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ORIGINAL ARTICLE

The co-existence of glucose-6-phosphate dehydrogenase deficiency among individuals with hemoglobinopathies and their effects on red cell indices .

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ABSTRACT

Background: Glucose-6-phosphate dehydrogenase deficiency and hemoglobinopathies are common among people of Duhok City in northern Iraq.

Objectives: The study was carried out to determine the co-existence of G6PD deficiency among individuals with thalassemia and sickle cell disorders.

Methods: A total of 84 diagnosed cases of hemoglobinopathies were enrolled in the study, 59 (70.24%) were diagnosed with thalassemia disorders and 25 (29.76%) with sickle cell disorders. About 3 ml of blood was collected by venipuncture from each participant and used to run G6PD and complete blood count. The data were analyzed using SPSS version 26. A manual procedure was used to estimate the G6PD enzyme activity of the participants using BIOLABO SA, G6PD assay kit (Maizy, France).

Results: G6PD deficit was detected in 15(17.90%) of the case, (10.71%) of SCD, and (7.14%) of beta thalassemia syndromes with a statistically significant association between G6PD enzyme level and clinical diagnosis. Statistically significant differences were seen in the mean values of Hb, MCH, and MCV amongst individuals with sickle cell disorders with and with G6PD deficiency, however, no statistically significant variations were seen in the mean values of red cell parameters among individuals with thalassemia disorders with and without G6PD deficiency except for MCV where the P-Value was 0.0004.

Conclusion: The study emphasized the high co-existence of G6PD deficit among individuals with hemoglobinopathy as well as significant differences were seen in some red cell indices between individuals with hemoglobinopathies with and without concomitant G6PD deficiency.

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INTRODUCTION:

The two genetic disorders that most frequently affect millions of people worldwide are hemoglobinopathies and red blood cell (RBC) G6PD deficiency[1]. G6PD deficiency is an X-linked recessive genetic disorder that mostly occurs in males in heterozygous conditions and in females in only homozygous conditions but can be partial in a heterozygous female[2]. G6PD in humans is an X-linked enzyme that plays an important role in the formation of reduced nicotinamide adenine phosphate, which is the only source of reducing power in red blood cells, where it is essential to keep equilibrium and to detoxify free oxygen species like hydrogen peroxide H2O2 and other compounds through reduced glutathione (GSH)[3] .

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is estimated to affect around 400 million people worldwide and is the most common enzymopathy in human beings[4]. It prevents red blood cells (RBCs) from oxidative damage by providing reducing energy to red cells through maintenance of the level of reduced co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH)[5]. The NADPH is again involved in keeping adequately the intracellular glutathione level (GSH) which acts as an antioxidant scavenger to counteract and remove the reactive oxygen species[6]. G6PD deficiency is usually associated with a spectrum of clinical manifestations including favism, neonatal jaundice, drug-induced red cell hemolysis, and chronic non-spherocytic hemolytic anemia[7]. The prevalence rate of G6PDD in Duhok city in a previously reported study was 10.9%, while for beta thalassemia trait and sickle cell carrier condition from the prior study were (3.7%) and (1.2%) respectively[8,9].

The geographical distribution of G6PD deficiency and hemoglobinopathies (sickle cell disease and thalassemia) is related to the past and present occurrence of malaria outbreaks because of a selective advantage against malaria infection[10],[11],[12]. Sickle cell disease (SCD) is an autosomal recessive genetic disorder brought on by the substitution of the amino acid glutamic acid by valine at position 6 of the beta-globin chains, located on chromosome 11[13]. Persons who are heterozygote for the sickle cell gene have hemoglobin (HbAS) and are usually asymptomatic, while homozygote individuals (HbSS) have lifetime acute and chronic serious complications[14].

Thalassemia is an autosomal recessive disorder of hemoglobin causing insufficient productions of at least one of the globin chains resulting in an imbalanced globin-chain production, ineffective erythropoiesis and finally to red cell hemolysis occurs, and leading to anemia[15]. The effect of G6PD deficiency on individuals with hemoglobinopathies is debatable. Many reports have highlighted the increased frequency of G6PD deficiency among individuals with hemoglobin disorders. This study's objective was to assess the prevalence

of G6PD deficiency among individuals with hemoglobinopathies in Duhok City residents.

Subjects and methods:

This cross-sectional study was carried out on 84 persons with hereditary hemoglobin abnormalities after ethical approvals were obtained from the Ethical Committee of the College of Health and Medical Techniques, Medical laboratory department, Duhok Polytechnic University. Consents were also obtained from the patients and, regarding children, consents were taken from the parents before they were recruited. The participants were informed that their information would be kept confidential and they had the right to withdraw their consent and terminate participation in the study at any time.

Data Collection, Transportation, and Storage. Three milliliters (3 ml) of blood were collected by venipuncture into EDTA anticoagulant tubes. Complete blood count (CBC) was done after appropriate shaking for 5-10 minutes using the automated blood count analyzer (Coulter; Sweden), G6PD enzyme assay samples were kept in the laboratory refrigerator at 2- 6○C until, and analyzed within the first 24 hours of collection.

