Original Research Article

Asymmetric Dimethylarginine Level in Children Undergoing Bone Marrow Transplantation

Abstract

Background: Asymmetric dimethylarginine (ADMA) is a toxic, non-proteinogenic amino acids formed by post-translational modification and is a uremic toxin that inhibits nitric oxide (NO) production The aim of this work was to assess the serum level of asymmetric dimethylarginine in children underwent bone marrow transplantation.

Methods: This prospective, randomized controlled study has been conducted on 20 children aged 2-18 years underwent bone marrow transplantation (Group I) and 20 healthy control children of matched age and sex (group II).

Results: The serum level of ADMA was significantly higher after bone marrow transplantation (p value <0.05).

Conclusions: Elevated ADMA level after bone marrow transplantation indicates that endothelial dysfunction is a main complication in those patients.

Keywords: Asymmetric dimethylarginine, bone marrow transplantation.

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Introduction:

Hematopoietic stem cell transplantations (HSCTs) are now an established treatment modality with definitive indications for many hematological disorders. However, HSCT requires tremendous resources, and it is increasingly challenging for transplantation experts to practice in the developing world and to reach a compromise between requirements and available resources [1].

Stem cell transplantation can be grossly classified as autologous when the stem cells are obtained from the patient or allogeneic when taken from a donor [2].

Expanding indications aside from acute leukemia and aplastic anemia (such as congenital disorders of the hematopoietic system, metabolic disorders, and autoimmune disease), extending donor availabilit, innovations in HSCT conditioning, better understanding of immunology, resulting in lower intensity conditioning regimens and development of new sources of stem cells have dramatically extended the availability of allogeneic transplantation [3].

Asymmetric dimethylarginine (ADMA) is a toxic, non-proteinogenic amino acids formed by post-translational modification and is a uremic toxin that inhibit nitric oxide (NO) production and play multifunctional roles in many human diseases [6].

Asymmetric dimethylarginine (ADMA) is a molecule that can inhibit the production of nitric oxide (NO) by blocking the activity of nitric oxide synthetase (NOS). It is regarded as a biomarker of endothelial dysfunction (ED) and is elevated in many human disease [6].

The aim of this work was to assess the serum level of asymmetric dimethylarginine in children underwent bone marrow transplantation complicated with PRES.

Patients and Methods:

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This prospective, randomized controlled study has been conducted on 20 children aged 2-18 years underwent bone marrow transplantation in Bone Marrow Transplantation Unit, Tanta University Teaching Hospital, and Nasser Institute.

An informed written consent was obtained from the guardian of the patients. The study was done after approval from the Ethical Committee Tanta University Hospitals.

Exclusion criteria were children with underlying neurological disease, children with underlying hepatic disease, children with underlying renal disease and children with diabetes mellitus. Twenty healthy control children of matched age and sex (group II).

All patients were subjected to complete history taking, thorough clinical examination, complete blood picture, liver and kidney functions tests, serum electrolytes (Na, k, Mg and ionized calcium), random blood sugar, cyclosporine trough level every three days till reaching acceptable trough level, then every one week, lactate dehydrogenase (LDH), and assessment of serum levels of ADMA by enzyme- linked immunoblot assay (Elisa).

Measurement of ADMA serum level:

ADMA assay: plasma ADMA was analyzed using commercial ADMA ELISA Kit [7].

Test principle: the kit uses a double-antibody sandwich (ELISA) to assay the level of Human ADMA in samples. Add ADMA to monoclonal antibody enzyme well which is pre-coated with Human ADMA monoclonal antibody, incubation; then, add ADMA antibodies labelled with biotin, and combined with streptavidin – HPR to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. then add chromogen solution A, B, the color of the liquid changes into the blue, and at the effect of acid, the color finally become yellow. the chroma of the color and the concentration of Human Substance ADMA of sample were positively correlated.

Reagents provided: 96 well plate with 8 stips: break –apart microtiter test strips with 8 ADMA antibody coated single wells, ADMA standard: soluble and concentrated human –

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ADMA, standard diluent, biotinylated ADMA antibody, HRP – conjugated streptavidin, 30x wash solution, stop solution 2 NH_2SO_4, Chromagen solution A and Chromagen solution B.

Reagent preparation: all reagents and samples were brought to room temperature (18-25) before use, the standard was diluted as Table 1 and wash buffer: 30 ml of wash buffer was diluted with distilled H_2O to yield 600 ml of 1x wash buffer.

