Red Blood Cell Fragility and Reticulocyte Count In Hemolytic Anemic Patients with and Without G-6PD Enzyme Deficiency

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Abstract:

Background: Erythrocyte G-6PD enzyme deficiency is an important cause of Hemolytic anemia with consequent increase in reticulocyte count. **Objective:** To assess the osmotic fragility of RBC status and reticulocyte count in G-6PD enzyme deficient patients with hemolytic anemia in order to find their hemolytic status. **Methods:** The cross sectional study was carried out in the Department of Physiology, BSMMU, Dhaka from July 2002 to June 2003 to observe the osmotic fragility of RBC status and reticulocyte count in patients with hemolytic anemia. For this, total number of 50 hemolytic anemic patients(Group-B) with age ranged from 5 to 30 years of both sexes were studied. Among them, 25 were without G-6PD deficient hemolytic anemia (group-B_1) and 25 were hemolytic anemia with G-6PD enzyme deficiency (group-B_2). Age & sex matched 30 apparently healthy subjects with normal blood G-6PD level were included to observe the baseline data (Group-A) and also for comparison. All the subjects were selected from Out Patient Department of Hematology, Bangabandhu Sheikh Mujib Medical University (BSSMU), Dhaka. Blood erythrocyte G-6PD enzyme level, osmotic fragility of RBC & reticulocyte count were measured by standard laboratory techniques. Analysis of data was done by unpaired Student 't'test. **Result:** Mean starting & completing points of hemolysis of RBC were significantly higher in Group B_2 vs GroupA and also with Group B_1 and similar higher levels of these values were also observed in Group B_1 than those of GroupA, but the differences between them were not statistically significant. Reticulocyte count was significantly higher in Group-B_2 vs Group B_1 and also with GroupA and similar higher levels of this values were also observed in Group B_1 vs GroupA which was also statistically significant. **Conclusion:** From this study, it may be concluded that, increased hemolysis of RBC with higher reticulocyte count occur in G-6PD deficient hemolytic anemic patients which may be due to membrane defect.

Key words: Osmotic fragility, Reticulocyte count, G-6PD enzyme, Hemolytic anemia.

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Introduction:

Erythrocyte G-6PD enzyme deficiency is one of the enzyme deficient disorder and is an important cause of anemia[1]. Acute hemolytic crisis may occur in G - 6PD deficiency due to some oxidative stress, such as intake of some anti-malarial drugs, ingestion of Fava beans, various types of bacterial & viral infection [2,3]. Hemolysis of RBC may occur even without prior administration of drugs in G6PD deficiency [4,5]. It can also lead to life threatening hemolytic crisis in childhood and in advanced age by interacting with specific drugs [6]. Hemolytic anemia induced by drugs is more common in patients with erythrocyte G-6PD enzyme deficiency [7]. Erythrocyte enzyme concentration has been significantly lowered in hemolytic anemia suffering from any type of infection[8]. Again, when erythrocyte G-6PD enzyme deficiency is present usually more marked hemolysis occurs in this group of anemic patients.[9] On the other hand, oxidative stress, ingestion of certain drugs also cause marked hemolysis in similar group of enzyme deficient patients with hemolytic anemia[10].

Various hematological changes occur in hemolytic anemia without and with G-6PD enzyme deficiency including osmotic fragility of RBC which depend on the integrity of its membrane[11]. Workers of different countries reported that osmotic fragility was increased in hemolytic anemia without and with G-6PD enzyme deficiency[12]. On the contrary, osmotic fragility of erythrocyte has been found to be decreased in drug induced hemolytic anemia associated with erythrocyte G-6PD enzyme deficiency[13]. Again, a normal osmotic fragility of erythrocyte has also been observed in similar group of patients[14]. However, the common clinical consequences of this enzyme deficiency are neonatal jaundice and sporadic hemolytic crisis[9].

Hemolysis of RBC is evidenced by increased reticulocyte count[12].The reticulocyte count has been increased in

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erythrocyte G-6PD enzyme deficient hemolytic anemia associated with acute viral hepatitis[15].On the other hand, the reticulocyte count has been decreased in drug induced hemolytic anemic patients[16].On the contrary normal reticulocyte count has also been reported[17].

