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ASSESSMENT OF G6PD AND CBC PARAMETERS AMONG SUDANESE PATIENTS WITH TYPE 2 DIABETES MELLITUS

Eltaher A. E. Ahmed[1]*, Mahmoud I. O. Hamad, Mohammed B. Omer[3] and Amira A. K. Humida[4]

[1]*ALNeelain University- Faculty of Medical Laboratory Sciences - Department of Hematology, Khartoum-Sudan.
[2]SHARQ EL NIEL College, Department of Histopathology and Cytology- Khartoum-Sudan.
[3]Ibn Sena University Faculty of Medical Laboratory Science Department of Hematology & Immunohematology, Khartoum-Sudan.
[4]ALNeelain University- Faculty of Medicine c - Department of Pathology, Khartoum-Sudan.

***Corresponding Author: Eltaher A. E. Ahmed**
ALNeelain University- Faculty of Medical Laboratory Sciences - Department of Hematology, Khartoum-Sudan.

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ABSTRACT

Back ground: Diabetes mellitus (DM) is a common and complicated disease with increasing prevalence especially in developing countries. Complications of DM impose heavy health and financial burden on society. The definition of diabetes mellitus has recently changed considerably. This condition is now defined as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion and/or action. **Objective:** This study aimed to assess the G6PD level and CBC indices among Sudanese patients with D.M. **Materials and method:** This was a prospective case-control hospital-based study conducted in Modern Medical Center in Khartoum, Sudan from December 2016- May 2017. This study included sixty diabetic patients as cases, control group included (40) non- diabetic volunteers. CBC and G6PD were assessed among both the case and control groups and frequency of abnormal result was determined. **Results:** In this study we found that the level of G6PD was low in all the sixty patients (mean, SD: 1279.00±680) and was normal in the control group (mean, SD: 1989.35±503.19) with a statistically significant difference (p.value of 0.00). Also found that there was insignificant correlation between G6PD level and Hb level with a p-value of (0.594). **Conclusion:** This study concluded that G6PD level was significantly decreased in diabetic patients when compared to control individuals, there was strong association between DM and G6PD deficiency, diabetic hyperglycemia may lead to serious complications and decrease G6PD activity**.** Also this study concluded that there was no significant difference in CBC parameters between different case and control groups.

KEYWORD: Complete blood count (CBC), Diabetes mellitus (DM), (G6PD), Hemoglobin (Hb).

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorders characterized by high levels of sugar (glucose) in the blood.[1] It is said to occur when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces and hence, an increased concentration of glucose in the blood known as hyperglycaemia.[1][2]

Type 1diabetes, previously called insulin-dependent diabetes mellitus or juvenile-onset diabetes, is due to a deficiency in endogenous insulin secretion secondary to destruction of insulin-producing beta cells in the pancreas. Although type 1 diabetes does have a peak incidence around the time of puberty, approximately 25% of cases present after 35 years of age. Type 2diabetes, formerly known as non-insulin-dependent or adult-onset diabetes mellitus, is characterized by insulin resistance with an insulin secretory defect leading to relative insulin deficiency. This group accounts for 90-95% of patients with diabetes and also has a strong genetic predisposition. Type 2 patients are usually, but not always, older than age 40 at presentation. Obesity is a frequent finding. Symptoms of diabetes are gradual and typically extreme thirst, frequent passing of water and heavy weight loss over a short period. Others include fatigue, frequent infections, itching and rashes as well as disturbed vision. However, some people show none of these symptoms. As a result, most people remain undiagnosed for a long time until when complications of the disease become evident. Some of which may lead to blindness, numbness/infections in feet, amputation of limbs, kidney failure, or heart disease.[3]

Other forms of diabetes mellitus include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several

forms of monogenic diabetes (Cooke and Plotnick, 2008; Lawrence et al., 2008).[4][5]

According to World Health Organization (WHO) and International Diabetes Federation (IDF), the following criteria are recommended for the diagnosis of diabetes mellitus: fasting plasma glucose ≥ 7.0mmol/l (126mg/dl) or 2-h plasma glucose (venous plasma glucose 2 hours after ingestion of 75g oral glucose load) ≥ 11.1mmol/l (200mg/dl). They further recommend that the oral glucose tolerance test is the most preferred diagnostic test for diabetes mellitus.[6] The test should be performed in the morning after an overnight fast of between 8 and 14 hours and after at least 3 days of unrestricted diet (≥ 150g carbohydrate per day) and unlimited physical activity. More so, the subject should remain seated and not smoke throughout the test. In recent times, glycated haemoglobin has also been recommended for the diagnosis of diabetes, with a threshold of ≥ 6.5%.[7]

Glucose-6-Phosphate Dehydrogenase (G6PD) is a cytoplasmic enzyme affecting the production of the reduced form of the extra mitochondrial Nico- tine-Adenosine-Dinucleotide Phosphate coenzyme G6PD activity, which is the only source of NAD P H.[8] The gene encoding G6PD is located in the telomeric region of the long arm of the X chromosome (band Xq28). More than 300 alleles with point mutation in the G6PD gene sequence have been identified.[9]

MATERIALS AND METHODS

This was a prospective case-control hospital-based study conducted to determine CBC and G6PD activity among diabetes mellitus cases.

