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"Role of G6PD enzyme in neonatal jaundice and its effects on RBC indices"

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Abstract: *Glucose-6-phosphate dehydrogenase deficiency is the most common clinically significant red blood cell enzyme defects in human biology. This cross sectional study was carried out to observe the G6PD status in 90 male, term neonates with jaundice, age ranged from 3 to 12 days (Group B) in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University from 1st July 2007 to 30th June 2008. Our aim was to identify G6PD status in male, term, neonates with jaundice and its effect on hematological parameters including, hemoglobin concentration, hematocrit value, reticulocyte count, RBC count and also different features of peripheral blood film . On the basis of total serum bilirubin level, study group was further divided into three groups: Group B1 (TSB <15mg/dl), GroupB2 (TSB 15-20mg/dl) and Group B3 (TSB>20mg/dl). For comparison, age and sex matched 30 apparently healthy neonates (Group A) were also studied. Study group was selected from in patient and control group from outpatient department of Dhaka Shishu Hospital. Erythrocyte G6PD level, serum bilirubin, ALT and hematological parameters like hemoglobin concentration, hematocrit, total count of RBC,MCV,MCHC, RDW reticulocyte count, were measured and peripheral blood film was examined on the 2nd day of their admission or visit. Erythrocyte G6PD level was measured by Spectrophotometric method by using kit of Randox. All other hematological and biochemical parameters were done by standard laboratory technique. For statistical analysis Anova, independent sample t test and Pearson's correlation coefficient test were performed as applicable by using SPSS for windows version-12. Study revealed that Hemoglobin concentration, hematocrit value and RBC count were significantly lower in both moderate ($p<0.05$) and severe ($p<0.01$) groups in comparison to those of control and mild group .Erythrocyte G6PD levels were significantly lower in moderate ($p<0.01$, $p<0.05$) and severe ($p<0.001$,$p<0.01$) hyperbilirubinemic group in comparison to those of control and mild group. Among them, reticulocyte count was significantly ($p<0.001$) higher in severe hyperbilirubinemic than those of control mild and moderate group. From this study, it is revealed that G6PD deficiency in neonates has changes some hematological factors like decreases Hemoglobin concentration, hematocrit value and RBC count and significantly increases reticulocyte count those who have severe hyperbilirubinemic.*

Keywords: *Glucose-6-phosphate dehydrogenase deficiency (G6PD), Red Blood Cell (RBC), Neonatal jaundice*

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I. Introduction

G6PD deficiency is one of the commonest inherited enzyme disorders affecting greater than 400 million world populations (Mehta et al. 2000). The prevalence of G6PD enzyme deficiency is high in Africa, The South East Asia, the Mediterranean the Middle East and also in population of the Indian sub-continent (Smith 2005). The enzyme glucose-6-phosphate dehydrogenase (G6PD) is one of the most important enzymes in the human body, present in various amounts in many cells, including red blood cells[1]. Around 5% of neonates who have G6PD deficiency has affected with jaundice after the first 24 hours of his birth .Neonatal Jaundice is a commonly encountered pediatric problem, usually visible in the first week of life. Approximately 60% of term infants usually develop jaundice at this period (Nelson 2007). It has been reported that hyperbilirubinemia results from excessive destruction of red blood cells. In addition, they also reported that decreased conjugation and excretion of bilirubin by the liver had major contribution to the development of neonatal hyperbilirubinemia in G6PD deficient neonates (Oluboyeda et al. 1979; Beutler 1995; Kaplan et al. 1996; Mehta

et al. 2000). Some researchers reported that most of these enzyme deficient neonates developed exaggerated jaundice without hemolysis same as non-deficient neonates (Chan 1996). In addition .Hematological parameters including, hemoglobin concentration, hematocrit value, total count of red blood cells, reticulocyte count and different features of peripheral blood film in neonate's withG6PD deficiency also influence on Blood group, treatment duration .(Hafez et al 1986; Arif and Bhutta 1998; Kaplan et al.2000; Dhillon et al. 2003; Mritunjay et al. 2005). Since the last two decades a good number of studies were undertaken to investigate the role of G6PD deficiency in neonatal jaundice and it was reported that G6PD deficiency is the second common cause of hyperbilirubinemia in neonates (Kaplan et al. 2001; Sgro et al. 2006; Castro et al. 2006).

