Original Article

Reticulocyte count and Mean corpuscular Hemoglobin 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 and decrease in MCH.

Objectives: To assess the Reticulocyte count and MCH in Erythrocyte G-6PD enzyme deficient with hemolytic anemia in order to find their status.

Methods: The cross sectional study was carried out in the Department of physiology, BSMMU, Dhaka from July 2002 to 2003 to observe the RC (Reticulocyte count) and MCH in patient with hemolytic anemia. For this, total number of 50 hemolytic anemic patients (Groups-B) with age ranged from 5 to 30 years of both sexes was studied. Among them, 25 were without G-6PD deficient hemolytic anemia (B_1) and 25 were hemolytic anemia with G-6PD deficiency(group-B_2).Age and sex matched 30 apparently healthy subjects with normal blood G-6PD were included to observe baseline data (Group-A)and also for comparison. All the subject were selected from out Patient Department of Hematology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. Blood erythrocyteG-6PD enzyme level,Reticulocyte count and MCH were measured by standard laboratory techniques. Analysis of data was done by unpaired Student't' test.

Results: Reticulocyte count was significantly higher Group B2 vs. Group A and also Group B1 which was statically significant. MCH was significantly lower Group B2 vs. Group A and also Group B1 which was statically significant.

Conclusion: From this study, it may be concluded that, increased hemolysis of RBC with high Reticulocyte count and low MCH occur in G-6PD deficient hemolytic anemic patients which may be membrane defect.

Key words: Reticulocyte count, Mean Corpuscular Hemoglobin , G-6PD enzyme, Hemolytic anemia.

Introduction:

Erythrocyte G-6PD enzyme deficiency is one of the enzyme deficits disorder and is an important cause of anemia.[1] Acute hemolytic crisis may occur in G-6PD enzyme deficiency due to some oxidative stress, such as intake some anti–malarial drugs,ingestionof Feva beans, various types of bacterial & viral infection.[2,3] Hemolysis of RBC may occur even without prior administration of drugs in G-6PD enzyme deficiency.[4,5] It can also lead to life threatening hemolytic crisis in childhood and advanced age by interacting with specificdrugs.[6] Hemolytic anemia induced by drugs is more common in patients with erythrocyte G-6PD enzyme deficiiency.[7] Erythrocyte enzyme concentration has been significantly lowered in hemolytic anemia suffering from any type of infection.[8] Again, when erythrocyte G-6PD enzyme eficiency its present usually more markedhemolysis occurs in this group of anemic patients.[9] On the other hand, oxidative stress, ingestion of certain drugs also causes 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 Reticulocyte count, MCH which depend on the integrity of its membrane.[11] Workers of different countries reported that Reticulocyte count was increased in hemolytic anemia with G-6PD enzyme deficiency. On the contrary, MCH has been found to be decreased in drug induced hemolytic anemia associated with G-6PD enzyme deficiency.[12,13] Again, a normal MCH 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 decreased MCH. The MCH has been decreased in G-6PD enzyme deficiency hemolytic anemia associated with viral hepatitis.[15] On the other hand, the MCH has been decreased in drug induced hemolytic anemic patients.[16] On the contrary, normal MCH has also been reported.[17]

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

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

Therefore, the present study was under taken to Reticulocyte count along with MCH in hemolytic anemic patients without and with erythrocyte G-6PD enzyme deficiency. The outcome of the study may be helpful to create awareness among the clinicians about 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 were included. Among them, 50 patients with hemolytic anemia were included in Group B_1. On the basis of G-6PD enzyme level subject B were further divided into Group B_1, consisted of 25 patients without this enzyme deficiency and Group B_2 consisted of 25 patients with this enzyme deficiency. Age and 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 enzyme deficient and non deficient patients were selected from personal contact. Patients with acute hemolytic episode or receive blood transfusion in the last 2 months and the thalassemia trait were from the study. For all the subject, G-6PD enzyme level, Reticulocyte count and MCH were measured. Erythrocyte G-6PD enzyme level was determined by Spectrophotometric method.[20] Calculation of Reticulocyte count and MCH were done by Hemoglobin, total count of RBC and packed cell volume. Hemoglobin concentration was determined by standard procedures of cyanmethehemoglobin method and the result was obtained by colorimetric measurement of color reaction with an absorbance maximum at 530 nm wave length.[21] Total count of RBC was determined by hemocytometry which is done by under microscope. Packed cell volume was determined by macrohaematocrit which is done by centrifugation of anticoagulated blood at a standard speed and the result was obtained by calculating the fraction of red cell column of the whole blood.[22] All of these tests were done in the Departmen of Hematology,BSMMU,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 (G-6PD enzyme deficiency) than that of healthy control(Group A) 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 statistically healthy control was also significant(P<0.001).(Table-I).

