ORAL SUPPLEMENTATION OF VITAMIN E REDUCES OSMOTIC FRAGILITY OF RBC IN HEMOLYTIC ANEMIC PATIENTS WITH G6PD DEFICIENCY

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Background: Vitamin E has role in maintaining the integrity of red cell membrane by preventing oxidation of polyunsaturated fatty acids, thus protects cells from oxidative stress-induced lysis in G6PD deficiency. Changes in osmotic fragility of RBC and some absolute values like MCV, MCH & MCHC may occur in haemolytic anaemic patients with G6PD deficiency. **Objective:** To observe the effects of vitamin E supplementation on these changes in order to evaluate the role of this anti-oxidant vitamin in reducing chronic haemolysis in G6PD deficient patients. **Research design and method:** A total number of 102 subjects with age ranged of 5 to 40 years of both sexes were included in the study. Among them 68 were G6PD enzyme deficient patients, of whom 34 were in supplemented group (experimental group) and 34 were in non-supplemented group (control group). The supplemented group received vitamin E supplementation for 60 consecutive days at a dose of 800 IU/day for adult and 400 IU/day for children ≤12 years (in a divided dose, i.e., 4 times daily). Age and sex matched 34 apparently healthy subjects with normal blood G6PD level were taken to observe the base line data (healthy control) and also for comparison. All the G6PD deficient patients were selected from Out Patient Department (OPD) of Haematology, Banglabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh from July 2005 to June 2006 and all healthy subjects were selected from personal contact. Blood G6PD level, osmotic fragility of RBC were measured by standard techniques and MCV, MCH, and MCHC were obtained by calculation. All the parameters were measured on day 1 of their first visit and also were on day 60 in deficient group. Data were compared among the deficient groups, also in supplemented group just before and after supplementation. Analysis of data was done by appropriate statistical method. **Result:** Mean starting and completing points of osmotic fragility of RBC were significantly higher but MCV. MCH, MCHC were significantly lower in patients suffering from haemolytic anaemia due to G6PD deficiency in comparison to those of the healthy control. After supplementation with vitamin E starting and completing points of osmotic fragility of RBC were significantly decreased whereas, MCV, MCH, MCHC were significantly increased towards those of healthy of healthy control in supplemented group of patients in comparison to those of their pre-supplemented (day-1) and non-supplemented groups both on day 1 and day 60. **Conclusion:** From this study it may be concluded that, disturbances of some of the haematological parameter like higher osmotic fragility of RBC and lower MCV, MCH, MCHC occur in G6PD deficient haemolytic anaemic patients, which returned towards normal after supplementation of vitamin E, which clearly indicates the role of this anti-oxidant vitamin in maintaining red cell membrane integrity and thereby decreases the rate of haemolysis in this group of patients. So, vitamin E can be supplemented along with other drugs for better management of the patients.

Keywords: Haemolysis, G6PD, Vitamin E, Osmotic fragility, Haemolytic anaemia

INTRODUCTION

Glucose 6-posphate dehydrogenase (G6PD) deficiency is the most common clinically significant enzyme defect in human biology and the common clinical manifestation of this enzyme defect is haemolytic anaemia.[1] Acute haemolytic crisis may occur in G6PD deficiency due to some oxidative stress, such as intake of some anti-malarial drugs, ingestion of fava beans, various types of bacterial and viral infection.[2–4] Haemolysis of RBC may also occur even without prior administration of drugs in G6PD deficiency.[5–7] Vitamin E is one of the major lipid soluble antioxidant. It prevents oxidation of polyunsaturated fatty acids and thus protects red blood cells from oxidative stress-induced lyses.[8] Again, deficiency of vitamin E is a common feature in genetic anaemia, including G6PD deficiency haemolytic anaemia due to its increased consumption.[8,9] Supplementation of vitamin E may have an important role in maintaining red cell membrane integrity by reducing osmotic fragility of erythrocyte,[10,11] and can minimize the severity of haemolysis in G6PD deficient patients.[12] Again, Vitamin E supplementation can restore the required amount of Vitamin E level in this group of patients, and thus may prevent haemolysis by improving red blood cells survival.[5,6,13] Normal red blood cell indices like MCV, MCH and MCHC may also be found in

peripheral blood film by oral supplementation of vitamin E.[11]