II. Objectives

General Objective:

To Role of G6PD enzyme in neonatal jaundice and its effects on RBC indices

Specific objective:

To measure G6PD Enzyme Deficiency among neonates in Bangladesh

III. Material And Method

This was a cross sectional study. Study was placed in Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh from July 2007 to June 2008. Ninety (90) Male term neonates aged 3-12 days who were hospitalized in inpatient department of Dhaka shishu hospital withjaundice are the study subject group and 30 healthy male term neonates without jaundice from outpatient department of DSH was the control group. Total numbers of 120 male, term neonates are the sample population. Sample populations are divided into 2 groups: Group A and Group B. Group A (Control) Consisted of 30 apparently healthy male, term neonates without jaundice. Group B (Study group) was consisted of 90 male, term neonates with jaundice. On the basis of total serum bilirubin level (TSB), Group B was further divided into three subgroups: GroupB1 (with TSB <15mg/dl.), Group B2 (TSB from 15 to 20 mg/dl) and Group B-3(with TSB >20mg/dl.). For Better understanding and study defined group B1 designated as mild, group B2 as moderate and group B3 as severe hyperbilirubinemia. Protocol approved by the Ethical Committee of the Department of Physiology, BSMMU and Ethical Committee of BICH DSH, Dhaka. After selection of the subjects, aims, objectives and detail procedure of the study and the benefit of the child, out of the study were explained to the parents or legal guardians by the investigator herself. The legal guardians/parents were encouraged for voluntary participation & they have allowed freedom to withdraw from the study even after participation whenever they feel like. Then written informed consents were taken from parents or legal guardians in a prescribed form. The case of jaundice was diagnosed by clinical examination and was confirmed by increased total serum bilirubin level corresponding to gestational age & body weight. Legal guardians/parents were interviewed for detail about gestational, delivery, medical and family history. Data were expressed as mean and standard deviation. Statistical analyses were performed by using SPSS (Statistical package of social service) for windows version-17. Anova test, Independent sample t test, Chi-Square test and Pearson's correlations coefficient test were performed as applicable. P value <0.05 was accepted as significant.

Inclusion criteria (for both groups):

Age 3-12 days, Sex-Male, Gestational age-more than 37 weeks of gestation are enrolled in both group but for control: Neonates without jaundice having bilirubin level <5mg/dl and for study group, Neonates with jaundice having serum bilirubin level >10mg/dl (Vitros 250 model Biochemistry auto analyzer).

Exclusion criteria (for both groups):

Age-less than 3 days & more than 12 days, Sex –female, Gestational age- less than 37 weeks of gestation, Rh incompatibility, Sepsis, Cephalohematoma

IIII. Results

Data analysis from the study clearly identified that 5.83% of the G6PD deficiency neonates have affect on some hematological factors where Average RBC count, Hematocrit, RDM is increasing in compare with increased G6PD level. The differences of these values among the groups were statistically significant ($p<0.01$. It clearly indicated that if G6PD is increased which affects some hematological factors like increased RBC count, Hematocrit, RDM and decreases Reticulocyte count. All the values were almost similar and no statistically significant differences were observed among the age groups. Therefore, all the groups were matched for age. Study identified that in controlled group there had no neonates who have erythrocytes G6PD deficiency. But in Study group, 7 subject population identified deficiency where 1 subject from Moderate group and 6 from Group 3 (Severe hyperbilirubinemia). Near 6% babies observed lo G6PD deficiency, which have low G6PD enzyme activity.G6PD 5% (6 subject population had the lowest Erythrocyte G6PD level) study subject have

ErythrocytesG6PD deficiency included in Group B3.The mean ((SD) erythrocyte G6PD level of the subjects were 280.90± 29.01, 263.77± 30.64 and 232.77± 77.58 mU/109 erythrocyte in Group-B1,Group-2, and Group-3 respectively. Mean erythrocyte G6PD levels were lower in Group B3in comparison to Group B1 and Group B2. All these values were within normal range except Group B3, which was below this range.

Table I: Distribution of G6PD deficiency in the study participants (n=120)

	n	%	Group A	Group B1	Group B2	Group B3
G6PD Deficiency (<245 mU/10^9 erythrocytes)	7	5.83	0.00	0.00	0.83	5.00
Normal G6PD (245-299 mU/109 erythrocytes)	93	77.50	18.33	17.50	22.50	19.17
G6PD Deficiency>300 mU/109 erythrocytes)	20	16.67	6.67	7.50	1.67	0.83

