ORIGINAL ARTICLE

Impact of Oxidative Status in Infant and Children with Cyanotic and Acyanotic Congenital Heart Diseases

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ABSTRACT

Background: There are few studies regarding the role of oxidant status (OS) in children with congenital heart defects. We believed that CHD has led to increased levels of oxidative stress and inflammation and decreased antioxidant potential. This study aimed to investigate the oxidative status and its impact on the heart in infants and children with congenital cyanotic and acyanotic heart diseases.

Methods: This case control study was carried out in cardiology Unit, Pediatrics department in Zagazig University Hospital from April 2017 to October 2017. 90 infants and children were included in the study, their age ranged from 2 months and 4 years they were divided randomly into three groups. Group 1: 30 children with cyanotic congenital heart defect, group 2: 30 children with acyanotic congenital heart defect and group 3: 30 healthy children as control group. All patients were evaluated clinically and investigated for routine laboratory in addition to Oxygen saturation measurement by pulse oximeter, Serum malondialdehyde and Echocardiography using Two-dimensional and M-mode measurements were obtained.

Results: There was high statistical significant difference among three studied groups regarding oxygen saturation, which was normal in control group and lower in patient group and levels of Serum malondialdehyde, was highest among cyanotic heart defect group.

Conclusions: Children with congenital heart diseases are susceptible to oxidative stress especially in those with cyanotic congenital heart diseases reflected by significant decrease of Partial Pressure of Oxygen and significant increase of Serum malondialdehyde (MDA) in cyanotic group when compared with the other 2 groups.

Keywords: Congenital heart defects (CHD), oxidative stress, prooxidant and antioxidant factorso, malondialdehyde.

INTRODUCTION

Congenital heart defect may be defined as an issue in the heart structure that is present at birth. Congenital heart defects are usually evaluated in two groups: cyanotic and heart defects of acyanotic nature. In the case of non-cyanotic heart defects, a structural deformity causes blood to flow from the left side of the heart to the right side of the heart. Individuals with shunting from left to right often retain normal levels of saturation of oxyhemoglobin in the systemic circulation[1]. Many defects do not need treatment, but medication or surgery are required for some complex congenital heart defects. Children with congenital heart defects are vulnerable to oxidative stress, cyanotic or acyanotic[2].

Oxidative stress comes from an imbalance between a human body's oxidant status and antioxidant defense mechanisms. Oxidative stress has been well established to induce apoptosis and the

generation of reactive oxygen radicals that may be responsible for cellular damage [3]. Understanding the mechanisms underlying cardiovascular disease pathogenesis (CVD) is extremely important, because CVD is the world's leading cause of morbidity and morbidity [4]. The present study aims to investigate the oxidative status and its impact on the heart in infants and children with congenital cyanotic and acyanotic heart diseases.

METHODS

This case control study was carried out in cardiology Unit, Pediatrics department in Zagazig University Hospital from April 2017 to October 2017. 90 infants and children were included in the study, their age ranged from 2 months and 4 years there were divided randomly into three groups. Group 1: 30 children with cyanotic congenital heart defect, group 2: 30 children with acyanotic congenital heart defect and group 3: 30 healthy children as control group. All patients were evaluated clinically and investigated for routine laboratory in addition to Oxygen saturation measurement by pulse oximeter, Serum malondialdehyde and Echocardiography using Two-dimensional and M-mode measurements were obtained. Inclusion criteria: All patients with congenital heart diseases are included in the study except those with exclusion criteria: Patient with the following diseases: Infection, Mal-nutrient, Genetic syndromes, Chronic renal diseases, Chronic liver diseases, Chronic pulmonary diseases, Electrolyte disturbance disorders and Bleeding disorders. Also patients taking drugs affecting the results such as anabolic drugs e.g., Iron, vitamins or other antioxidants.

All study participants had been subjected to complete history taking, clinical examination regarding the temperature of the Vital signs, respiratory rate, blood pressure and heart rate.

Any patient had tachypnea, working ala nasi, tachycardia, grunting and inter costal retraction was considered to have respiratory distress. Color: cyanosis, pallor or jaundice. weight and height.