An increase in osmotic fragility of RBC may occur in haemolytic anaemia with G6PD deficiency.[14] Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) may also decrease in this group of haemolytic anaemic patients.[15,16]

However, the common clinical consequences of this enzyme deficiency are neonatal jaundice and sporadic haemolytic crisis,[2] can be minimized by vitamin E supplementation. In our country many people are suffering from haemolytic anaemia due to G6PD deficiency. Unfortunately, most of them are treating without knowing the actual cause. Study of the changes in osmotic fragility of RBC and MCV, MCH, MCHC is important as it may reflect the haemolytic crisis in G6PD deficient patients. Evaluation of supplementation of vitamin is equally important in these cases.[10,11]

In Bangladesh there is lack of adequate information about deficiency of G6PD enzyme among the anaemic patients. Only one study regarding the haematological parameters of G6PD enzyme deficient patients has been reported in our country.[17] But no published data regarding effects of vitamin E supplementation in these G6PD enzyme deficient patients are available. For this, the present study was aimed at to observe some aspects of haematological parameters in G6PD deficient haemolytic anaemic patients both before and after supplementation of vitamin E, in order to explore its role in preventing red cell lyses and thereby maintains the normal haematological status in these enzyme deficient patients. The output of the study may be helpful to create awareness about the deficiency of G6PD enzyme in anaemic patients as well as the role of vitamin E in minimizing the risk of complications. Moreover it can provide information to clinicians for better management of these patients.

METHODS

The present Prospective interventional study was carried out in the Department of Physiology, BSMMU, Dhaka from July 2005 to June 2006. In this study, a total number of 102 subjects with age ranged from 5 to 40 years of both sexes were included. Among them 68 were patients of haemolytic anaemia with blood G6PD level below the normal reference range,[18] of whom 34 were in supplemented group (experimental group) and 34 were without supplementation and was considered as non-supplemented group (control group). The supplemented group received vitamin E supplementation for 60 consecutive days at a dose of 800 IU/day for adult and 400 IU/day for children ≤12 years; in a divided dose, i.e., 4 times daily.[6,19] Age and sex matched 34 apparently healthy subjects with normal blood G6PD level were taken to observe the baseline data (healthy control) and also for comparison. All the G6PD deficient patients were selected from Out Patient Department (OPD) of Haematology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, and all the healthy subjects were selected from personal contact. Blood G6PD level, osmotic fragility of RBC and red cell indices (MCV, MCH, MCHC) were done in all the subjects on day-1 of their 1st visit and in G6PD enzyme deficient groups of subjects also on day-60. Data were compared among healthy control, supplemented, non-supplemented and also within supplemented groups just before and after supplementation. All the subjects belonged to middle and lower middle socio-economic status. Patients with acute haemolytic episode or received blood transfusion in the last two months and β thalassaemia trait were excluded from the study. The objectives and benefits of the study were explained to all the subjects to ensure their voluntary participation and a written informed consent was taken from each subject prior to the study.

Two ml of blood was taken in an EDTA test tube for determination of erythrocyte G6PD level and the haematological parameters. The remainder was transferred in a test-tube where serum was separated for estimation of bilirubin level.

Erythrocyte G6PD enzyme level was determined by spectrophotometric method[20] and the haematological parameters were estimated by standard laboratory technique.[21,22] All of these tests were done in the Department of Physiology, BSMMU, Dhaka. Data were expressed as Mean±SD. Independent-samples (unpaired) *t*-test and paired-samples *t*-test were done as the tests of significance wherever applicable. The statistical analysis was done by using SPSS 12 and *p*-value <0.05 was considered as significant.

