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Asymmetric Dimethyl Arginine Level in Children with Sickle Cell Disease: A Cross Sectional Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Sickle cell disease (SCD) consists of a group of hemoglobinopathies in which individuals inherit hemoglobin variants derived from single point mutations. Asymmetric dimethylarginine (ADMA) contributes to limiting Nitric Oxide (NO) bioavailability in SCD. The aim of the present study was to assess the level of the Asymmetric Dimethyl Arginine in children with sickle cell.
Methods: This cohort cross-sectional study was carried out on 60 children which were divided in to 3 equal groups. Group I: SCD children with sickle retinopathy. Group II: SCD children without retinopathy. Group III: healthy control children who were selected from the outpatient clinic.
Results: There was a significant increase in ADMA level among participants withSCD. There was a positive significant correlation between ADMA level and family history as well as the incidence of hepatomegaly. There was no significant correlation between ADMA level and demographic and laboratory parameters except LDH.
Conclusions: The level of ADMA is elevated in children with sickle cell anemia. High plasma ADMA level is a risk for hepatomegaly in children with sickle cell anemia.

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1. INTRODUCTION

Sickle cell disease (SCD) consists of a group of hemoglobinopathies in which individuals inherit hemoglobin variants derived from single point mutations, that cause morphological abnormalities in the red blood cells (RBCs). RBCs of SCA individuals are less flexible since the polymers lead to morphological and biochemical changes and hence they impair the blood flow causing vaso-occlusion (VO) [1].

However, no longer valid is the simplistic explanation of sickle cells being solely responsible for causing vascular blockage or vaso-occlusion once red cells assume the pathognomonic sickle cell shape following exposure of the cell to deoxygenation. While vaso-occlusion is central to the understanding of the disease and can cause local hypoxemia with ensuing direct tissue injury and inflammation, the single gene mutation seen in sickle cell disease leads to complex physiologic changes. These changes result in the protean clinical manifestations of the disease [2].

SCD patients exhibit a wide range of clinical manifestations described through multiple studies. These clinical features are defined by chronic anemia, sepsis, hemolysis and recurrent acute vaso-occlusive crises. The last is characterized by pain and systemic inflammatory response that may be severe, episodic and unpredictable. In addition to different clinical manifestations, laboratory parameters are also important biomarkers useful for the patients' follow-up due to the possibility to monitor anemia, hemolysis, leukocytosis, endothelial dysfunction and to predict many clinical manifestations [1,3].

Nitric oxide (NO) plays a fundamental role in maintaining a normal vasomotor tone. Recent clinical and experimental data suggest that NO may play a role in the pathogenesis and therapy of sickle cell disease (SCD) [4]. Asymmetric dimethylarginine (ADMA) contributes to limiting NO bioavailability in SCD. ADMA is derived from the irreversible post-translational methylation of arginine residues by protein arginine methyltransferases (PRMT) and are released as free amino acids upon proteolysis. ADMA competitively inhibits NO synthase (NOS) enzymes, thereby limiting NO production [5].

This recent knowledge on the pathophysiology of sickle cell disease (SCD) has emphasized the role of hemolysis and nitric oxide (NO) depletion on the occurrence of acute and chronic complications. This new paradigm raises the possibility of innovative therapeutic approaches, including arginine supplementation [6].

The aim of the present study was to assess the level of the Asymmetric Dimethyl Arginine in children with sickle cell.

2. PATIENTS AND METHODS

The study included 60 children and were divided in to 3 equal groups. Group I: SCD children with sickle retinopathy. Group II: SCD children without retinopathy. Group III: healthy control children who were selected from the outpatient clinic. We excluded children with SCD who had diabetes mellitus or abnormal hepatic or renal function .

All patients included in this study were subjected to complete demographic and medical history with special emphasis on disease duration, family history of chronic hemolytic anemia and transfusion history (age of first blood transfusion, frequency, and dose of transfusion per month) and history of splenectomy, clinical examination including anthropometric measures, abdominal (liver, spleen and ascites) and neurological assessment were performed. Routine lab investigations such as CBC, lactate dehydrogenase, Seru ferriti, liver function tests, renal function tests, fasting and postprandial blood sugar, and plasma level of ADMA using a commercial ADMA ELISA Kit (Immundiagnostik AG) were also performed.