Laboratory Studies: All participants in the study had been subjected to complete history taking, clinical examination regarding the Vital signs temperature, respiratory rate, blood pressure and heart rate.Complete blood count including hemoglobin. C-reactive protein (CRP) to role out infection. Oxygen saturation measurement by pulse oximeter. Serum malondialdehyde (MDA): 0.5 ml each of the plasma was mixed with 2.5 ml of 20% 9 (v/v) trichloroacetic acid (TCA) and centrifuged for 10 min at 3000 rpm. The supernant was decanted, and treated with 0.2 g/dl TBA 3ml. 0.5ml of each plasma were mixed with 2.5 ml of 20 percent 9 (v/v) trichloroacetic acid (TCA) and

centrifuged at 3000 rpm for 10 min. The supernant was decanted and treated 3 ml TBA with 0.2 g/dl.

Echocardiography Examination:

All patient were subjected to trance- thoracic echocardiography using portable Echo sonosite (Sonosite 180 Elite sonoheart). For ejection fraction (EF) percent, fraction shortening (FS) percent, Lt ventricular mass and Lt ventricular size, two-dimensional, M-mode measurements were obtained. Pulsed -wave Doppler (PWD) echocardiography was used to measure the front filling (E-wave), atrial contractility (A-wave) and (E/A) ratio of mitral and tricusped peak. The pulmonary artery systolic pressure was estimated from the peak tricuspid regurgitation velocity (TRV) (m/s) using continuous wave Doppler and modified Bernoulli equation

$Spap = 4(v)^2 + RA$ pressure

Spap= systolic pulmonary artery pressure

V= velocity of tricuspid regurg. Jet .

RA pressure = Right arterial pressure.

Ethical Clearance: Written Informed consent for participation in the study was obtained from the patient's parents. Approval for the study was obtained from the Departments of Pediatrics and Clinical Pathology, Zagazig University Hospitals, following approval by the Institutional Review Board (IRB). The work was carried out in conformity with the World Medical Association Code of Ethics (Helsinki Decleration).

STATISTICAL ANALYSIS

Data was collected, coded, revised, and entered into version 20 of the Statistical Package for Social Science (IBM SPSS). For the qualitative data, mean, standard deviations and ranges for quantitative data with parametric distribution and interquartile range (IQR) median for quantitative data with non-parametric distribution, the data were presented as number and percentages. The chi-square test was used to compare two groups with quantitative data, and the exact Fisher test was used instead of the Chi-square test when the expected count in any cell was found to be less than five. Independent t-tests were used, and the Mann-Whitney test was used to compare two groups. The comparison of more than two groups with quantitative data and parametric distribution was made using the one way variance analysis (ANOVA) test and the Kruskall-Wallis test was used in the comparison of more than two quanti groupings.

RESULTS

Table (1) showed that groups were matched regarding sex and age and there was no statistical significant difference. There was a high statistical significant difference among three groups regarding BMI, which was normal among control group. Table (2), showed the distribution of

congenital heart diseases in cyanotic group that 40% of the patients were TOF, 23.3% TGA, 16.6% tricuspid atresia, 6.7% DORV, 6.7% ebestien and 6.7% single ventricle. Also it show the distribution of congenital heart diseases in acyanotic group that 43.33% of the patients were VSD, 23.33% PDA,10% ASD, 10% ASD+VSD and 13.33% ASD+PDA+VSD. Table (3), showed that there was a high statistical significant difference among three studied groups regarding levels of hemoglobin, heart rate and respiratory rate, which all were higher among cyanotic HD group. There was no significant difference among three studied groups regarding levels of platelet and WBCs. Table (4), showed that there was a high statistical significant difference among cyanotic and acyanotic HD groups regarding levels of EF, FS and Lt ventricular mass and PAP which were

higher in cyanotic HD. Table (5), showed the high statistical significant difference among three studied groups regarding oxygen saturation, which was normal among control group and levels of MDA, was highest among cyanotic HD group. Table (6), showed, as regard PO2 there were only significant difference between control vs cyanotic group and between cyanotic vs acyanotic group. As regard MDA, there were significant difference between each two group. Table (7), showed that there was a high statistical significant negative correlation between MDA and each of age, BMI, PO2 and EF and FS while there was a high statistical significant positive correlation with left ventricular mass and Lt ventricular size in cyanotic CH group. There was a high statistical significant negative correlation between MDA level and HB and PO2 level in control group.

