Original Article

The impact of oxidative stress on Glucose-6-Phosphate Dehydrogenase level and prevalence of anemia among diabetic patients

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ABSTRACT

Objective: The present study was conducted to assess the impact of oxidative stress on glucose 6 phosphate dehydrogenase (G6PD) and prevalence of anemia among diabetic patients.

Methodology: The study involved 100 adult patients attending Buraidah Central Hospital and 30 healthy controls. Blood samples were collected and analyzed for (G6PD) activity, total antioxidant status (TAS), fasting blood sugar (FBS), hemoglobin (HGB),red cell(RBCs) count, hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC), hemoglobin A1c (Hb A1c), blood urea, serum creatinine, and microalbuminuria.

Results: It showed significant correlation between G6PD deficiency and low TAS among diabetics and significant correlation between low hemoglobin concentration (females < 120 g/L, males <130 g/L), G6PD deficiency and low concentration of TAS. The prevalence of anemia was 22% in diabetics.

Conclusion: It can be concluded that there is significant impact of oxidative stress (reduced TAS) on reduced G6PD level and the low HGB concentration in diabetic patients, that means oxidative stress of diabetes mellitus is possible cause of G6PD anemia.

KEY WORDS: Oxidative stress, TAS, G6PD, Diabetes mellitus, Anemia.

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INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme that catalyses the first reaction in the pentose phosphate pathway, providing reducing power to all cells in the form of NADPH (reduced form of nicotinamide adenine dinucleotide phosphate). NADPH enables cells to counterbalance oxidative stress that can be triggered by several oxidant agents, and to preserve the reduced form of glutathione. Since red blood cells do not contain mitochondria, the pentose phosphate pathway is their only source of NADPH; therefore, defense against oxidative damage is dependent on G6PD.[1]

The prevalence of Diabetes mellitus is increasing worldwide; this condition is likely to affect approximately 300 million people by 2025.[2]

Improved medical care and scientific advances in the treatment of patients have helped to prolong their lifespan. However, increased survival potentially predisposes such patients to long-term complications and consequent reductions in quality of life.[3]

One of these long-term complications is anemia, a condition characterized by reduced hemoglobin levels (<130 g/l in males and <120 g/l in females).[4] Patients with diabetes mellitus might be especially vulnerable to the adverse effects of anemia in the presence of cardiovascular disease and hypoxia-induced organ damage.[5] Chronic anemia can adversely affect psychological and physical development, cognitive function, appetite and exercise tolerance,[6] and might cause fatigability, malaise, dyspnea and heart palpitations. Anemia is a modifiable risk factor; its correction substantially improves quality of life for patients with chronic kidney disease and might help to arrest its progression.[7] The etiology and pathogenesis of anemia in diabetes mellitus is multi factorial. Chronic hyperglycemia might result in abnormal red blood cells, oxidative stress, and sympathetic denervation of the kidney related to autonomic neuropathy. These factors promote a hypoxic environment in the renal interstitium, which leads to impaired production of erythropoietin by the peritubular fibroblasts. Inappropriately low erythropoietin level is an important cause of early anemia in patients with diabetes mellitus.[8]

The present investigation was designed to study the impact of oxidative stress on G6PD level and the prevalence of anemia in diabetic patients.

METHODOLOGY

The prevalence of diabetes mellitus was 7% hence the sample size was calculated from the formula

$$ss = \frac{Z\,2 * (p) * (1\text{-}p)}{c\,2}$$

Z= standard error of test= 2, P = prevalence of diabetes, C = part of test = 0.05

Sampling: Blood samples were collected by venepuncture into heparin and EDTA-containing vacutainers as well as urine sample from 100 adult diabetic patients attending Buraidah central Hospital, and from 30 adult non- diabetics apparently healthy, selected at random as control.

Inclusion criteria: Test samples were collected from diabetics, their fasting blood glucose level more 140 mg/dl, and HbA1c percentage more than 8%. Controls and patients were not suffering from nephropathy: blood urea below 45 mg/dL, serum creatinine below 1 mg/dl, and microalbuminuria level below 30 mcg/mg. The cut off for defining G6PD deficiency was the concentration of G6PD below 118 mU/gHGB

Sample preparation: The heparinized, and EDTA samples were labeled by serial number, date of collection. Samples were mixed well and tested within one hour for HbA1c estimation, G6PD enzyme measurement, and reticulocyte count. Thereafter, tested by coulter (Sysmex company, Japan) to determine the HGB, RBCs count, HCT, MCV, MCH and MCHC.

