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Evaluation of Steroid Hormones Level, Vitamin D3, K and Some Minerals in The Sera of Patients Infected with Glucose-6-Phosphate Dehydrogenase Deficiency

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ABSTRACT

Background: The deficiency of glucose-6-phosphate dehydrogenase enzyme (Glucose-6-phosphate dehydrogenase G6PD) is one of the genetic blood diseases. It is a disease related to the sex of the newborn, meaning that this disease is genetic disease linked to sex, and usually affects males more than females.

Objective: The study aimed to evaluate the activity of some fatty acids and glucose-6-phosphate dehydrogenase enzyme in the blood of glucose-6-phosphate dehydrogenase deficient patients.

Subjects and methods: The study was a group of 118 blood samples, 58 samples from healthy people (control), distributed as 34 males and 24 females in addition to 60 patients with glucose-6-phosphate dehydrogenase deficiency. The subjects were collected from the Outpatient Clinics in Baghdad City and Al-Dhuluiya district, Salah Al-Din district between 20/11/ 2020/ and 15/3/2021. The current study evaluated glucose-6-phosphate dehydrogenase enzyme activity, steroid hormones (Testosterone and dihydrotestosterone), Vit. D3, K, Ca, Cu and Zn in the serum of studied subjects.

Results: There was a significant decrease in G-6-P-D in the blood serum of patients compared to the healthy group. Also, the results showed significant decrease in testosterone, DHEA, vit D3 and vit. k in the blood serum of patients compared to the healthy group. Additionally, the results showed that there was significant decrease in Zn in patients' blood serum compared to the healthy group. However, the results revealed an increase in Cu in the blood serum of patients when compared to the healthy group. Furthermore, the results revealed that there was no significant change in Ca between patients and healthy people.

Keywords: G-6-P-D, Steroid hormone, Vit. D, Vit K.

INTRODUCTION

One of the genetic blood diseases is the deficiency of glucose-6-phosphate dehydrogenase enzyme (G6PD), a disease related to the sex of the newborn, meaning that this disease is a genetic disease linked to sex, and usually affects males more than females, as a result of genetic makeup for both sexes [(1)].

So, many researchers depend on measuring the effectiveness of enzymes in both pathological and natural cases, and benefit from them in diagnosing and treating diseases [(2)]. It is one of the oxidation-reduction enzymes Oxidoreductas EC 1.1.1. 49 that was described for the first time by **Warburg and Christion [(3)]** 1931 and it is one of the cytoplasmic enzymes that circulates in all cells of the body, especially in red blood cells [(4)].

Testosterone is one of the steroid hormones, which belongs to the family of androgens and is derived from cholesterol [(5)]. It is also a fat-soluble hormone. Its molecular weight is 288.42 g/mol and it has a structural formula consisting of 19 carbon atoms with a ketone group in position 3, a double bond at position 4 and a hydroxyl group at position 17 [(6)]. The main source of its manufacture and secretion is the testes, and it is also secreted from the ovaries in women with a small percentage secreted by the adrenal gland [(7)].

Although vitamin D is called a vitamin, it is not an essential dietary vitamin in the strict sense because all mammals can synthesize enough of it from sunlight. In this case, it must be obtained from the diet by an organism. Vitamin D, like other compounds (called vitamins) was discovered in an effort to obtain a nutritional substance that was lacking in diseases such as rickets (a form of osteomalacia in childhood). Furthermore, vitamin D, like other vitamins in the modern world, is added to basic foods such as milk to prevent diseases caused by its deficiency [(8)].

MATERIAL AND METHODS

Sample Collection: 60 blood samples were collected from patients of both sexes with glucose-6-phosphate dehydrogenase deficiency from Outpatient Clinics in Baghdad city and Al-Dhuluiya/Salah Al-Din district and healthy control group included 34 males and 24 females through the period from 20/11/2020 to 15/3/2021.

Estimation of G-6-P-D in all group

The enzyme activity in red blood cells was estimated using the diagnostic kit supplied by the French company, as the principle of the examination depends on the speed of release of NADPH by the enzyme and the absorbance of the formed compound that was measured at the wavelength of 340 nm[(9)]. The activity of glucose-6-phosphate dehydrogenase enzyme was calculated according to the following equation [(10)].

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$$\text{IU/g Hb} = \frac{(\Delta Abs/\min \times 5000)}{Hb\ g/dL}$$

Estimation of Steroid hormones level in all group: In this method, a ready-made assay kit was used to estimate the concentration of total testosterone hormone and DHEA in the blood serum by the direct ELISA test method.

Estimation of Vitamins level: Vitamin D was measured using the analysis kit Bio active supplied by American company, while vitamin K was measured according to the equipment Mybiosource supplied by the American company.

Estimation of mineral level in all group: Calcium concentration in the blood serum was measured according to the ready-made analyzer kit by the French company Spectrum [(11)]. The level of copper in the blood was estimated by means of the diagnostic kit supplied by the Egyptian Spectrum company [(12)]. Zinc was detected by a Colorimetric test [(13)], using a special kit to detect zinc (Zinc colorimetric test Mark, Gisse, Rome-Italy).

Ethical consent:

The study protocol was assessed and approved by the Ethics Committee of College of Education for Women, University of Tikrit, Tikrit, Iraq. Informed consents were taken from the patient's relatives or the patient himself when he was still conscious with keeping the patients` records confidential in all stages of the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical Analysis

The SPSS statistical program was used to compare the outcomes of patients and controls using the T test at a probability level of ($P \leq 0.05$).