Table (1): Demographic data of the three studied groups.

		Cyanotic HD (n=30)		A cyanotic HD (n=30)		Control (n=30)		(KW test)	p-value
		N	%	N	%	N	%		
Sex	Male	16	53.3	17	56.7	14	46.7	0.623	
	Female	14	46.7	13	43.3	16	53.3		0.732
Age month								10.1*	
Mean ±SD		6.7 ± 5.05		6.3 ± 5.14		6.4 ± 5.91			0.259
BMI								9.32	0.000**
Mean ±SD		18.2 ±3.21		21.91± 3.002		22.58 ± 4.52			

**P-value < 0.005 is high significant

Table (2): Types of CHD presented among studied group.

Cyanotic HD	N (n=30)	%
Tetralogy of fallot (TOF)	12	40
Transposition of great arteries (TGA)	7	23.3
Tricuspid atresia	5	16.6
Double outlet RT ventricle	2	6.7
Ebestein	2	6.7
Single ventricle	2	6.7
ACYANOTIC HD	N (n=30)	%
VSD	13	43.33
PDA	7	23.33
ASD	3	10
ASD+PDA	3	10
ASD+PDA+VSD	4	13.33

Table (3): Clinical and laboratory data of the three studied groups.

	Cyanotic HD (n=30)	A cyanotic HD (n=30)	Control (n=30)	F test*	p-value
HR(beat/min) Mean ±SD	148.5 ± 16.5	130.5 ± 11.14	90.5±14.95	124.1	0.000**
RR(breath\min) Mean ±SD	47.07 ± 7.21	34.91± 6.29	22.38±5.45	110.3	0.000**
HB(gm/dl)	17.14±2.4	18.4 ±1.11	11.55±0.89	114.6	0.000**
WBC(mm)	11.6±0.5	11.4±0.5	12.3±0.5	110.5	0.491
PLATELETS(X10/mm)	419.9±19.8	413.7±19.6	422.2±17.1	169.2	0.213

**P-value < 0.001 is high significant

Table (4): Echocardiographic measurements (ECHO) among cyanotic and a cyanotic HD groups.

	Cyanotic HD (n=30)	A cyanotic HD (n=30)	T -test	p-value
EF \% x ±SD	41.1 ± 7.13	48.7 ± 4.51	4.97	0.000**
F S\% x± SD	7 1.0±1.6	7 7.6±0.169	4.87	0.000
Lt ventricular mass(mm) x±SD	186.4 ± 17.1	174.4 ± 6.68	3.57	0.001**
Lt ventricular size(mm)x±SD	4.7 ± 0.737	4.9 ± 0.601	0.883	0.381
PAP(mmHg)x ±SD	44.33 ± 5.96	40.73 ± 5 .81	4.71	0.000*
E(x±SD)	0.067±0.004	0.071±0.004	1.43	0.316
A(x±SD)	1.69±0.11	1.94±0.120	2.44	0.119
E\A (x±SD)	0.074±0.003	0.114±0.002	5.89	0.2 65

**P-value < 0.001 is high significant

Table (5): Oxygen saturation and levels of malonaldehyde (MDA)among the three studied groups.

	Cyanotic HD (n=30)	A cyanotic HD (n=30)	Control (n=30)	F test*	p-value
PO2(%)	77.87 ± 7.48	92.7 ± 17.1	98.17 ±1.94	28.2	0.000**
MDA(nmol/ml) Mean ±SD	51.56 ± 12.56	24.45 ± 2.73	12.57 ±2.86	207.1	0.000**

**P-value < 0.001 is high significant

Table (6): Least significance difference (LSD) among three studied groups regarding oxygen saturation and MDA levels.

Variables	Groups		Mean difference	P -value
PO2 (%)	Control	Cyanotic HD	20.3	0.000**
		A cyanotic HD	5.5	0.05
	Cyanotic	A cyanotic	-14.8	0.000**
MDA(nmol\ml)	Control	Cyanotic HD	-38.98	0.000**
		A cyanotic HD	-11.86	0.000**
	Cyanotic	A cyanotic	27.12	0.001**

**P-value < 0.001 is high significant

Table (7): Pearson`s correlation between MDA and other demographic and clinical parameters among cyanotic and control cases.