The heparinized samples were centrifuged at 1000 x g for 10 minutes and plasma was harvested to be tested for TAS measurement, blood glucose level, blood urea, plasma creatinine, and blood total and direct bilirubin. The reagents for biochemical reactions, and G6PD measurement were obtained from Randox laboratories LTD, Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom BT29 4QY.

HbA1c was assayed using reagent obtained from Biosystems S. A. Costa Brava 30, Barcelona. Spain. Reticulocyte cells were counted using flowcytometer as described.[9] Microalbumiuria was measured as the ratio of albumin to creatinine in urine sample using commercial kits from Randox laboratories LTD, Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom BT29 4QY.

Statistical Analysis: Data were analyzed statistically using student t-test to find significant difference between means of patients and controls, and also to correlate between hematology parameters and G6PD and TAS using Pearson correlation, .Significance is taken at and below the 0.05 level. Permission from the faculty, and hospital management were taken, and verbal consent from study units on explanation of study objectives was taken.

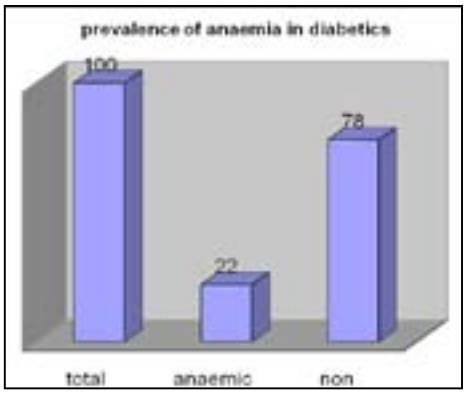

Fig-1: Shows the prevalence of G6PD anemia (HGB for females < 120g/L, <130 g/L for males) among diabetics.

Table-I: Glucose 6 phosphate deydrogenase (G6PD), total antioxidantstatus (TAS), fasting blood sugar (FBS) , total bilirubin, direct bilirubin, blood urea, serum creatinine, microalbuminuria (means ± S.E.M) in Diabetic patients (n=100) and healthy controls (n=30).

	Units	*Healthy controls*	*Patients*
G6PD level	m U/g hemoglobin	131.05 ± 2.57	70.41** ± 6.87
TAS serum level	mmol/L	1.7 ± 0.2	0.04** ± 0.005
fasting blood sugar	mg/dL	95.5 ± 2.78	145.3** ± 12.79
Total bilirubin	mg/dL	0.8 ± 0.07	3.75 **± 1.06
Direct bilirubin	mg/dL	0.2 ± 0.08	2.1*± 0.44
Blood urea	mg/dL	31.8 ± 0.34	32.0 ± 0.33
Plasma creatinine	mg/dL	0.9 ± 0.08	0.7 ± 0.05
Microalbuminuria	mcg/mg	25.3 ± 1.9	27.6 ± 1.1

* $p < 0.05$ ** $p < 0.001$

RESULTS

This study comprised of 100 patients and 30 controls. As shown in Table-I the level of G6PD, TAS, fasting blood sugar, total bilirubin diabetics are highly significant ($p \leq 0.001$) in comparison with healthy controls and direct bilirubin is significant ($p \leq 0.05$) .

Hematological changes are presented in Table-II. There was a significant decrease ($p \leq 0.001$) in HGB concentration and MCH value ($p \leq 0.05$) in all diabetic patients compared with controls, and highly significant ($p \leq 0.001$) increase in HbA1c, and reticulocyte count.

The data in Table-III show significant correlation between low HGB concentration and G6PD, and TAS deficiency at level 0.05 (2-tailed), and also there is highly significant ($p \leq 0.001$) correlation between decreased G6PD, and TAS. The prevalence of G6PD anemia is shown in Fig.1 which comprises 22% among diabetic patients.