Sixty diabetic patients were included as cases, 40 healthy people were recruited as control group which were aged matched with the case group. This study conducted in Modern Medical Center in Khartoum, Sudan from December 2016- May 2017. This study was approved by ethical committee of the faculty of medical laboratory sciences, Al-Neelain University, and informed consent was obtained from each participant before sample collection and ethical conduct was maintained during data collection and throughout the research process. All statistical analyses were performed by SPSS software version (20). Continuous variables were expressed as mean and standard deviation. In the analytical results, p-value <0.05 was considered significant.

Hematological assay

5ml of blood were collected into EDTA container and each sample mixed gently to estimate then CBC and G6PD. CBC was performed using sysmex for estimation of Hb, RBCs, PCV, and RBCs indices.

G6PD activity was performed using full automation by Mindary Bs 480 which was already calibrated according to manufacture standardized procedure and the G6PD done within the calibration frequency period 21 days. Was dissolved a vial of R2 - NADP with 1 mL of R1 - BUFFER, and was mixed gently avoids foaming then was Let the reagent reach the room temperature before use. Was closed immediately after handling. The Reagents have to be used correctly, to avoid contamination. An incompetent handling relieves us from any responsibility. If the absorbance/minute is higher than 0.060 was used half sample volume and multiply the result x 2, the sensitivity limit, that is the minimum concentration that can be distinguished by zero, is 27 U/L.

RESULTS

The study included 100 participants, 60 diabetic patients as case group and 40 healthy people as control groups; it was conducted during the period from December to May, 2017 at the Khartoum state.

Table (1): Frequency of G6PD in case study.

N.V of G6PD > 1300 U/L

G6PD(u/L)	Frequency
<1300u/L(low activity)	66
>1300u/L(Normal activity)	34

The frequency of G6PD among cases group was more frequent G6PD <1300u/L (66) and less frequent G6PD >1300u/L (34).

Table (2): Frequency of Hb g/dl and mean G6PD in correlate to Hb in case study.

Hb(g/dL)	Frequency	MeanG6PD
<7	0	0
7-9	0	0
9-12	38	977.9
12-14	24	1472.8
14-16	30	1376.8
16-18	8	1241.1

The frequency of Hb among cases group was classified into <7g/dL and 7-9g/dL were (0) patient, in range between9-12g/dL was 38 patients, in range between12-14g/dL was 24 patients, in range between14-16g/dL was 30patients, and in range between16-18g/dL was 8patients. The mean result of G6PD in correlated to Hb g/dl was (977.9(low), 1472.8, 1376.8, and 1241.1(low)) in patients with Hb in range 9.1-12g/dl, 12.1-14, 14.1-16, and 16.1-18, respectively).

The frequency of age in case group was more in patients with age more than 50(68) and the age less than 50 was (32). The frequency of gender among case was males (50) and female (50). Table (3).

Table (3): Frequency of age and gender in case study.

		Frequency
Age	<50	32
	>50	68
Gender	Male	50
	Female	50

Table (4) Mean comparison among the study and control group.

Parameters	Case (Mean±SD)	Control (Mean±SD)	P-value
RBC	4.82±0.60	4.93±0.54	0.355
Hb	13.34±2.05	13.39±1.88	0.913
PCV	40.94±5.52	41.05±4.87	0.906
MCV	85.02±5.21	84.48±6.32	0.629
MCH	27.72±2.42	26.86±2.52	0.076
MCH c	32.74±2.44	31.47±4.85	0.088
B. glucose	164.42±77.39	105.00±20.00	0.000
G6PD	1279.00±679.89	1989.35±503.19	0.000

The mean result of RBCs was insignificant within normal range in case (4.82±0.60) when compare to control (4.93±0.54) with p.value 0.355, the mean of Hb was insignificant within normal range in case (13.34±2.05) when compare to control (13.39±1.88) with p.value 0.913, the mean of PCV were insignificant within normal range in case (40.94±5.52) when compare to control (41.05±4.87) with p.value 0.906, the results of MCV were insignificant within normal range in case (85.02±5.21) when compare to control (84.48±6.32) with p.value 0.629, the mean of MCH were insignificant within normal range in case (27.72±2.42pg) when compare to control (26.86±2.52pg) with p.value 0.076, and the mean of MCHC were insignificant within normal range in case (32.74±2.44%) when compare to control (31.47±4.85%) with p.value 0.088, the mean of glucose was significant increased in case (164.42±77.39) when compare to control group (105. 00±20.00) with p.value 0.00, and the mean result of G6PD was significant decreased in case (1279.00±679.89) when compare to control (1989.35±503.19) with p.value 0.00.