Variables	MDA	
	r	P-value
Cyanotic		
Age (mon)	-0.405	0.002
BMI	-0.099	0.000
PAP(mmHg)	0.001	0.649
PO2(%)	-0.0756	0.000
EF(%)	-0.0823	0.000
F S (%)	-0. 675	0.000
Lt ventricular mass (mm)	0.0874	0.001
Lt ventricular size (mm)	0.318	0. 000
Control		
Age(mon)	0.408	5.9
BMI	0.206	6.1
HB(mmg	-0.235	0.000
PO$_2$(%)	- 0. 318	0.000

DISCUSSION:

Congenital heart disease has an approximate incidence rate of 8 per 1000 live births and thus, it is deemed to be one of the most important causes of mortality in infancy and it is the most common congenital defect at birth [5]. In the past, many CHD patients could not reach adulthood, but recent advances in medical treatment, interventional approaches, surgical methods, and postoperative care in early life have resulted in a rise in the number of adults with such defects. It is now expected that more than 90% of patients born with CHD will survive to adolescence and adulthood. Currently, the number of adults with CHD is estimated to be equal to or more than that of children with CHD [6] .Pathogenesis of congenital heart defects is complex and involves genetic, inflammatory and autoimmune mechanisms. In patients with uncorrected CHD, increased pulmonary pressure leads to vascular remodeling and endothelial dysfunction, secondary to an imbalance in vasoactive mediators which promotes vasoconstriction, inflammation thrombosis, cell proliferation, impaired apoptosis and fibrosis [7] .The altered hemodynamic condition has been investigated for children with congenital heart defects. Consequently, early disruption of the reduction-oxidation balance and the presence of oxidative stress in the pulmonary vasculature was deemed essential for endothelial dysfunction and vascular remodeling [8]. Free radical are reactive compounds that are produced normally in the body. They can have positive or negative effects in our body. To limit the harmful effect, the organism need a strong antioxidant system. The antioxidant system consists of enzymes such as catalase, glutathione peroxidase, and superoxide dismutase, and non-enzyme such as vitamin A, vitamin C, vitamin, Uric acid and glutathione. An imbalance between the oxidant and antioxidant systems can lead to state of oxidative stress and various disease [9]. It is thought that oxidative stress plays a particularly important role in the development of cardiovascular pathology and hence the by-products, such as MDA, formed as a consequence of OS products, have a potential use as disease progression markers and

consequently are the focus of current biomedical research.To our best knowledge, there are only a few reports in the literature concerning the role of OS in children with congenital heart defect (CHD) We hypothesized that CHD leads to an increase in the level of oxidative stress and inflammation and to a decrease in the antioxidant capacity. As regard prevelance of congenital heart diseases according to sex, there was statistical difference in our study between males and females which was higher in males as in study done by Animasahun et al. [10] as prevelance was higher in males.

The acyanotic patients were found to have significantly higher levels of BMI compared to cyanotic patients. In comparing the acyanotic group with the control, BMI levels were not significantly different as in the Pirinccioglu et al. [11] study Because BMI in the acyanotic group was higher than cyanotic, this may be due to factors that interfere with the growth of the baby: excessively rapid heart beat, High respiratory rate, reduced appetite, high energy requirements, decreased intake of food due to rapid breathing and exhaustion, frequent respiratory infections such as bronchitis or pneumonia, low digestive tract nutrient absorption and hypoxia.