In Bangladesh, many people are suffering from hemolytic anemia due to G-6PD deficiency.Unfortunately,most of them are treated without knowing the underlying cause.

In our country, there is lack of adequate information about deficiency of G-6PD enzyme in the hemolytic anemic patients and a few published data regarding the effects of G-6PD enzyme deficiency patients are available in our country[18,19] and also from different other countries[14,15].

Therefore, the present study was undertaken to observe the osmotic fragility of RBC status along with reticulocyte count in hemolytic anemic patients without and with G-6PD enzyme deficiency.The outcome of the study may be helpful to create awareness among the clinicians about the presence of G-6PD enzyme deficiency and also to do the needful in avoiding various complications due to this deficiency in hemolytic anemia.

Methods:

The present cross-sectional study was carried out in the Department of Physiology, BSMMU, Dhaka.For this,a total number of 80 subjects with age range from 5 to 30 years of both sexes were included. Among them, 50 patients with hemolytic anemia were included in Group B. On the basis of G-6PD enzyme level subjects of group B were further divided into GroupB_1, consisted of 25 patients without this enzyme deficiency and Group B_2, consisted of 25 patients with this enzyme deficiency(Group B_2) . Age & sex matched 30 apparently healthy subjects with normal blood G-6PD enzyme level were taken to observe the baseline data (Control) and also for comparison. All the G-6PD deficient & non deficient patients were selected from OPD of Hematology, BSSMU, Dhaka, and all the healthy subjects were selected from personal contact.Patients with acute hemolytic episode or receive blood transfusion in the last 2 months and the â thalassemia trait were excluded from the study. The objectives and benefit of the study were explained to all the subjects to ensure their voluntary participation and a written informed consent was taken from each subjects prior to the study. For all the subjects,G-6PD level, osmotic fragility of RBC and reticulocyte count were measured. Erythrocyte G-6PD enzyme level was determined by Spectrophotometeric method[20],osmotic fragility of RBC by Colorimetrically[21]and reticulocyte count by Brilliant cresyl blue method[22]. All of these tests were done in the Department of Hematology, BSSMU, Dhaka. Data were expressed as Mean ± SE. Statistical analysis of the results were done by unpaired Student 't' test by using SPSS program version 12.

Results:

Mean erythrocyte G-6PD enzyme level was significantly ($P<0.001$) lower in G-6PD enzyme deficient group(GroupB_2) than that of healthy control(GroupA) and hemolytic anemia without G-6PD enzyme deficiency(Group B_1). Again, this enzyme level was within normal range in Group B_1 and the difference of this value with healthy control was also statistically significant($P<0.001$), (Table-I).

The mean starting & completing points of osmotic fragility of RBC were significantly ($P<0.001$) higher in G-6PD deficient (GroupB_2) and non deficient (Group$B_{1)}$ in comparison to those of healthy control(Group A). On the other hand, though starting and completing points of hemolysis were slightly higher in GroupB_1 than those of Group A but the differences were not statistically significant (Table-II).

The reticulocyte count was significantly($P<0.001$) higher in hemolytic anemia with(Group B_1) and without(Group B_2) G-6PD enzyme deficiency compared to that of healthy control (Group A).Again,this count was significantly ($P<0.001$) higher in Group B_1 than that of Group B_2 (Table-III).

Table-I

Erythrocyte Glucose–6 Phosphate Dehydrogenase Enzyme levels in different groups of subjects (n = 80)

Groups	n	RBC level (mU / 10^9 erythrocyte) Mean (± SE)
A	30	119.79±1.69 (101.60 – 140.20)
B1	25	130.42 ± 2.80 (109.00 – 168.30)
B2	25	41.28 ± 3.99 (16.40 – 91.10)

Statistical Analysis

Groups	df	t value	P value
A vs B	53	-5.01	<0.001
***A vs B_2	53	18.76	<0.001
***B_1 vs B_2	48	-18.30	<0.001***

Data were expressed as mean ±SE. Figures in parenthesis indicate ranges.