G6PD assay: G6PD in RBCs is released by a lysing agent present in the reagent. The released G6PD catalyzes Glucose-6-phosphate to 6- phosphogluconate with simultaneous reduction of Nicotinamide adenine dinucleotide phosphate (NADP) to reduced NADPH. The rate of increase in nicotinamide adenine dinucleotide phosphate NADPH concentration measured at 340 is proportional to the G6PDH activity in the specimen. BIOLABO SA, G6PD assay kit (Maizy, France) was used and the manual procedure of the provided kit was followed according to the manufacturer's instructions.[16]

Exclusion criteria: included those with a history of blood transfusion in the last three months.

Statistical analysis: was done using Statistical Package for Social Sciences (SPSS version 26 software) for the calculation of means, standard deviation, Chi-square test, and independent t-test for the calculation of differences between the participants. Statistical significance was considered if the p-value is ≤ 0.05.

RESULTS:

A total of eighty-four individuals diagnosed with different types of hemoglobin disorders were enrolled in this study and screened for G6PD enzyme deficiency. The present study was carried out in the Department of Medical Laboratory Technology in our institute college over the period from February 2022 to February 2023 after taking the consent form from the patients and the Jeen Center for blood diseases.

A total of 43 (51.2%) participants were males and 41 (48.8%) were females. The age of the participants ranged from 1 to 60 years. Hemoglobin disorders were confirmed by High-Performance Liquid Chromatography (HPLC) instrument. Most of the participants, 59 (70.24%) were diagnosed with thalassemia disorders of alpha or beta types and the others

had sickle cell disorders 25(29.76%).

Table 1 shows the categorization of G6PD groups into normal, deficient, and high based on G6PD enzyme activities and according to the reference values provided with the enzyme assay Kit (10.1-14.1 U/gmHb). Those with enzyme activity < 10.1 U/gmHb were considered deficient while those with enzyme levels between 10.1-14.2 U/gmHb and greater than 14.2 were regarded as normal and high respectively. G6PD deficiency was detected in 15(17.90%) of all enrolled cases with hemoglobinopathies, of whom nine (10.71%) were males and 6 (7.14%) were females with no statistical significance between the two genders, with a Chi-square of 2.449 and a P value of 0.294 as shown in table 1.

The co-existence of G6PD deficiency observed was slightly higher among individuals with sickle cell disorders compared to those with thalassemia syndromes, 9 (60%) vs 6(40%), and a statistically significant association was observed between G6PD enzyme state and clinical diagnosis of hemoglobinopathy with a Chi-square of 10.887 and the P value of 0.028 as shown in Table 2.

As demonstrated in Table 3, a comparison of RBC count, hemoglobin (Hb), Mean cell hemoglobin (MCH), Mean cell volume (MCV) and Red cell distribution width (RDW%) showed statistically significant differences in the mean value of Hb, MCH and MCV amongst individuals with sickle cell disorders with and with G6PD deficiency, while comparison between individuals with thalassemia disorders with and without G6PD deficiency showed no statistically significant variations in the mean values of red cell parameters among these persons except for MCV where the P Value was 0.0004.

Table 1. Showing gender distribution of participants based on their G6PD enzyme state.

	G6PD status					
Gender	Deficient	Normal	High	Total	Chi-square	P Value
Female	6	17	18	41(48.80%)	2.449	0.294
Male	9	11	23	43(51.20%)		
Total	15	28	41	84(100%)		

Table 2. Thedistribution of clinical diagnosis of participants based on their G6PD enzymestate.

	G6PD status					
Diagnosis	Deficient	Normal	High	Total	Chi-square	P Value
AT	0	3	7	10(11.91%)	10.887	0.028
BT	6	16	27	49(58.33%)		
SCD	9	9	7	25(29.76%)		
Total	15	28	41	84(100%)		

$G6PDD = Glucose\ 6hosphate\ dehydrogenase\ deficiency$

Figure 1 Gender distribution of participantsin correlation with their G6PD enzyme state. F=Female, M= Male

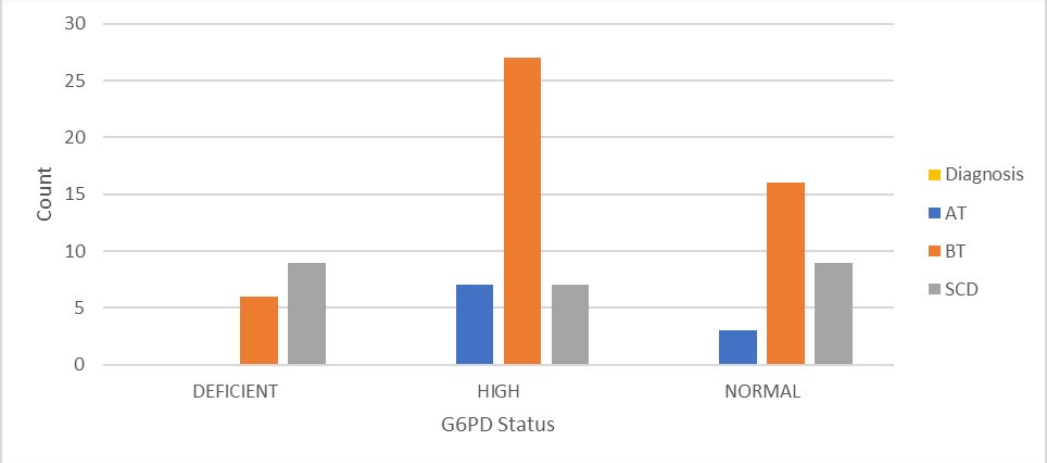

Figure 2 The distribution of clinical diagnosis of participants based on their G6PD enzyme state. AT Alphathalassemia, BT Beta thalassemia, SCD Sickle Cell Disorder