Figure I: Group comparison based on G6PD enzyme level

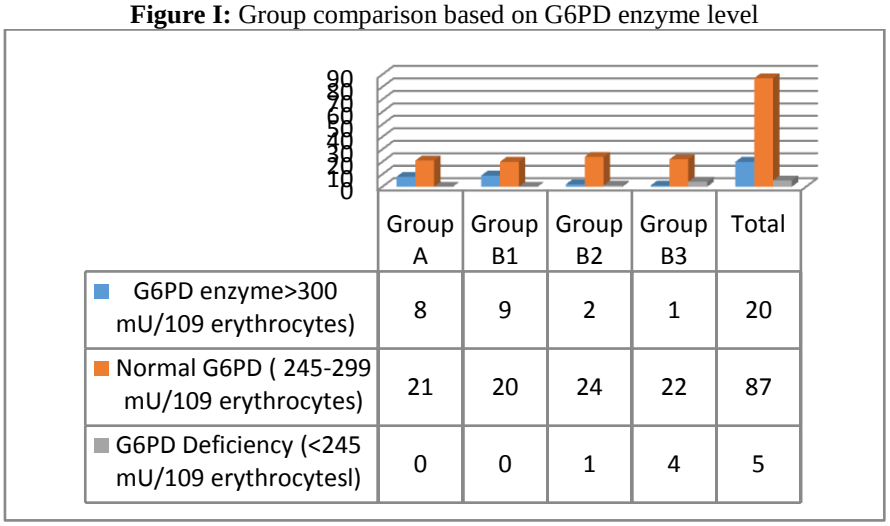

Table II: Study group were divided based on TSB level (n=90)

	N	Mean Age	Mean Weight
Group-B1	30	7.33 days	2.34 kg
Group-B2	30	7.40 days	2.36 kg
Group-B3	30	7.30 days	2.34 kg

Table III: Erythrocyte G6PD level in groups (n=90)

Groups	n	Mean ± SD	Range
Group-B1	30	280.90± 29.01	245-344
Group-B2	30	263.77± 30.64	137-311
Group-B3	30	232.77± 77.58	49-304

Table IV: Distribution of Hematological findings of the study groups (n=120)

	Group A	Group B1	Group B2	Group B3
RBC count	4.65	4.23	3.99	3.98
Hemotocrit	48.85	42.90	42.54	41.49
RDW	15.20	15.22	15.96	15.97
Reticulocyte Count	1.45	2.63	3.14	5.70

Table V: Distribution of Hematological findings based on G6PD enzyme (n=120)

	RBC Count (Average)	Hematocrite	RDM	Reticulocyt
G6PD Deficiency (<245 mU/109 erythrocytesl)	4.1	42.27	17.19	6.71
Normal G6PD (245-299 mU/109 erythrocytes)	4.14	43.32	15.19	3.08
G6PD enzyme>300 mU/109 erythrocytes)	4.69	47.66	15.05	2.71

Table VI: Distribution of MCV, MCH and MCHC in different groups of neonates **(**n=120)

Groups	**n**	**MCV (fl)**	**MCH (pg)**	**MCHC (g/dl)**

A	30	103.96 ± 8.56 (81.6 – 120.3)	35.62 ± 3.11 (25.2- 40)	34.13 ± 2.32 (30.8- 38.7)
B_1	30	99.58 ± 7.16 (80.42- 111.5)	35.33 ± 2.97 (26.6- 42.3)	35.22 ± 2.19 (30.7- 38.7)
B_2	30	101.85 ± 7.08 (88- 113.9)	36.45 ± 1.81 (32.2-39.4)	35.71 ± 2.10 (30.5- 37.9)
B_3	30	98.29 ± 13.36 (56.4- 119)	35.47 ± 3.14 (25.2 – 39.6)	35.71 ± 1.76 (31.8- 38.7)

Statistical analysis

Groups		p value	
A vs B_1vs B_2vs B_3	0.100^{ns}	0.401^{ns}	0.036*
A vs B_1	0.056^{ns}	0.789^{ns}	0.067^{ns}
A vs B_2	0.302^{ns}	0.169^{ns}	0.106^{ns}
A vs B_3	0.055^{ns}	0.934^{ns}	0.045*
B_1vs B_2	0.222^{ns}	0.083^{ns}	0.342^{ns}
B_1vs B_3	0.644^{ns}	0.856^{ns}	0.791^{ns}
B_2vs B_3	0.203^{ns}	0. 0.145^{ns}	0.206^{ns}

Data were expressed as Mean ± SD. For statistical analysis, one-way ANOVA was performed for comparison among the groups and independent sample t- test was done for comparison between the groups. Figures in parentheses indicate ranges.

Table VII: Distribution of the subjects by peripheral blood film in different groups (n = 120)

Groups	n	Normal No (%)	Abnormal No (%)
A	30	30(100%)	0 (0%)
B_1	30	24(80%)	6 (20%)
B_2	30	20(60.67%)	10 (30.33%)
B_3	30	10(30.33%)	20 (60.67%)

Statistical analysis

Groups	p value	
A vs B_1	0.414^{ns}	_
A vs B_2	0.157^{ns}	_
A vs B_3	0.002**	_
B_1vs B_2	0.546^{ns}	0.317^{ns}
B_1vs B_3	0.016*	0.006**
B_2vs B_3	0.068^{ns}	0.068^{ns}

Data were expressed as number of normal and abnormal peripheral blood films. For statistical analysis, Chi-square test was performed for comparison between the groups. Figures in parentheses indicate percentages.