DISCUSSION

Diabetic patients were selected in this study depending on absence of nephropathy, normal blood urea, serum creatinine, and who had no microalbminuria in their urine as shown in Table-I, because the major cause of oxidative stress in diabetes mellitus is nephropathy. Fortunately, most G6PD-deficient individuals are asymptomatic throughout their life, and unaware of their status. The illness is generally manifested as acute hemolysis, which usually arises when red blood cells undergo oxidative stress triggered by agents such as drugs, infection, or the ingestion of fava beans.[10,11]

The present study shows that there was a significant ($p \leq 0.001$) deficiency in red cell G6PD, and plasma TAS level in diabetic patients as compared to controls (Table-I). The precise mechanism by which increased sensitivity to oxidative damage leads to hemolysis is not fully known,[12] however as G6PD is major antioxidant in RBCs, so reduced activities of G6PD in diabetics beside the reduction of TAS as shown in (Table-I) may play a role in destruction of RBCs by diabetes mellitus oxidative stress resulting in anemia (low HGB concentration) which is significant ($p < 0.001$) as shown in Table-II. Acute hemolysis in G6PD deficiency is characterized by increased total bilirubin, direct bilirubin as shown in Table-I, and increased reticulocyte count (Table-II) and characterized clinically by fatigue, back pain, pallor, and jaundice.[12]

There was a significant correlation ($p < 0.05$) between deficiency of G6PD, TAS and decreased HGB

Table-II: Comparative Hematological values (means ± S.E.M) in diabetic patients (n=100), and control (n=30).

	Units	*Healthy controls*	*Patients*
G6PD level	m U/g hemoglobin	131.05 ± 2.57	70.41** ± 6.87
RBCs count	1012 L-1	5.0 ± 0.08	4.7 ± 0.14
HGB	g/L	150 ± 2.2	13.2** ± 4.5
HCT	L/L	45.5 ± 2.78	45.3 ± 12.79
MCV	fL	85.5 ± 1.17	84.5 ± 2.07
MCH	Pg	28.8 ± 0.28	27.4*± 0.44
MCHC	g/dL	31.8 ± 0.34	32.0 ± 0.33
Hb A1c	%	4.1 ± 1.3	9.3** ± 1.9
Reticulocyte count	%	1.4 ± 0.2	4.4* ± 0.9

RBCs: count-Red cells count; HGB: hemoglobin; HCT: hematocrit; MCV: mean cell volume; MCH: mean cell hemoglobin; MCHC: mean cell hemoglobin concentration; G6PD: glucose6 phosphate dehydrogenase. HbA1c.
*The significance level at $p < 0.05$ ** The significance level at $p < 0.001$

Table-III: Correlation between Hematology values, glucose 6 phosphate dehyrogenase (G6PDa), and total antioxidant status (TASa)

	RBCs count	*HGB*	*HCT*	*MCV*	*MCH*	*MCHC*	*G6PD*	*TAS*
RBCs count	1.0							
HGB	0.304**	1.0						
HCT	0.048	0.132	1.0					
MCV	0.025	0.388**	0.067	1.0				
MCH	0.031	0.655**	0.007	0.657**	1.0			
MCHC	0.064	0.598**	0.037	0.211*	0.752**	1.0		
G6PD	0.092	0.235*	0.113	0.020	0.053	0.158	1.0	
TAS	0.201	0.904*	0.690	0.507	0.553	0.454	0.911**	1.0

**correlation is significant at the 0.01 level (2-tailed)

*correlation is significant at the 0.05 level (2-tailed) [a]Pearson correlation

concentration (anemia) as shown in Table-III. The lifespan of red blood cells might be decreased in patients with diabetes mellitus.[13] Red blood cells are affected by various disturbances in the hematopoietic milieu, such as chronic hyperglycemia.

These abnormalities contribute to oxidative stress in the red blood cells and might modulate their flexibility, which makes them prone to being trapped and sequestered in the reticulo-endothelial system.[14] Furthermore, diabetes mellitus has been associated with impaired red-blood-cell deformability, a hemorrheologic perturbation that promotes microvascular complications and anemia. A number of studies have demonstrated that red blood cells have a role in the vascular damage associated with diabetic vasculopathy.[15] All these factors can contribute to microvascular disturbances in diabetes mellitus.

In a cross-sectional study by Thomas *et al*, approximately 25% of patients who attended a diabetes clinic were anemic. While in the present study about 22% of the patients are anemic presented in Fig I. Anemia is an independent risk factor for the development and progression of cardiovascular diseases,[16] congestive heart failure,[17] and chronic kidney diseases,[18] and a potential contributing factor to the development and progression of diabetic retinopathy[19] and other diabetic complications.[20]

Further studies are required to investigate the relationship between the exact impact of oxidative stress in diabetic patients. It can be concluded that in diabetic patients there is a correlation between the low HGB concentration and reduced G6PD, and TAS level, that means diabetes mellitus oxidative stress is possible cause of G6PD anemia.

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