lower GSH levels were significantly not survived and associated with mortality.

5. Correlation of studied markers with each other

Table (5) represents correlation analysis of studied markers (IL-6, MDA, CAT, SOD, and GSH) with each other depending on correlation coefficient (rs). The presented data recorded that: MDA showed significant negative correlation with CAT, SOD, and GSH levels. CAT showed significant positive correlation with SOD and GSH. SOD showed significant positive correlation with GSH.

6. Correlation of studied markers with sepsis severity

Table (6) represents correlation analysis of studied markers (IL-6, MDA, CAT, SOD, and GSH) with sepsis severity depending on correlation coefficient (rs). The presented data recorded that sepsis grade (sepsis, sever sepsis, and septic shock) showed significant positive correlation with IL-6 [Figure 6(A)] and significant negative correlation with GSH [Figure 6(B)].

7. Logistic regression analysis to predict mortality

Table (7) shows logistic regression analysis for prediction of mortality using age, gender, sepsis grade, IL-6, MDA, CAT, SOD, and GSH as covariates. The presented data showed that higher concentrations of IL-6, MDA and lower GSH level were suggested to be independent predictors of death in uni- and multivariate analyses.

Table (6): Correlation of studied markers with sepsis severity

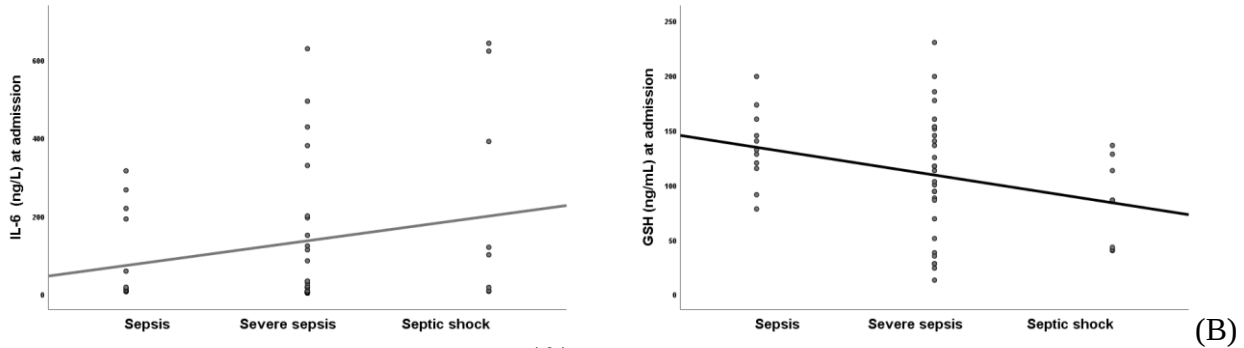
	Sepsis severity	
	rs	***p***
IL-6	0.233	**0.025**
MDA	0.033	0.757
CAT	-0.049	0.648
SOD	-0.133	0.214
GSH	-0.213	**0.045**

rs, correlation coefficient; p value is significant <0.05.

Table (7): Logistic regression analysis to predict mortality

	Univariate				**Multivariate**			
	p	**OR**	**95% CI**		***p***	**OR**	**95% CI**	
Age	0.638	1.025	0.924	1.137				
Gender	0.736	1.134	0.545	2.357				
Sepsis grade at day 1	0.284	0.691	0.352	1.359				
IL-6	0.025	1.399	1.097	1.301	0.029	1.400	1.198	1.602
MDA	0.014	1.626	1.182	1.971	0.027	1.556	1.157	1.865
CAT	0.338	0.932	0.807	1.076				
SOD	0.511	0.995	0.978	1.009				
GSH	0.047	0.994	0.990	0.998	0.048	0.986	0.990	0.998

OR, odds ratio; CI, confidence interval; p value is significant <0.05.



(A) (B)

Figure (6): Correlation of IL6 (A) and GSH (B) with sepsis severity

4. Discussion

Sepsis is the leading cause of death worldwide in the pediatric population resulting in an estimated 7.5 million deaths annually **[10].** It encompasses the top four causes of childhood mortality as reported by the World Health Organization **(WHO)**: severe pneumonia, severe diarrhea, severe malaria, and severe measles. In the United States alone, there are 72,000 children hospitalized for sepsis

with a reported mortality rate of 25% and an economic cost estimated to be $4.8 billion **[11].**

The present work aimed to study the serum levels of some antioxidants such as catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) and the anti-inflammatory marker IL-6 and lipid peroxidation markers (MDA) in pediatric septic ICU children in comparison to healthy controls.

The present study was conducted on 48 sepsis cases [their median age was 1.0 year, ranged from 0.3 to 11 years. Both males and females were included, they were 27 males (56.3%) and 21 females (43.8%)] and 20 healthy control children [their median age was 1.0 year, ranged from 0.3 to 11 years. Both males and females were included, they were 12 males (60%) and 8 females (40%)]. From our results, the studied markers such as oxidative stress, and inflammatory in the blood samples of septic patients and healthy control were confirmed to be significantly higher in septic patients versus controls, these results are in accordance with the observation of Cancelier et al., **[12]**. Especially, malondialdehyde (MDA), were increased as well as IL-6 levels in patients with sepsis, and the results were confirmed clinically highly probable, compared to the healthy group. These results are in agreement with the findings of **[12, 13].** However, the level of GSH was significantly reduced in sepsis patients compared to the levels in healthy control. These results are in agreement of the findings of **[13].** CAT and SOD activities did not differ significantly between sepsis cases and controls as in concert with these finding, in newborn sepsis **[14]**. According to our results, in septic patients, the induced oxidative stress and antioxidant defenses distributed. Leading to oxidative damage in the body and the elevation of the oxidative markers compared to healthy controls **[15]**. Moreover, the levels MDA and IL-6 in septic patients showed a highly significant correlation with severity of sepsis, while, a positive correlation was detected between these markers and mortality of sepsis **[16]**. Since some signs of oxidative stress have a strong correlated with markers of antioxidant defense, so, the relationship between markers of oxidative stress and severity of sepsis will have strong significance in the Pediatric intensive care unit **[17]**. Despite at present, CAT and SOD activities and GSH level in septic children showed opposite relation with clinical severity degree of sepsis and motility **[18, 19].** These data are agreement with Plotnikov et al., **[20]** who found that the activation of prooxidant markers and overproduction of ROS due to sepsis, paralleled by elevation the activity of antioxidant systems, resulting in detrimental cellular effects, as demonstrated by increased markers of oxidative damage. Comparison of multivariate logistic analysis for MDA, IL-6 and GSH appeared that MDA and IL-6 are better predictor of admission of septic children.

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