RESULTS

Mean erythrocyte G6PD levels were significantly ($p<0.001$) lower in G6PD enzyme deficient group of patients when compared to that of healthy control (Table-1). The mean starting and completing points of osmotic fragility of RBC were significantly ($p<0.001$) higher in both the G6PD deficient groups in comparison to those of healthy control group on day-1. After supplementation of vitamin E (i.e., on day-60) starting and completing points of osmotic fragility of RBC were significantly ($p<0.001$) decreased in comparison to those of their pre-supplemented (day-1) and also of non-supplemented groups both on day-1 and day-60 and returned almost toward the values of healthy control (Table-2).

Patients with G6PD deficiency had significantly ($p<0.001$) lower MCV, MCH and MCHC compared to those of healthy control. These values were increased significantly ($p<0.001$) toward the values of healthy control in G6PD deficient group following vitamin E supplementation (Table-3).

Table-1: Erythrocyte G6PD level in different study groups (Mean±SD) (n=102)

Groups	N	$U/10^{12}$ RBC	U/g Hb
A	34	191±18.8 (161–226)	6.69±1.19 (5.00–9.60)
B_1	34	105±9.38 (90–121)	3.29±0.34 (2.38–3.90)
C_1	34	105±10.09 (85–122)	3.31±0.33 (2.60–3.84)

Statistical analysis of Table-1

Groups			*p*-value	
A	Vs	B_1	0.000^{***}	0.000^{***}
A	Vs	C_1	0.000^{***}	0.000^{***}
B_1	Vs	C_1	0.747^{ns}	0.893^{ns}

Group A= Healthy subjects for baseline and control; **Group B**= Haemolytic anaemic patients with G6PD deficiency (Control) non-supplemented group; **Group C**= Haemolytic anaemic patients with G6PD deficiency (Experimental) supplemented group; B_1 and C_1= On day 1; B_2 and C_2= On day 60

Table-2: Osmotic fragility of RBC in different study groups Mean±SD (n=102)

Groups	n	Starting point (%)	Completing point (%)
A	34	0.48±0.03 (0.45–0.55)	0.31±0.04 (0.25–0.35)
B_1	34	0.6±0.04 (0.5–0.65)	0.42±0.032 (0.35–0.45)
B_2	34	0.6±0.04 (0.5–0.65)	0.41±0.03 (0.35–0.45)
C_1	34	0.59±0.04 (0.5–0.65)	0.42±0.028 (0.35–0.45)
C_2	34	0.5±0.04 (0.4–0.55)	0.32±0.04 (0.25–0.35)

Statistical analysis of Table-2

Groups			*p*-value	
A	Vs	B_1	0.000^{***}	0.000^{***}
A	Vs	C_1	0.000^{***}	0.000^{***}
A	Vs	B_2	0.000^{***}	0.000^{***}
A	Vs	C_2	0.245^{ns}	0.295^{ns}
B_1	Vs	C_1	0.314^{ns}	0.546^{ns}
B_2	Vs	C_2	0.000^{***}	0.000^{***}
B_1	Vs	B_2	0.374^{ns}	0.711^{ns}
C_1	Vs	C_2	0.000^{***}	0.000^{***}

Group A= Healthy subjects for baseline and control; **Group B**= Haemolytic anaemic patients with G6PD deficiency (Control) non-supplemented group; **Group C**= Haemolytic anaemic patients with G6PD deficiency (Experimental) supplemented group; B_1 and C_1= On day 1, B_2 and C_2= On day 60 (Values in parentheses indicate ranges)

Table-3: MCV, MCH and MCHC in different study groups Mean±SD (n=102)

