Indian J Hematol Blood Transfus (Oct-Dec 2019) 35(4):742–749
https://doi.org/10.1007/s12288-019-01109-6

ORIGINAL ARTICLE

Asymmetric Dimethylarginine Levels and Its Correlation to Cerebral Blood Flow in Children with Sickle Cell Anemia

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Received: 23 February 2018 / Accepted: 3 March 2019 / Published online: 13 March 2019

Abstract Asymmetric dimethylarginine (ADMA) level may play a role in the pathogenesis of cerebrovascular stroke in Children with Sickle Cell Anemia (SCA). To assess the plasma level of ADMA in children with SCA and its correlation to cerebral blood flow. This is a cross sectional study was carried out on 30 children with homozygous SCA under follow up in the Out Patients Clinic, Pediatric Department at Tanta University Hospital and 30 healthy children as a control group. Both groups had undergone the following investigations: Complete blood count, lactate dehydrogenase enzyme, and plasma level of ADMA by a commercial ADMA ELISA Kit. Trans-cranial Doppler were done for both groups. ADMA plasma level was significantly higher in-patient group in comparison to the control group ($p < 0.001$), with a mean value 1.43 ± 0.20 µmol/l, 0.48 ± 0.16 µmol/l respectively. The time-averaged mean maximum velocities for middle cerebral artery, anterior cerebral artery, inferior cerebral artery and posterior cerebral artery were significantly different between patient and control group, $p < 0.05$. Trans-cranial Doppler data revealed that, 86.7% of patients have low velocity (< 70 cm/s) and 13.3% having very low velocity (< 10 cm/s) while control group have normal velocity. There was a significant negative correlation between ADMA plasma levels and cerebral blood flow. Elevated ADMA levels may have a role in the pathogenesis of the decreased cerebral blood flow in children with SCA.

Keywords Sickle cell anemia · TCD · Asymmetric dimethyl arginine

Introduction

Sickle cell anemia (SCA) is a consequence of inserting Valine in the position of glutamic acid as a result of point mutation in the β-globin gene on chromosome 11. The result is the hemoglobin polymerization, erythrocyte rigidity, hemolysis, increased expression of adhesion molecules on erythrocytes and endothelial cells, interactions with leukocytes, increased levels of circulating inflammatory cytokines, and enhanced micro vascular vaso-occlusion leading to recurrent attacks of painful crises [1].

Asymmetric dimethylarginine (ADMA) is a natural amino acid present in plasma and urine. ADMA as an endogenous inhibitor of the arginine-nitric oxide (NO) pathway. It is synthesized when arginine residues in the nuclear proteins are methylated through the action of the protein arginine methyltransferases (PRMTs). The concentration of ADMA in plasma is variable in the pediatric population [2]. In any vascular disease characterized by reduction of nitric oxide bioavailability, ADMA is mentioned as of great importance. It inhibits NOS and transporters of cationic amino acid that supply this enzyme by its substrate-arginine from plasma [3].

ADMA is a competitive inhibitor to NOS isoforms which is countered by the high concentration of L-arginine. ADMA induces what is called "*arginine paradox*" through

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the observation, it was found that L-arginine improves NO production in vivo and not in vitro [4].

Sickle cell anemia is characterized by a reduction of nitric oxide bioavailability, ADMA is mentioned as of great importance. It inhibits nitric oxide synthase (NOS) and transporters of cationic amino acid that supply this enzyme by its substrate, L-arginine from plasma by this way it contributes to inhibition of NO biogenesis, which is expected to have a harmful effect, especially on blood vessels, muscles and kidney. Therefore, ADMA and its mechanisms of transport are put to regulate the endothelial function [5].

An ischemic stroke is a life-threatening complication of SCA, occur in over 10% of children with homozygous Sickle Cell Anemia (HbSS) by the age of twenty. Silent strokes also cause significant morbidity. It's estimated that 17% of SCD children under the age of 14 have silent strokes and the rate increases to 23% by the age of 18, with the size and number of lesions increasing. Strokes can impair intellectual ability, attention, visual skills, language, and long-term memory. Early detection through screening and brain imaging is of the utmost importance to prevent recurrences [6].

Transcranial Doppler (TCD) studies used by The Stroke Prevention Trial in Sickle Cell Anemia (STOP Trial) to evaluate intracranial arterial hemodynamics, to screen and identify children at greatest risk of ischemic stroke [7]. Cerebral endothelial dysfunction related to the decreased nitric oxide bioavailability and increased ADMA as a competitive inhibitor of nitric oxide synthetase may predispose to the development of cerebral stroke in the SCA.