The most frequent congenital heart defects among the acyanotic group in our study, were VSD, PDA and ASD and this agreed with Davari et al., [12] study, in which VSD, PDA, ASD were the most common heart defects.As regard types of cyanotic cardiac lesions of our studied patients were include cases of Fallot tetralogt, TGA, tricuspid atresia, DORV and Single ventricle, that agreed with, Animasahun et al. [10] study in which the included congenital cyanotic heart defects were, Fallot tetralogt, DORV, TGA. We found that isolated heart defects were more common than multiple defects in our study This agreed with, Animasahun et al. [10] study, in which, isolated lesions were more than multiple lesions. Study of Pirinccioglu et, al, [11] had the same results. In our study, there is high statistical significant difference among three studied groups regarding levels of heart rate and respiratory rate, which were all higher among cyanotic HD group. This may be due to hypoxia leading to acidosis which stimulate

sympathetic nervous system leading to increase heart rate, also acidosis stimulate respiratory centre leading to increase respiratory rate[13]. There was also a high statistically significant difference in the level of hemoglobin between three studied groups which was higher in the cyanotype community than the others. This was may be due to hypoxia which stimulate erthropioesis. This agreed with Pirinccioglu et, al [11] which had the same results. In our study, there was a high statistical significant difference among cyanotic and acyanotic HD groups regarding levels of EF, FS and Lt ventricular mass and PAP which were higher among cyanotic group. It was due to the cardiac hypoxia caused by the disproportion between supply and demand for oxygen. Because of high coronary arteriovenous differences, the myocardium can not bring about substantial improvements in the supply of oxygen by increasing the extraction of oxygen from the blood. A chronic oxygen shortage results in increased pulmonary vasoconstriction, which redistributes pulmonary blood flow from low to high PO2 regions. Chronic pulmonary vasoconstriction may lead to pulmonary hypertension, which may increase the afterload on the right ventricle, eventually leading to heart failure [14].

In our study there is high statistical significant difference among the three studied groups regarding the levels of MDA, which was higher among cyanotic CHD group [15]. Pirinccioglu et al. agreed with our study as they found that the level of oxidative stress in patient with cyanotic congenital heart disease was significantly high their findings were explained by hypoxia which increase oxygen free radical. Also, found that lipid peroxidation, as evaluated by MDA level was enhanced in congenital cyanotic heart disease due to imbalance between prooxidant and oxidant reactions associated with congenital heart defect pathology in infants.

Also Pirinccioglu, et al, [11] found that MDA level was significantly increased in patient with CHD than normal and also higher in patient with cyanotic CHD than acyanotic CHD this result was due to reduced TACs (Total Antioxidants Capacity) leading to increased free radicals, which caused oxidative

stress which lead to more lipid peroxidation. They postulated that cyanotic CHD hypoxia was expected to consume the antioxidant reserve capacity, leading to increased oxidative stress susceptibility. In contrary to our finding, Eman and elHamid found that lipid peroxide MDA was significantly increased in acyanotic group than both cyanotic and control group and no significant difference between cyanotic and control group. This may be due to pathological changes in pulmonary vasculature in acyanotic group more than cyanotic group leading to increase MDA level in a cyanotic group.

In our study, there was high statistical significant negative correlation between MDA and PO2 in cyanotic group Also in our study, there was negative correlation between MDA level and PO2 in a cyanotic group. This due to effect of hypoxia on oxidant and pro oxidant balance which lead to oxidative stress and more lipid peroxidation leading to increase in MDA in the serum. Also in our study, there was high statically significant negative correlation between MDA level and HB and PO2 level in control group.

Limitations of our study: Further studies on large scale are required with larger sample volumes and longer follow-up durations should be carried out to determine the impact of oxidative stress on vascular wall function in infant and children with CHD.

CONCLUSIONS:

Children with congenital heart diseases are susceptible to oxidative stress especially in those with cyanotic congenital heart diseases reflected by significant decrease of Partial Pressure of Oxygen (PO2) and significant increase of Serum malondialdehyde (MDA) in cyanotic group when compared with the other 2 groups. In congenital heart defects serum malondialdehyde may be used as a new diagnostic biomarker.

RECOMMENDATIONS:

The serum malondialdehyde can be used as an oxidative stress biomarker in congenital heart defects, the findings may provide new etiological clues about the underlying mechanism of congenital heart defects if done with further investigations at molecular level.

conflict of interest: there are no conflicts of interest in this study.

Financial disclosure:

There are no financial conflicts of interest to disclose. This study did not receive any kind of financial support funding, grants or sponsorship. There is also no commercial or financial relationship that might have the potential of being viewed as a conflict of interest and will bias the results of our research in any way

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