Groups	n	MCV (fl)	MCH (pg)	MCHC (g/dl)
A	34	91±6.65 (73–105)	31±1.53 (26–34)	34±2.13 (28.5–40)
B_1	34	85±10.78 (65–104)	27±2.94 (22–33)	32±2.1 (26–33)
B_2	34	85±9.37 (67–100)	27±2.72 (22–31)	32±2.24 (26–35)
C_1	34	85±9.01 (65–101)	28±2.83 (22–34)	32±1.87 (28–33)
C_2	34	90±6.64 (77–104)	30±2.42 (26–35)	33.5±0.76 (32.5–36)

Statistical analysis of Table-3

Groups			*p*-value		
A	Vs	B_1	0.025^{*}	0.000^{***}	0.010^{*}
A	Vs	C_1	0.015^{*}	0.000^{***}	0.018^{*}
A	Vs	B_2	0.019^{*}	0.000^{***}	0.000^{***}
A	Vs	C_2	0.967^{ns}	0.851^{ns}	0.773^{ns}
B_1	Vs	C_1	0.895^{ns}	0.773^{ns}	0.842^{ns}
B_2	Vs	C_2	0.012^{*}	0.000^{***}	0.000^{***}
B_1	vs	B_2	0.615^{ns}	0.397^{ns}	0.136^{ns}
C_1	vs	C_2	0.002^{**}	0.000^{***}	0.002^{**}

Group A= Healthy subjects for baseline and control; **Group B**= Haemolytic anaemic patients with G6PD deficiency (Control) non-supplemented group. **Group C**= Haemolytic anaemic patients with G6PD deficiency (Experimental) supplemented group. B_1 and C_1= On day 1; B_2 and C_2= On day 60 (Values in parentheses indicate ranges)

DISCUSSION

The present study revealed that patients with G6PD deficiency have significantly higher osmotic fragility of RBC along with significantly lower values of red cell indices like MCV, MCH, MCHC in comparison to those of healthy control. These findings are in consistent with those of some other researchers of different countries.[14–16] On the contrary, no remarkable change in osmotic fragility of RBC and red cell indices were reported.[15,23]

Again, in this study after 60 days supplementation of vitamin E osmotic fragility in G6PD deficient patients was significantly decreased and it was almost close to those of healthy control. Similar observations were also reported.[24] Red cell indices (MCV, MCH, MCHC were significantly increased and moved towards normal value in the present series of patients. This finding is similar to that of some other researchers.[11,13] On the other hand, no remarkable changes in these values were observed in other studies,[25] which might be due to short duration and low dose of vitamin E supplementation in their studies.

In G6PD deficiency oxidation of polyunsaturated fatty acid on the RBC membrane may increase its susceptibility to hemolysis.[25] In addition, abnormal degradation of haemoglobin, disordered cellular metabolism may also be responsible for early destruction of RBC in G6PD deficient patients.[11] Therefore, early destruction of RBC is the consequence of higher osmotic fragility of RBC in oxidative stress.[26,27] In addition, decreased level of MCV, MCH, MCHC in haemolytic anaemia with G6PD deficiency is a consequence of excessive haemolysis, more marked under oxidative stress.[27–29]

Therefore, increased osmotic fragility of RBC in G6PD deficiency indicates the presence of membrane defect in the present series of patients. Moreover, decreased MCV, MCH and MCHC might be due to nutritional deficiency resulting from increased nutritional demand imposed by fragile RBC in this type of patients.

Vitamin E acts as an anti-oxidant by scavenging free radicals, thus prevents premature destruction of RBC.[19,25] Therefore, supplementation of vitamin E restores osmotic fragility of RBC and thus increases RBC survival.[9,24] However, following vitamin E supplementation decreased osmotic fragility of RBC and shifting of MCV, MCH and MCHC towards normal in the patients of present study are suggestive of protective role of vitamin E supplementation in this group of patients.

CONCLUSION

This study concludes that increase in osmotic fragility and decrease in red cell indices may occur in G6PD deficiency and vitamin E supplementation helps to return these values towards normal. Determination of vitamin E level, red cell half-life and long time supplementation of vitamin E with larger sample size may be helpful to draw any definite conclusion.

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