Subjects and Methods

After research ethics committee approval, an informed written consent was obtained from all participants in this research. This study was conducted between July 2016 and July 2017. This study was carried out in Hematology and Oncology Unit, Pediatric Departments, Tanta University on 60 children who were divided to: Group (A): Thirty child with sickle cell anemia who were attendant of Hematology and Oncology unit of Pediatric Departments, Tanta University Hospital, their age ranged from 6 to 18 years. Group (B): Thirty healthy age and sex matched children as a control group. Patients with other types of chronic hemolytic anemia or with history and signs of neurological diseases were excluded from the study.

All children in this study were subjected to clinical, laboratory investigations, including: complete blood count (CBC), lactate dehydrogenase (LDH), Hb electrophoresis, plasma levels of ADMA use commercial ADMA ELISA Kit (Immundiagnostik AG, Stubenwald-Allee 8a, D 64625 Bensheim made in German). And trans-cranial Doppler (TCD). Patients were positioned dorsally and oriented about the procedures; they were asked to not laugh, speak, hold breath or sleep. The middle cerebral artery (MCA), anterior cerebral artery (ACA), posterior cerebral artery (PCA) was evaluated. Ultrasound signal was deepened by 2 mm each time and the highest speed was chosen, in accordance with STOP study.

The mean maximum velocities in the right and left middle cerebral arteries (MCA) were recorded, focusing attention on the highest mean flow velocities, i.e. time-averaged mean maximum velocity (TAMM) on either side of the MCA. Maximum flow velocity was recorded at aninsonation depth of 40–60 mm. The findings were categorized as normal (70–169 cm/s), conditional (170–199 cm/s), abnormal ($\geq$ 200 cm/s) or abnormally low ($<$ 70 cm/s) velocities, according to the Stroke Prevention Trial in Sickle Cell Anemia Protocol. The frequency of flow velocity asymmetry defined as an inter-hemispheric flow velocity difference of at least 30 cm/s between segments in the MCAs, was also analyzed. A 2-MHz pulsed Doppler ultrasonography (Model No: CX Cart. Manufactured for Philips Ultrasound, Part No.: 453561373772, Made in USA) was used.

Statistical Analysis of the Data [8]

Data was fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) [9]. Qualitative data were described using numbers and percent. The Kolmogorov–Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. The significance of the obtained results was judged at the 5% level.

Results

Patients demographic, clinical and laboratory data are shown in (Tables 1, 2, 3). The ADMA level was significantly higher in patient than in the control group with a mean value of 1.43 $\pm$ 0.20 µmol/l and 0.48 $\pm$ 0.16 µmol/l, respectively (Table 3).

- The TAMM velocities for MCA, ACA, ICA and PCA are significantly different between patient and control group, $p < 0.05$ (Table 4).
- The TAMM velocities for PCA shows significant asymmetry between the right and left sides (P1 = 0.021*) (Table 5).
- Regarding the range of velocity by TCD, 86.7% of study patients have low velocity ($<$ 70 cm/s) and

Table 1 Demographic data of studied groups

	Patients (n = 30)		Controls (n = 30)		*p* value
	No.	%	No.	%	
Sex					
Male	20	66.7	19	63.3	0.787
Female	10	33.3	11	36.7	
Age (years)					
Range	6.0–3.0		6.0–15.0		0.491
Mean ± SD	8.03 ± 1.96		8.43 ± 2.49		
Median	7.50		8.0		
IQ	3.00		2.00		
Height (cm)					
Range	114.0–155.0		118.0–160.0		0.034*
Mean ± SD	129.17 ± 11.72		137.80 ± 11.95		
Median	126.0		135.50		
IQ	15.5		17.25		
Weight (kg)					
Range	20.0–60.0		22.0–65.0		0.003*
Mean ± SD	31.10 ± 10.67		39.40 ± 11.93		
Median	27.50		37.0		
IQ	11.0		15.00		
BMI (%)					
50th	9	30.0	0	0.0	< 0.001*
75th	15	50.0	0	0.0	
90th	6	20.0	10	33.3	
Range	15.0–25.0		18.0–31.0		< 0.001*
Mean ± SD	17.93 ± 2.79		23.30 ± 3.83		
Median	17.0		22.0		
IQ	3.00		6.00		

BMI body mass index

Table 2 Clinical characteristics of patient group

	No.	%
Onset of disease (months)		
Range	7–72	
Mean ± SD	28.5 ± 18.80	
Common presentation		
Bone pain	16	53.3
Infection	8	26.7
Acute chest syndrome	4	13.3
Diarrhea	2	6.7
Hyper hemolytic crises	2	6.7
Chronic abdominal pain	1	3.3
Aplastic crises	2	6.7
Crises per year	1	3.3

13.3% having very low velocity (< 10 cm/s) while 100% of control group have normal velocity (Table 5).