DISCUSSION:

Red blood cells depend on the G6PD enzyme to survive, due to its insufficiency, the red cell lacks the reducing power needed for oxidation protection [17]. In the Duhok community, hemoglobinopathies and G6PD deficiency are commonly diagnosed during daily practice.

The current study revealed an overall co-existence rate of G6PD deficit in 17.9% (15/84) cases, with G6PDD being detected in 36% (9/25) of cases with sickle cell disorders (SCD) and 12.24% (6/49) of cases with thalassemia syndromes. As a result, our findings are higher than those reported by Narayan Gautam et al. in 2019. (7) On the other hand, the co-

Table 3. Comparison of hematological parameters between sickle cell and thalassemia disorders with low and normal G6PD enzyme levels.

Clinical diagnosis	RBC count	Hb gm/dl	MCH pg	MCV fl	RDW%
SCD + G6PDD Mean ± SD	5.32 ± 0.50	15.48 ± 1.55	29.03 ± 1.08	81.69± 2.86	12.875 ± 0.948
SCD without G6PDD Mean ± SD	5.73 ± 2.89	10.41 ± 3.36	26.00 ± 3.56	76.49 ± 6.01	17.033 ± 7.475
t-test	0.413	4.202	2.305	2.264	1.550
P Value	0.684	0.0004	0.0333	0.036	0.136
Thalassemia syndromes + G6PDD Mean ± SD	4.50 ± 1.33	9.52 ± 1.75	21.86 ± 3.78	69.0 ± 8.86	20.14 ± 4.66
Thalassemia syndromes without G6PDD Mean ± SD	5.13 ± 0.80	10.00 ± 1.62	19.54 ± 2.40	62.68 ± 5.94	19.31± 5.11
t-test	1.584	0.629	1.975	2.154	0.347
P Value	0.119	0.532	0.054	0.036	0.730

existence rate of G6PDD among SCD was lower than that reported by Samuel et al. 2019[10]. In this study the co-existence of G6PDD was higher among males than females, the finding is similar to that reported by Gibbs et al. 1980[18].

Furthermore, the study also found that 36% of individuals with sickle cell disorders have G6PDD, the figure exceeds those observed in the Taiz region, where there was an incidence of 22.6% G6PD deficiencies amongst SCD patients, this may be due to variation in the sample size and the geographical distribution of these diseases[19]. In this study, the prevalence of G6PDD among individuals with thalassemia was 11.32% (6/53), the results are comparable to those reported by Sakorn 2013[20].

According to the current study, the overall variations in G6PD enzyme level were as follows: Deficiency was observed in 36% and 12.24% of cases of SCD and BTT, respectively, while an increased G6PD level was established in 28.0% SCT and 55.10% BTT. The results diverge from those of a study done by Gautam et al. 2019[21] that claimed G6PD deficiency was found in 20.93% SCD and 4.8% BTT and an elevated G6PD level was only found in 5.3% SCT and 4.8% BTT. It's likely that there are differences between the normal range of enzyme activities and the prevalence of G6PDD.

The qualitative tests that are widely used to check for the condition in clinical settings can identify males and females with G6PD deficiency, which aids in directing the choice of the most efficient treatments. In order to reliably diagnose those who have SCD or thalassemia in conjunction with G6PD deficiency, more research employing DNA techniques is required as well as planning for studying the clinical presentations of the combined pathologies.

CONCLUSION:

The study revealed a high co-existence rate of G6PD deficiency among individuals with hemoglobinopathy with significant differences in some red cell indices between individuals with hemoglobinopathies with and without concomitant G6PD deficiency and especially those with sickle cell disorders.

DECLARATIONS:

Authors' contributions:

Contributor Role	Degree of Contribution		
	Lead	Equal	Supporting
Conceptualization	SSA		DAM
Data curation	SSA		DAM
Formal analysis	SSA		DAM
Funding acquisition	SSA		DAM
Investigation	SSA		
Methodology	SSA		DAM
Project administration	SSA		DAM
Resources	SSA		
Software	SSA		
Supervision	SSA		DAM
Validation	SSA		
Visualization	SSA		
Writing-original draft	SSA		DAM
Writing-review & editing	SSA		DAM

Conflict of interest: None

Ethical Approvals: Ethical approvals were obtained from the Ethical Committee of the College of Health and Medical Techniques, Medical laboratory department, Duhok Polytechnic University.

Data Availability: Duhok Directorate General of Health, Azadi Teaching Hospital, Laboratory department, Duhok governorate

Funding Resources: None

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