Table VIII: Distribution of study subjects by presence of different abnormal cells type in peripheral blood film (n=90)

Abnormal cells	Groups		
	B_1 (n=30) No (%)	B_2 (n=30) No (%)	B_3 (n=30) No (%)
Nucleated redblood cell	1(3.33%)	2(6.67%)	7(23.34%)
Target cells	3(10%)	3(10%)	9(30%)
Aniso-poikilocytic cells	3(10%)	5(16.67%)	7(23.34%
Pinocyte	0(0%)	0(0%)	2(6.67%)

Table IX: Correlations of different hematological parameters with G6PD level in different groups of neonates (n=120)

Parameters	Group A		Group B_1		Group B_2		Group B_3	
	r	p	r	p	r	p	r	p
Hematological factors								
Red blood cell	+.116^{ns}	0.541	+.537**	0.002	+.209^{ns}	0.269	-.054^{ns}	0.776
Reticulocytes	+.434*	0.015	+.241^{ns}	0.200	+.039^{ns}	0.837	-.214^{ns}	0.257
Hemoglobin	+.046^{ns}	0.811	+.520**	0.003	+.146^{ns}	0.441	+.002^{ns}	0.993
Hematocrit	+.094^{ns}	0.620	+.524**	0.004	+.122^{ns}	0.522	-.017^{ns}	0.931

MCV	+.115^{ns}	0.545	+.300^{ns}	0.107	+.102^{ns}	0.592	+.244^{ns}	0.193
MCH	+.154^{ns}	0.415	-.022^{ns}	0.908	+.284^{ns}	0.128	+.305^{ns}	0.101
MCHC	-.056^{ns}	0.767	-.208^{ns}	0.271	-.081^{ns}	0.672	-.023^{ns}	0.902

Pearson's correlation coefficient (r) test was performed as the test of significance. ** = $p<0.01$. * = $p<0.05$.ns = Not significant. n = Number of subjects. Hematological values in G6PD deficient hyperbilirubinemic neonates (n=7).

V. Discussion

Differences of enzyme level were found between groups Group-B1vsGroup-B2 and Group-B2vsGroup-B3 but between Group-B1vs Group-B3difference was statistically significant ($p<0.01$).The mean ((SD) RBC , Hematocrit ,RDW and Reticulocyte count of the subjects were shown in group A, Group B1, Group B2 and Group B respectively. Mean hematological indices RBC count, Hematocrit ,RDW were lower in all three study groups in comparison to control group and the differences of these values among the groups were statistically significant ($p<0.05$).Mean hematocrit were lower in study groups GroupB1, Group B2 and Group B3 in comparison to control group A. However, the differences of these values among the groups were statistically significant ($p<0.01$). Again, significantly lower level of hematocrit were observed between the groups A vs B2($p<0.01$), A vsB3($p<0.01$) and also between A vsB1 ($p<0.05$).Whereas, these values were almost similar in study groups and no significant difference were found between groups B1 vs B2 , B1vs B3 and B2 vs B3 . Mean reticulocytes count were higher in groups Group B1, Group B2 and GroupB3 than that of Group A, and the differences of these values among the groups were statistically significant ($p<0.001$). Again, significantly ($p<0.001$) higher count of reticulocytes were observed between groups A vs B1, A vs B2 and A vs B3.On the other hand, reticulocytes count were slightly higher in group B2 in comparison to group B1 but difference was not significant . Whereas, significantly ($p<0.001$) higher reticulocytes count were observed between groups B1 vsB3 and B2 vs B3. It shown the hematological factors like RBC count, Hematocrit ,RDM and Reticulocyte count with the lowest G6PD level which compares with three different situation : G6PD deficiency (<245mU/109 erythrocytes),Normal G6PD (245-299 mU/109 erythrocytes), G6PD enzyme>300 mU/109 erythrocytes).Average RBC count, Hematocrit, RDM is increasing in compare with increased G6PD level. Then differences of these values among the groups were statistically significant ($p<0.01$. It clearly indicated that if G6PD is increased which affects some hematological factors like increased RBC count, Hematocrit, RDM and decreases Reticulocyte count.

VI. Limitations Of The Study

We conducted an observational study in one Centre with limited sample size. So, study result can't reflect the scenarios of the whole country. Case-control study can identify the risk factors more accurately.

VII. Conclusion

In the present study, maximum numbers of G6PD deficient neonates were with severe hyperbilirubinemia which is suggestive of excess hemolysis of RBC in this group of neonates. Significant changes in hematological parameters and their positive correlations with G6PD level are further supported the occurrence of hemolysis in this group of neonates. Therefore, from this study it can be concluded that G6PD deficiency in neonates has co-related with some hematological indices and hyperbilirubinemia. It is most likely due to hemolysis of red blood cells. Therefore, early detection of this enzymopathy and close surveillance of the affected neonates may be important in reducing the complications of severe hyperbilirubinemia.

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