- Our results show a negative correlation between ADMA level (increased) and the velocity of cerebral blood vessels (decreased). Figure 1(a–d).

Discussion

Stroke is a stronger predictor of early death than any other chronic complication of SCD [10].

Our results showed that all patients were affected in their growth and nutrition (body mass index was below 90th percentile), which is coinciding with Senbanjo et al. [11].

In this study, the plasma ADMA level was significantly higher in patient than control group ($p < 0.001$). Similar results were found by El-Shanshory et al. [12] and Czarnecka et al. [13]. There are many causes of elevated ADMA concentrations in the SCA.; increased proteolysis associated with increased RBC_S turnover [14].

Table 3 Laboratory data of the studied groups

CBC	Patients (n = 30)	Controls (n = 30)	*p* value
Hemoglobin (g/dl)			< 0.001*
Range.	5.50–10.40	11.5–14.80	
Mean ± SD	8.55 ± 2.09	12.73 ± 2.04	
Median	7.65	11.50	
IQ	1.55	2.05	
MCV (fl)			0.725
Range	77.0–92.7	82.4–95.0	
Mean ± SD	85.32 ± 10.27	94.09 ± 4.50	
Median	85.60	90.2	
IQ	10.33	6.48	
MCH (pg)			0.748
Range	19.03–31.50	22.0–35.50	
Mean ± SD	27.18 ± 3.28	27.42 ± 2.50	
Median	27.5	27.55	
IQ	3.05	3.53	
MCHC (%)			0.012*
Range	26.08–34.70	28.60–36.30	
Mean ± SD	31.95 ± 1.67	33.21 ± 2.08	
Median	27.50	27.45	
IQ	2.03	3.05	
Platelets/cmm (thousand)			0.041*
Range.	100.0–540.0	255.0–430.0	
Mean ± SD	388.77 ± 113.19	340.37 ± 54.86	
Median	32.20	33.40	
IQ	137750	88500	
WBCs/cmm (thousand)			< 0.001*
Range	4.10–22.13	7.40–12.40	
Mean ± SD	13.63 ± 4.19	9.61 ± 1.36	
Median	415.0	340.0	
IQ	4567	2412	
Reticulocytic count (%)			< 0.001*
Range	0.20–23.30	0.20–2.50	
Mean ± SD	3.89 ± 5.23	1.34 ± 0.64	
Median	13.62	9.55	
IQ	1.00	1.00	
LDH (u/l)			< 0.001*
Range	380.0–630.0	123.0–270.0	
Mean ± SD	484.67 ± 63.94	202.60 ± 38.68	
Median	470.0	200.0	
IQ	88.0	68.0	
ADMA (umol/l)			< 0.001*
Range	1.12–1.78	0.14–0.79	
Mean ± SD	1.43 ± 0.20	0.48 ± 0.16	
Median	1.43	0.46	
IQ	0.42	0.24	

CBC complete blood count, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *WBCs* white blood cells, *LDH* lactate dehydrogenase, *ADMA* asymmetric dimethylarginine

Table 4 Comparison between the two studied groups according to transcranial cranial Doppler