Chart 1: Standard number and their constitutiion

Standard number	Concentration	Constitution
Standard No.5	2.4 nmol/ml	120 µl original standard + 120 µl standard diluents
Standard No.4	1.2nmol/ml	120µl standard NO.5 + 120 µl standard diluents
Standard No.3	0.6nmol/ml	120 µl standard No.4 + 120 µl standard diluents
Standard No.2	0.3nmol/ml	120 µl standard No.3 + 120 µl standard diluents
Standard No.1	0.15nmol/ml	120 µl standard No.2 + 120 µl standard diluents

Assay procedure: all reagents and samples were brought to room temperature (18-26 C) before use, 5o µl of chromogen solution A and 5o µl of chromogen solution B were added to blank well, 50 µl standard dilution and 50 µl of streptavidin –HPR were added to each standard well, 40 µl of sample, 10 µl of biotinylated ADMA- antibody and 50 µl streptavidin-HPR were added to each test well, the plate was incubated for 60 minutes at room temperature without shaking, washing: membrane was removed carefully, and liquid was drained, 50 µl chromogen solution A then 50 µl chromogen B were added to each well, gently mixed, incubated for 20 minutes at 37C away from light, 50 µl of stop solution were added into each well to stop the reaction (the color changed immediately from blue to yellow) and the optical density of each well was determined within 15 minutes, using a micro-plate reader set to 450 nm.

Statistical analysis

Statistical analysis was done by SPSS v27 (IBM©, Chicago, IL, USA). Shapiro-Wilks test and histograms were used to evaluate the normality of the distribution of data. Quantitative

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parametric data were presented as mean and standard deviation (SD) and were analysed by ANOVA (F) test with post hoc test (Tukey). Quantitative non-parametric data were presented as median and interquartile range (IQR) and were analysed by Kruskal-Wallis test with Mann Whitney-test to compare each group. Qualitative variables were presented as frequency and percentage (%) and were analysed utilizing the Chi-square test. A two tailed P value < 0.05 was considered statistically significant.

Results:

Table 1 shows there was no statistically significant differences between studied groups as regard sex and age.

Table 1: Comparison of demographic data of studied groups

		Group I (n = 20)	Group II (n = 20)	t. test	p. value
Age (years)	**Min. – Max.**	2.50 – 18.0	3.0 – 12.0	H= 1.031	0.597
	Median (IQR)	8.0 (5.40 – 11.50)	7.50 (6.0 – 10.0)		
Sex	**Male**	11 (55.0%)	12 (60.0%)	χ^2= 0.417	0.812
	Female	9 (45.0%)	8 (40.0%)		

Data are presented as frequency (%).

Table 2 shows that the most common transplanted patients in our study were thalassemic patients and represent 60% in followed by SAA 10%.

Table 2: Diagnosis of transplanted groups

Diagnosis	Group I (n = 20)
Thalassemia	12 (60.0%)
Sickle thalassemia	2 (10.0%)
Fanconi anemia	2 (10.0%)
SAA	2 (10.0%)
PRCA	1 (5.0%)
CVID	1 (5.0%)

Data are presented as frequency (%), SAA: severe aplastic anemia, PRCA: pure red cell aplasia, CVID: common variable immunodeficiency

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Table 3: Comparison between the three studied groups according to ADMA level

ADMA (nmol/ml)	Group I (n = 20)	Healthy control Group II (n = 20)	p
Min. – Max.	2.10 – 3.20	0.18 – 1.60	<0.001*
Mean ± SD.	0.34±2.59	0.33±0.67	
Median (IQR)	2.55 (2.30 – 2.85)	0.66 (0.46 – 0.75)	

ADMA: asymmetric dimethylarginine

Discussion

Hematopoietic stem cell transplantation (HSCT) is a well-established standard of care for many haematological and non-haematological disorders. However, this treatment modality requires tremendous resources and puts the recipient at high risk for a variety of complications both during and after the HSCT [8]. Some of these complications are related to endothelial disfunction following HSCT [9]. The mechanism underlying the development of endothelial dysfunction includes endothelial damage caused by oxidative stress [10]. That was the rationale behind the study of ADMA as a marker for detection of endothelial damage. ADMA is a competitive endogenous inhibitor of NOS with a key role in the pathophysiology of endothelial dysfunction [11].

In our study the range of collecting blood for ADMA analysis was from day 1.0 – 145.0 in group I and the mean value of the day of transplant for collecting blood sample for ADMA analysis was 28.25± 34.20. Similar to our study, a study by ***Gaziev et al*** on a total of 281 consecutive pediatric patients with thalassemia (n = 222) or SCD (n = 59) from 38 different countries underwent allogeneic HCT and documented that overall 31 children developed endothelial dysfunction presented as posterior reversible encephalopathy syndrome (PRES), associated with CNIs (11%) and observed that the median time to PRES onset was 49 days (range, 4 to 208 days) from the start of CNIs therapy [15]. Additionally a study done by

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Németh et al reported that ADMA is an endogenous inhibitor of nitric oxide synthase, marker and mediator of endothelial dysfunction [16].

In our study, ADMA was significantly higher in patients who underwent HSCT as compared with healthy controls. Also, *El-Shanshory et al* reported that ADMA level may play a role in the pathogenesis of cerebrovascular stroke in Children with sickle cell anemia. Elevated ADMA levels may have a role in the pathogenesis of the decreased cerebral blood flow in children with sickle cell anemia [17].

Conclusions:

Elevated ADMA level after bone marrow transplantation indicates that endothelial dysfunction is a main complication in those patients.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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