Table-II

Osmotic fragility of RBC (Starting and completing points) in different groups of subjects(n=80)

Groups	n	Osmotic Fragility Of RBC	
		Starting point(%) Mean(SE±)	Completing point(%) Mean(SE±)
A	30	0.51±0.00	0.28±0.01
		(0.50 – 0.60)	(0.20 – 0.35)
B_1	25	0.52±0.01	0.29±0.01
		(0.50 – 0.60)	(0.20 – 0.40)
B_2	25	0.57±0.02	0.35±0.01
		(0.45 – 0.70)	(0.25 – 0.40)

Statistical analysis

Groups	df	P value	P value
A vs B_1	53	>0.01 ns	0.50 ns
A vs B_2	53	<0.001***	<0.001***
B_1 vs B_2	48	<0.001***	<0.001***

Data were expressed as mean ±SE. Figures in parenthesis indicate ranges.

Table-III

Reticulocyte counts in different groups of subjects (n=80)

Groups	n	Reticulocyte count (%) Mean (± SE)
A	30	1.85 ±0.09
		(1.20 – 3.00)
B_1	25	5.50 ± 0.11
		(5.00 – 6.60)
B_2	25	6.86 ± 0.23
		(5.50 – 8.50)

Statistical Analysis

Groups	df	t value	P value
A vs B	53	-21.58	<0.001***
A vs B_2	53	-25.87	<0.001
***B_1 vs B_2	48	-5.39	<0.001***

Data were expressed as mean ±SE. Figures in parenthesis indicate ranges.

Discussion:

The patients with G-6PD deficiency had significantly higher osmotic fragility of RBC and reticulocyte count in comparison to those of healthy control. These findings are consistent with those of some investigators of different countries[23]. On the contrary, no remarkable change in osmotic fragility of RBC had also been reported by some other group investigators[24].

Changes in red cell membrane integrity may be the possible cause of early destruction of RBC in G-6PD deficient in hemolytic anemia[25]. It has been suggested that abnormal degradation of hemoglobin may occur in G-6PD deficient hemolytic anemia[26]. Disturbance of intracellular metabolism may also be the another possible underlying cause in this type hemolytic anemia[16].

The higher reticulocyte count in erythrocyte G-6PD enzyme deficiency may be due to shorter red cell life span[27]. In addition, it has also been suggested that lower level of reduced glutathione in erythrocyte of G-6PD deficiency limits their ability to resist oxidative stress and leads to premature destruction of RBC and there by leads to higher reticulocyte count[28-29].

All the above mentioned suggestions may also be the underlying cause of excess hemolysis of RBC in the G-6PD deficient hemolytic anemic patients of present series and it is also supported by increased reticulocyte count in this series of patients.But it is difficult to comment on all the above mentioned factors as they were not studied.

Conclusion:

Therefore, this study concludes that in G-6PD enzyme deficiency, excess hemolysis of RBC occur possibly due to membrane defect.

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References:

1. Firkin F, Chesterman C E, Penington D, Rush B. The red cell : Basic aspect of anemia. The hemolytic anemia. In : de-Gruchy's clinical hematology in medical practice, 5th ed. Delhi ; Oxford universities press; 1996. P20-172 .

2. Buetler E.Glucose -6 – phosphate dehydrogenase deficiency. Blood 1994 ; 84 : 3613-3636

3. Galiano S, Gaitani GF, Barabino A. Favism in the African Type of glucose -6 – phosphate dehydrogenase deficiency. (A). BMJ 1990; 300 : 236-2

4. Luzzatto M, Mehta A, Meloni T. Hemoglobinuria & heptoglobin in G6PD deficiency. Br J Haematol 1995; 91 : 511-512

5. Gostman I, Muszkat M. glucose -6 – phosphate dehydrogenase deficiency is associated with increased initial clinical severity of acute viral hepatitis. j Gastroenterol Hepatol 2001 : 16 (1): 1239-1243

6. Corash L, Speilberg S, Bartsocas C, Boxer L, Steinherz R, Sheetz M, Schlessleman J, Schulman JD. Reduced chronic hemolysis during high dose vitamin E administration in Mediterranean type Glucose- 6 – phosphate dehydrogenase deficiency. N Eng J Med 1980 ; 303 : 416-420.