TCD (TAMV cm/s)	Patients (n = 30)	Controls (n = 30)	*p* value
MCA			
Right			
Range	3.24–67.30	84.50–108.90	< 0.001*
Mean ± SD	34.06 ± 18.61	92.15 ± 9.62	
Median	35.75	89.35	
IQ	38	19	
Left			
Range	2.25–55.90	94.60–106.30	< 0.001*
Mean ± SD	31.89 ± 15.72	92.88 ± 18.66	
Median	32.05	90.15	
IQ	35	23	
p_1	0.734	0.614	
ACA			
Right			
Range	4.22–59.47	98.15–102.60	< 0.001*
Mean ± SD	33.81 ± 15.24	92.10 ± 8.77	
Median	35.98	88.24	
IQ	25	15	
Left			
Range	2.10–63.70	74.36–99.33	< 0.001*
Mean ± SD	33.37 ± 16.28	86.22 ± 8.14	
Median	34.22	87.28	
IQ	29	15	
p_1	0.465	0.165	
ICA			
Right			
Range	4.23–46.24	98.90–99.60	< 0.001*
Mean ± SD	26.42 ± 12.28	87.23 ± 8.18	
Median	27.74	87.90	
IQ	21	16	
Left			
Range	3.87–47.30	82.54–98.50	< 0.001*
Mean ± SD	26.66 ± 13.48	85.48 ± 8.65	
Median	27.31	87.60	
IQ	21	14	
p_1	0.491	0.465	
PCA			
Right			
Range.	2.78–49.80	88.33–108.99	< 0.001*
Mean ± SD	26.81 ± 11.83	83.55 ± 16.28	
Median	28.10	87.25	
IQ	17	14	
Left			
Range	1.87–50.80	93.12–109.45	< 0.001*
Mean ± SD	24.13 ± 12.73	88.53 ± 8.08	
Median	23.83	87.51	
IQ	6	13	

Table 4 continued

TCD (TAMV cm/s)	Patients (n = 30)	Controls (n = 30)	*p* value
p_1	0.030	0.171	

TAMV time-averaged mean maximum velocity, *MCA* middle cerebral artery, *ACA* anterior cerebral artery, *ICA* inferior cerebral artery, *PCA* posterior cerebral artery

Table 5 The percentage of the transcranial doppler velocities between the studied groups

TAMV (cm/s)	Patients (n = 30)		Controls (n = 30)		*p* value
	No.	%	No.	%	
Normal velocity	0	0.0	30	100.0	
Low velocity	26	86.7	0	0.0	< 0.001*
Very low velocity	4	13.3	0	0.0	

TAMV time-averaged mean maximum velocity

Furthermore, SCA is characterized by disturbances of intravascular wall which induce the expression of endothelial type-1 protein arginine methyltransferase, a catalyst of arginine methylation [15]. In addition, Engin [16] reported the presence of a large store of protein – incorporated ADMA near the vascular endothelium. This store may be released under certain pathological conditions. Also, intact RBC_S play an important role in storage of ADMA, whereas upon their damage they release a large amount of free ADMA due to proteolysis of methylated proteins [17]. The impaired metabolism of ADMA following inhibition of DDAH of oxidative stress triggered by several risk factors such as, hypoxia and elevated levels of pro-inflammatory cytokines which down regulate Dimethyl arginine dimethylaminohydrolase (DDAH). Decreased DDAH activity associated with endothelial dysfunction [18].

The current study showed that there were significant differences in cerebral blood flow between SCD patients and controls, even without any antecedent of previous