2.1 Statistical Analysis

Statistical presentation and analysis of the present study were conducted using SPSS. Quantitative variables were expressed as mean and standard deviation (SD) and were statistically analyzed by student t- test, Chi-square, linear correlation coefficient and analysis of variance [ANOVA] tests. $_P$ value ≤ 0.05 was considered statistically significant.

3. RESULTS

The patient groups and control group were matched as regard to the age, sex and

consanguinity with no significant difference Table 1.

Comparison between the three studied groups according to asymmetric dimethylarginine (ADMA) plasma level revealed significant difference between studied groups Table 2.

There were a positive significant correlation between ADMA level and family history as well as incidence of hepatomegaly Table 3.

There were no significant correlation between ADMA level and demographic and laboratory parameters except LDH units per liter Table 4.

4. DISCUSSION

In our study, there was no significant difference between males and females as regards the anthropometric measures, which reflects the growth and nutritional status. The results showed that both males and females had weights and heights below the standard for age and sex. Similar reports were obtained by Senbanjo et al. [7] who reported that growth retardation and under–nutrition are common in children with sickle cell disease.

Our study showed that the plasma ADMA level was significantly higher in patients than the control group. This comes in agreement with El-Shanshory et al. [8] who determined the plasma concentration for arginine metabolites in SCD patients and control group (ADMA mean plasma level), which was significantly higher in patients than in controls.

Elevated plasma ADMA concentrations leads to reduce NO bioavailability. Many causes of elevated ADMA concentrations occur in SCD [9]. The first cause is due to increased release of free ADMA by increased proteolysis associated with increased RBC turnover at a rate up to 20 times normal [10]. Furthermore, SCD is characterized by disturbance in the vascular wall which induces expression of endothelial type-1 protein arginine methyltransferase, a catalyst of arginine methylation [11].

Table 1. Demographic data and anthropometric measurements of patients

		Group I No=20		Group II No=20		Group III No=20		F	P-value
Age (years)	Range	4 - 14		3 – 12		5 - 14		2.226	0.117
	Mean ±SD	7.90 ± 2.69		7.50 ± 2.98		9.25 ± 2.55			
Weight (kg)	Range	15 - 50		13 – 39		17 - 50		1.577	0.216
	Mean ±SD	25.05 ± 8.92		24.50 ± 8.58		29.05 ± 9.03			
Height (cm)	Range	101 - 158		95 – 149		104 - 157		2.319	0.108
	Mean ±SD	125.80 ± 15.42		122.25 ± 18.06		133.05 ± 14.84			
		N	**%**	**N**	**%**	**N**	**%**	**(X^2)**	**P-value**
Sex	Male	11	55	11	55	15	75	2.256	0.324
	Female	9	45	9	45	5	25		
Consanguinity	Negative	8	40	10	50	12	60	1.600	0.449
	Positive	12	60	10	50	8	40		
Family history	Negative	8	40	6	30	-	-	0.440	0.507
	Positive	12	60	14	70	-	-		

Table 2. Asymmetric Dimethyl Arginine level of studied groups

ADMA (µmol/L)	Groups			ANOVA		TUKEY'S Test		
	Group I No=20	Group II No=20	Group III No=20	F	P-value	p_1	P_2	P_3
Range	0.7 - 2.3	0.21 - 1.55	0.03 - 0.49	39.036	<0.001*	0.002*	<0.001*	<0.001*
Mean ±SD	1.23 ± 0.48	0.80 ± 0.40	0.20 ± 0.15					

p_1: p value for comparing between group I and II
p_2: p value for comparing between group I and III
p_3: p value for comparing between group II and III