7. Hafez M, Amar ES, Zedan M, Hammad H, Sorour AH, Desouky SA, Gami N. Improved erythrocyte survival with combined vitamin E and selenium therapy in children with glucose-6-phosphate dehydrogenase deficiency and mild chronic hemolysis. J pediatr 1986 ; 108 : 558-561

8. Chen Bh, Tsai JL, Tsai LY, Chao MC. Comparison of serum copper, magnesium, zinc and calcium levels between G6PD and normal Chainese adult. Kaoshiung J Med Sci 1999; 15 : 646-650

9. Beutler E, Derin R J, Alving A S. The hemolytic effect of primaquine IV. An in vitro test for sensitivity of erythrocytes to primaquine. J lab Clin Med 1955; 45:40.

10. WHO, Glucose-6-phosphate dehydrogenase deficiency. 1989; 3 : 11-14

11. Mengal EC, Metz E, Yancy S W. Anemia during acute infection. Arch intl Med 1964; 119 : 287-90.

12. Chau T, Lai S T, Lai J Y, Yuien H. Hemolysis complicating acute viral hepatitis in patient with normal or deficient glucose 6- phosphate dehydrogenase activity. Scand J infect 1997; 29 : 551- 3

13. Clearfield RH, Brody I J , Tumen J H. Acute viral hepatitis, glucose- 6 –phosphate dehydrogenase deficiency and hemolytic anemia. Arch Int Med 1969; 123 : 689-91

14. Chan T K, Todd D. Hemolysis complicating viral hepatitis in patients with glucose -6 – phosphate dehydrogenase deficiency. Br Med J 1975; 1:131-3

15. Ackerman Z, Albin J, Shouval D. Active immunization against hepatitis is now warranted in glucose -6 – phosphate dehydrogenase deficiency subjects. Am J Med 1996; 413

16. Meloni G, Meloni T.Glyburide induced acute hemolysis in a G-6-PD deficient patient with NIDDM. Br J Haematol 1996; 92: 159-60

17. Milne DB 2001. Trace elements. In : C.A. Burtis & E.R. Ashwood, (eds). Tietz text book of clinical chemistry, 5th edition. Philadelphia : WB saunders company, 2001 pp : 215-247

18. Faruky S. Study on glucose – 6 phosphate dehydrogenase status in pregnancy with bad obstetric history & precelamsia. M. Phil. thesis 2009; BSMMU, Dhaka.

19. Sultana N. Effect of vitamin E supplementation on some aspect of hematological parameters & serum levels of copper and zinc in hemolytic anemic patients with G – 6PD deficiency. M. Phil. Thesis. 2006; BSMMU, Dhaka.

20. Kornberg A, et al. Method in enzymology 1. New York academic press ; 1955. P 323.

21. Luzzatto L, Ropper D. Investigation of hereditary hemolytic anemias ;Membrane abnormalities : In S.J.V Dace, Sm Lewis, practical hematology Oxford : Heineman professional publishing limited, 1994 pp c: 215-247

22. Dacie S J V, Lewis S M. Preparation & staining methods for blood & bone marrow films. In: Practical hematology, 8th ed. ELBS; 1994. P83 – 96.

23. Grattagliano I, Russmann S, Palmeiri VO, Juni P, Portineasa P, Palasciano G, Lauterburg BH,. Low membrane proteine sulfhydrils but not G6PD deficiency prediet ribavirin induced hemolysis in hepatitis C. Hepatol 2004: 39 (5) : 1248-1255.

24. Zinkham HW, Lenhard ER. Metabolic abnormalities of erythrocyte from patient with congenital nonspherocytic hemolytic anemia. J Pediatr 1959 : 80 : 319-336

25. Mengel Ac, Metz E, Yancy SW.Anemia during acute infections. Arch int Med 1967; 119 : 287-290

26. Rubins J, Young El. Hereditary spherocytosis. JAMA 1977 ; 273 : 797- 798

27. Marks PA. Glucose-6-phosphate dehydrogenase in mature erythrocytes. Am J Chin Pathol 1967 ; 47 : 287.

28. Salen G, Goldstein F, Hauran F, Writs WC, Acute hepatitis in patients with G6PD deficiency. Ann intl Med 1966; 65 : 1210-1220.

29. Kattamis AC, Tjartjatou F. The hemolytic process of viral hepatitis in children with normal or deficient G6PD activity. J Ped 1970 ; 77 : 422-30.