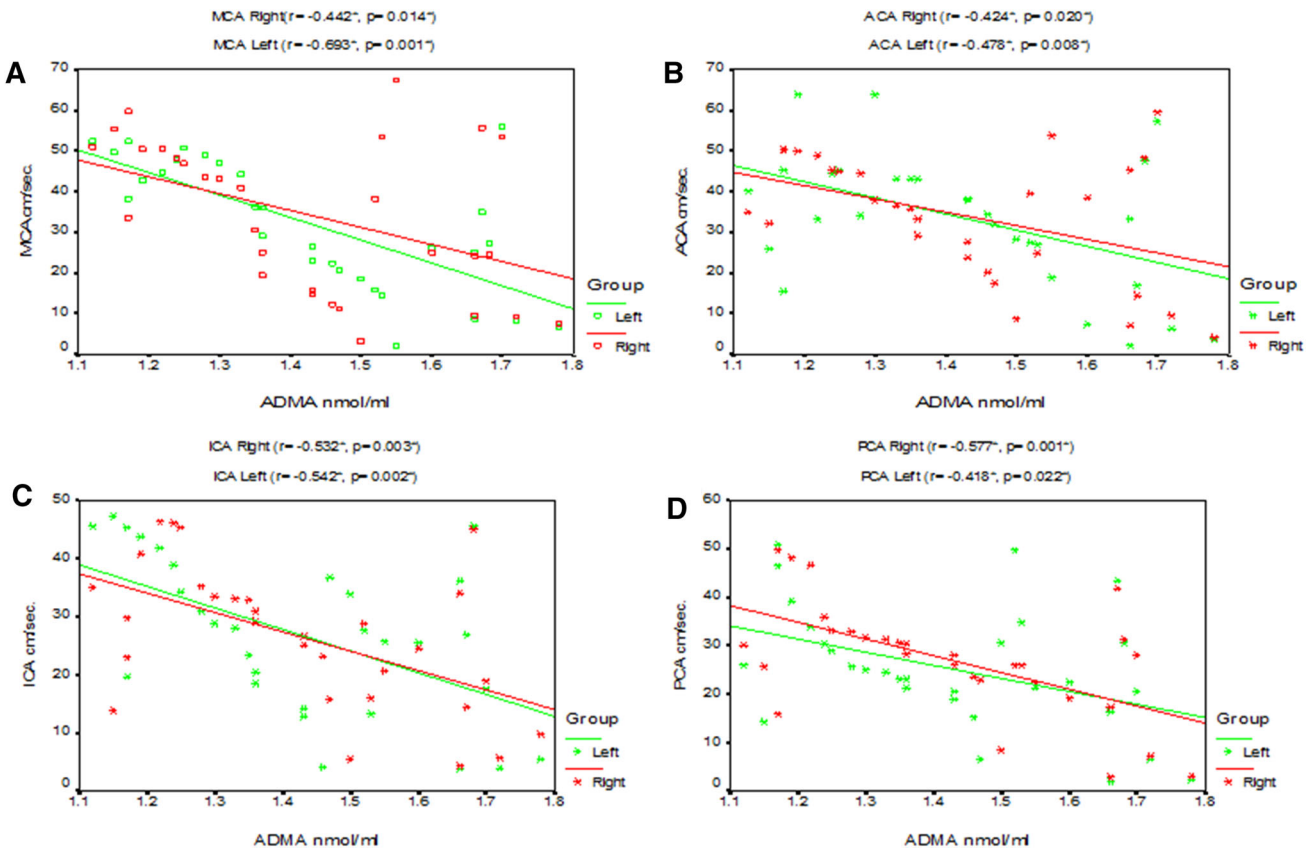

Fig. 1 Correlations between asymmetric dimethylarginine (ADMA) level and **a** middle cerebral artery, **b** anterior cerebral artery, **c** inferior cerebral artery, **d** posterior cerebral artery

cerebrovascular disease. TAMM analysis showed the decreased velocity in the patient group compared to control group in all studied vessels. Buchanan et al. [19] describe in their study, 5 children with sickle cell anemia, whose antecedent screening TCD velocities were measured to be $\leq$ 70 cm/s, but All patients in that study developed some form of cerebral insults, an overt cerebral infarction, silent stroke or transient ischemic attack. Based on these cases, low TCD velocities may identify another group of children at risk for cerebrovascular disease. So TCD velocities $<$ 70 cm/s in major vessels (MCA, ACA, and ICA) be considered another type of "abnormal," prompting more sensitive evaluations.

TCD results showed that 86.7% of the patients had low velocity ($<$ 70 cm/s) and 13.3% had very low velocity ($\leq$ 10 cm/s). This result agreed with Mazzucco, et al. [20] Very low TCD velocities may indicate severe arterial stenosis and associated with high risk of stroke. In both these cases, MRA could help distinguish technical problems from the advanced occlusive disease.

Our results showed significant asymmetry between the right and left PCA (p = 0.03), this result coincides with the study of Laranja et al. [15] they describe that 1.0% of their patients had abnormally low TCD velocities and significant asymmetry between the right and left sides by a frequency of 7.6%.

According to the STOP (stroke prevention trial in sickle cell anemia study) criteria, patients with abnormal values of time-averaged mean velocities of maximum blood flow (TAMM) $>$ 200 cm/s, detected by transcranial Doppler sonography (TCD), should undergo blood transfusion to reduce the risk of ischemic stroke [21].

Based on TAMM $<$ 70 cm/s identifies groups of children at risk for cerebrovascular disease, so considered another type of "abnormal," prompting more sensitive evaluations (Such as a brain magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) for the presence of central nervous system vascular disease, hence patients with normal TAMM might harbor silent strokes on MRI or MRAscan [21].

In the agreement with Li et al. [22] there is a significant negative correlation between the level of ADMA and transcranial Doppler as the increased the level of ADMA associated with decreased cerebral velocity screened by transcranial Doppler.

Conclusion

The level of ADMA is elevated in children with sickle cell anemia, which may have a role in the pathogenesis of the decreased cerebral blood flow in children with SCA.

Limitations

The number of patients included in this study are small, also we need more evaluations for the presence of cerebral stroke by MRAscan.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Standard All procedures performed in studies involving human participants were in accordance with the ethical standards of the institution and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all the guardians of children participants included in the study.

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