Table 3. Correlation of ADMA with different parameters

		ADMA		T-Test or ANOVA	
		N	Mean±SD	T or F	P-value
Sex	**Male**	22	1.03±0.51	0.246	0.807
	Female	18	1.00±0.48		
Consanguinity		22	0.98±0.50	0.495	0.623
Family history		26	0.89±0.48	2.309	0.026*
Pallor		7	0.92±0.74	0.561	0.578
Jaundice		0	0.00±0.00	-	-
Hepatomegaly		6	1.41±0.46	-2.244	0.031*
Splenomegaly		5	1.24±0.88	-1.110	0.274
Splenectomy		5	1.30±0.43	-1.409	0.167
Indication	Hemolytic crisis	4	1.25±0.48	-0.466	0.673
	Splenic sequestration crisis	1	1.50±0.00		
Frequency blood transfusion	Every 2 months	10	1.08±0.48	0.556	0.578
	Every 3 months	26	0.96±0.42		
	Every 4 months	4	1.22±0.91		

Table 4. Correlation of ADMA with different demographic and laboratory parameters

Correlations		
	ADMA	
	r	**P-value**
Age	0.214	0.185
Weight (kg)	0.184	0.255
Height (cm)	0.231	0.152
RBCs count	-0.094	0.566
HB	-0.028	0.863
Hct	0.039	0.810
MCV	0.094	0.564
MCH	-0.213	0.186
MCHC	-0.078	0.631
Platelet	-0.168	0.301
TLC	0.050	0.759
Retics	-0.031	0.847
Serum ferritin	-0.078	0.634
ALT	-0.162	0.318
AST	0.176	0.277
Bilirubin	0.200	0.217
Albumin	-0.053	0.744
Urea	0.308	0.053
Creatinine	0.040	0.805
Fasting blood glucose	0.062	0.706
Postprandial blood glucose	-0.005	0.978
LDH units per liter	0.468	0.002*
Disease duration (Years)	0.182	0.261
Age of splenectomy	0.617	0.268

In addition, Engin et al. reported the presence of a large store of protein –incorporated ADMA in close proximity to the vascular endothelium. This store may be released under certain pathological conditions. That study also demonstrated that blood itself is a potential contributor to the control of plasma ADMA levels [12]. Visser et al.also conclude that intact RBCs play an important role in the storage of ADMA, whereas upon its damage release large amount of free ADMA due to proteolysis of methylated proteins [13].

The second cause is due to impaired metabolism of ADMA following inhibition of DDAH by oxidative stress triggered by several risk factors such as, hypoxia and elevated levels of proinflammatory cytokines which down-regulate DDAH. Decreased DDAH activity associated with endothelial dysfunction Lüneburg et al. [14].

In this study we found high levels of ADMA in the SCD patients, which comes in the agreement with Kato et al. who reported that increased products of hemolysis included asymmetric dimethylarginine (ADMA) which promotes vasomotor dysfunction, and systemic vasculopathy, including pulmonary hypertension and stroke [15].

Kim –Shapiro and Gladwin reported that nitric oxide is thought to be important in maintaining vasomotor tone, limiting platelet aggregation, inhibiting ischemia –reperfusion injury, and modulating endothelial adhesion molecule expression [16].

There was no significant correlation between ADMA and other parameters including hemoglobin, platelet count, WBCs, and reticulocyte count which is in the agreement with Teplan et al. who reported that there was no correlation in ADMA levels and WBC count [17]. These results disagree with Goette et al. who showed that leukocytosis associated with increased Myeloperoxidase (the expression of leukocytes) activity during G-CSF therapy appeared to be responsible for the systemic release of ADMA, which impaired eNOS activity [18].

Wells et al. suggest that ADMA plays a private role in the development of diabetic nephropathy demonstrating as oxidative stress has been an essential factor in the pathogenesis of diabetic nephropathy [19] and ADMA is an important stimulator for oxidative stress.

We can suggest that ADMA, a strong and independent predictor of endothelial dysfunction, is increasingly a focus of interest in the microvascular complications.

5. CONCLUSIONS

Our study revealed that level of ADMA is elevated in children with sickle cell anemia. High plasma ADMA level is a risk for retinopathy and other microvascular complications in children with sickle cell anemia.

CONSENT

Written consent was taken from children's guardians.

ETHICAL APPROVAL

This cohort cross-sectional study was carried out from February 2020 to August 2020at Pediatrics Department at Tanta University Hospital after approval from Ethical Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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