The Reticulocyte count was significantly (P<0.001) higher in G-6PD deficient (Group B_2) in comparison to those of healthy control. On the other hand, though MCV was lower in Group B_1 than those of Group A but the differences were not statistically significant (table-II).

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

Table-I: Erythrocyte Glucose-6 Phosphate Dehydrogenase Enzyme levels in different groups of subjects (n=80)

Groups	n	RBC level (Mu/10^9erythrocyte) Mean(±SE)
A		119.79±1.69 (101.60-140.20)
B_1	25	130.42 ±2.80 (109.00-168.30)
B_2	25	41.28±3.99 (16.40-91.10)
Statistical Analysis		
Groups	**df**	**t value**	**P value**
A vs B_1	53	-5.01	<0.001
***A vs B_2	53	18.76	<0.001
***B_1vs B_2	48	-18.30	<0.001***

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

Group A: Apparently healthy subject

Group B1:Hemolytic Anemia without G-6PD deficiency

Group B2: Hemolytic Anemia with G-6PD deficiency

Table-II: Reticulocyte count in different groups of subjects (n=80)

Groups	n	Reticulocyte count(%) Mean(±SE)
A	30	1.46 ± 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_1	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 range

Group A: Apparently healthy subject

Group B_1: Hemolytic Anemia without G-6PD deficiency

Group B_2: Hemolytic Anemia with G-6PD deficiency

Table-III: Mean corpuscular Hemoglobin in different groups of subjects (n=80)

Groups	n	MCH(pg) Mean(±SE)
A	30	29.17 ±0.29 (25.00 - 32.29)
B_1	25	26.76 ±.78 (20.69-30.57)
B_2	25	19.25±.32 (16.67-23.08)
Statistical Analysis		
Groups	**df**	**t value**	**P value**
A vs B_1	53	22.88	<0.001***
A vs B_2	53	3.10	<0.01***
B_1 vs B_2	48	-8.88	<0.001***

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

Group A: Apparently healthy subject

Group B_1: Hemolytic Anemia without G-6PD deficiency

Group B_2: Hemolytic Anemia with G-6PD deficiency

Discussion: The patients with G-6PD enzyme deficiency had significantly lower MCHC in comparison to those of healthy control. These findings are consistent with those of some investigators of different countries.[23] On the other hand, MCH had significantly lower in patients without G-6PD enzyme deficiency than that of healthy control. These findings had also been reported by some other group investigators.[24] The MCH had significantly lower in patients with and without G-6PD enzyme deficiency due to excessive destruction of affected erythrocyte.[25]

Changes in red cell membrane integrity may be the possible cause of early destruction of RBC in G-6PD enzyme deficient in hemolytic anemia.[26] It has been suggested that abnormal degradation of hemoglobin may occur in G-6PD enzyme deficient hemolytic anemia.[27] Disturbance of intracellular metabolism may of also be the another possible underlying cause in this type of hemolytic anemia.[16]

Extensive studies on the occurrence of severe anemia in Erythrocyte G-6PD enzyme deficient patients indicate that such erythrocyte are prone to rapid and easy destruction by reticuloendothelial system. Abnormal degradation of hemoglobin, disturbances in intracellular metabolism or changes in membrane integrity is the possible underlying causes of early destruction of Erythrocyte G-6PD enzyme deficient erythrocyte in hemolytic anemia. Erythrocyte depends upon the pentose monophosphate shunt for the production of energy to drive various associated cell processes and Erythrocyte G-6PD initiates this pathway. A deficiency of this enzyme leads to lower level of reduced hemoglobin, glutathione or NADPH.As a result, intracellular stability of the affected erythrocytes may be impaired due to disturbances metabolism and such cells undergo destruction more rapidly than normal cell. In addition, it has also the lower level of reduced glutathione Erythrocyte G-6PD enzyme deficiency erythrocytes limit their ability to resist oxidative stress and leads to premature destruction. In present study, a comparative evaluation of hematological indices occurrence of significant anemia in deficient group. though the exact mechanism involved for this markedly increased hemolysis is not clear, it appears to be due to changes in the erythrocyte membrane permeability is most likely to be the cause of hemolysis as evidence higher MCV which due to excess hemolysis.Again Erythrocyte G-6PD enzyme is essential for maintain of the integrity of red cell membrane. Erythrocyte G-6PD enzyme deficiency might lead to more hemolysis. This is supported by markedly lower level in hemolytic anemic Erythrocyte G-6PD enzyme deficiency.

All the above mentioned suggestions may also be the underlying cause of excess hemolysis of RBC in the G-6PD enzyme deficient hemolytic anemic patients of present series. 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 possible due to membrane defect.

Acknowledge: The authors of the study acknowledge the financial support the students research grant of BSMMU and also the research grant of Bangladesh University Grant Commission.

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