Table (5): Mean comparison across gender distribution.

Parameters	Male (Mean±SD)	Female (Mean±SD)	P-value
RBC	4.98±0.55	4.66±0.61	0.054
Hb	13.95±1.89	12.74±2.04	0.034
PCV	42.32±5.28	39.55±5.52	0.076
MCV	84.35±4.65	85.69±5.73	0.368
MCH	28.01±2.41	27.43±2.44	0.406
MCH C	33.45±2.32	32.03±2.39	0.039
B. glucose	170.04±51.91	158.80±53.83	0.022
G6PD	1203.67±685.89	1354.33±679.33	0.012

The mean result of RBCs was insignificant within normal in case when correlated to gender male and female (4.98±0.55 and 4.66±0.61, respectively) with p.value 0.054, the results of Hb were significant within normal in case in correlated to gender male (13.95±1.89g/dL) and female (12.74±2.04g/dL) with p.value 0.034, the mean results of PCV were insignificant within normal in case male (42.32±5.28%) and female (39.55±5.52%) with p.value 0.076, the mean results of MCV were insignificant within normal in case male (84.35±4.65fl) and female (85.69±5.73fl) with p.value 0.368, the mean results of MCH were insignificant within normal in case male (28.01±2.41pg) and female (27.43±2.44pg) with p.value 0.406, the mean results of MCHC were significant within normal in case male (33.45±2.32%) and female (32.03±2.39%) with

p.value 0.038, The mean results of glucose were significant increased in case in correlated to gender male (170.04±51.91) and female (158.80±53.83) with p.value 0.022, and the mean of G6PD was significant decreased in male group (1203.67±685.89) than female (1354.33±679.33) with p.value 0.012.

DISCUSSION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common hereditary disorders in humans.

The frequency of G6PD among cases group was more frequent as (66%) of them showed low activity of G6PD (<1300u/L) while 34% showed normal activity of G6PD (>1300u/L).

That corresponds to a study conduct by Promise Emeka 2013[10], who found out of the total number of respondents sampled, 69.9% were carriers of G6PD deficiency gene and 30.10% normal G6PD, showing that diabetic patients had a significant ($p < 0.05$) presence of the disease.

G6PD deficiency recorded among diabetic patients in this study, this result was corresponding to a study conduct by Antonio Pinn, G6PD deficiency was found in 21 (5.4%) diabetic patients and 33 (8.5%) controls (P =0.09).

The mean result of G6PD in the present study was significantly decreased in case (1279.00±679.89) when compared to control (1989.35±503.19) with p.value 0.00. That agrees with Festus OO, 2012[12], the results of G6PD were significantly decreased when compared the case (1253) to control group (1444) with p.value <0.05. Also agreement with the reports of Wan et al, (2002) and Gwo-Hwa et al., (2002)[13] who reported G6PD was analyzed among 237 patients with diabetes and 656 healthy subjects, significantly lower activity of G-6-PD in type-2 diabetic individuals. Hence, unsuitable control of blood glucose may decreases G-6-PD activity and increases diabetes mellitus complications.

The finding of this study was however at variance with the work of Gaskin RS et al. (2001)[14], where they observed a slightly increase in the activity of G-6-PD in patients with type-2 diabetes mellitus.

In this study we found association between G6PD deficiency and glucose level P.value 0.000, this result was corresponding to a study conducted by M. B. Adinortey 2011[15], who concluded that there were the association between G-6-PD deficiency and diabetes mellitus and that G-6-PD deficiency (moderate and severe) is a risk factor for diabetes mellitus.

The mean of PCV was within normal range in case (40.94±5.52) when compared to control (41.05±4.87) with insignificant difference between the two groups p.value 0.906.

This study agrees in PCV results with Musa, B. O. P.[16] The results of PCV in that study were significant within normal range when they compare case (37%) to healthy control (40%) with p.value <0.05.

The results of Hb were significant within normal in case in correlation to gender male (13.95±1.89g/dL) and female (12.74±2.04g/dL) with p.value 0.034, that agrees in Hb level with previous study done by Mahboobeh Sadat Hosseini.[17] The results of Hb in correlated to gender male(14g/dL) and female(12.7g/dL) were within normal range.

CONCLUSION

We concluded from this study that G6PD were significantly decreased in diabetic patients type 2 when compared to control, diabetic hyperglycemia may lead to serious complications and decrease G6PD activity. For recommendation, further future studies should be conducted with more individuals such that events recorded will be substantial enough to predict statistically significant trends in relation to G6PD deficiency in diabetes in Sudan.

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