RESULTS

Measurement of the G-6-P-D, Testosterone, EDHA, Vit D, Vit K, Zn, Ca and Cu levels in the samples under study all were included in table (1) and figures (1, 2, 3, 4, 5, 6 and 7).

Evaluation of G-6-P-D activity of all groups:

The G-6-P-D enzyme activity in the blood serum of patients was found to be significantly lower compared to healthy subjects (Table 1). When comparing the activity of G-6-P-D enzyme by sex, the results showed that the activity of G-6-P-D enzyme was significantly decreased in male patients compared to healthy males (367.3 ± 44.1 vs 2303.2 ± 65.1 IU/gm respectively). Also, the results showed a decrease in the activity of G-6-P-D enzyme in female patients compared to healthy (442.1 ± 25.2 vs 2354.0 ± 58.2 IU/gm respectively). When comparing the activity of G-6-P-D between both sexes in patients, the results showed no significant differences as well as among healthy group (Figure 1).

Evaluation of Steroid hormone in serum blood of all groups:

Regarding testosterone and DHEA, there was a significant decrease in testosterone and DHEA levels in blood serum of patients compared to healthy controls (Table 1). When comparing the level by sex, the results revealed that the level of DHEA decreased significantly among male patients compared to healthy males (114.7 ± 16.2 vs 277.6 ± 27.7 ng/ml respectively). Also, there was a decrease in the level of DHEA hormone in female patients compared to healthy females (200.8 ± 28.2 vs 253.2 ± 27.5 ng/ml respectively). It was also found that the level of the happiness hormone decreased in males compared to females, as shown in figure (2).

Evaluation of the steroid Vitamins D and K in serum blood of all groups:

The results in table (1) showed a significant decrease and in the levels of Vit. D, Vit. K in the blood serum of patients compared to healthy controls.

Concerning comparison for the levels of vitamins by sex, the results revealed that the level of vitamin D decreased significantly in male patients compared to healthy males (22.31 ± 4.899 vs 54.85 ± 6.430 ng/ml respectively). The findings revealed a decrease in the level of vitamin D in female patients compared to healthy females (20.73 ± 3.600 vs 45.63 ± 7.340 ng/ml respectively) as shown in figure (3). A comparison was also made for the level of vitamin K by sex, and the results revealed that the level of vitamin K decreased significantly in male patients compared to healthy males (3.442 ± 1.272 vs 4.635 ± 0.547 nmol/liter respectively). Also, the results showed that there were no significant differences in the levels of vitamin K among sick and healthy females (4.302 ± 0.609 vs 4.263 ± 0.504 nanomol/liter respectively) as shown in figure (4).

Evaluation of Minerals in serum blood of all groups:

The results in table (1) showed a significant decrease in the levels of (Zn) in the blood serum of patients compared to healthy controls. **Concerning the level of calcium by sex**, as the results showed that the calcium level did not show a significant difference between male patients and healthy males (8.6500 ± 0.3402 vs 8.6048 ± 0.4155 mmol/dl respectively). Also, the results showed no significant differences in calcium level in female patients compared to healthy females (8.8700 ± 0.6270 vs 8.8180 ± 0.5000 mmol/dL respectively) as shown in figure (5).

As regards the level of zinc according to sex, zinc level was significantly decreased among male patients compared to healthy males (13.31 ± 4.428 vs 79.45 ± 12.58 mg/dl respectively).

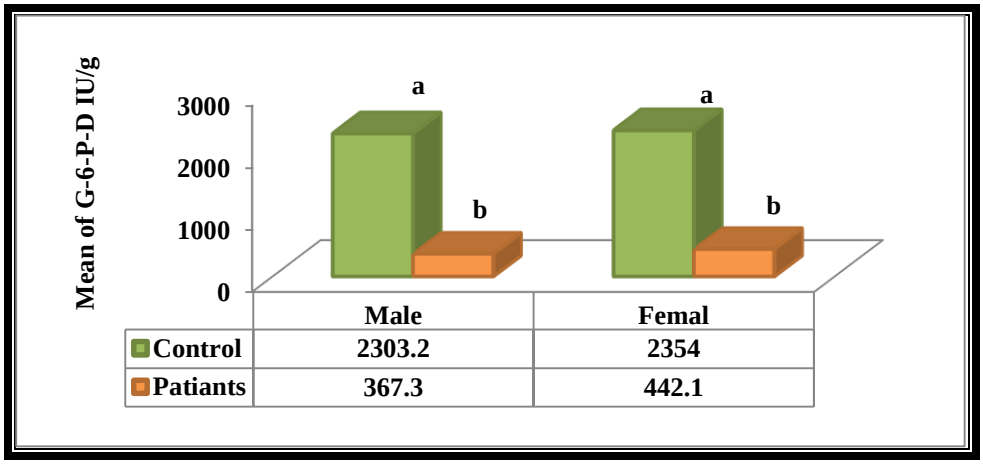
Also, the results showed a decrease in zinc level in female patients as compared to healthy females (54.90 ± 12.20 vs 84.01 ± 12.69 mg/dL respectively). In addition, the results showed a decrease in zinc level in males compared to females, as shown in figure (6).

Regarding level of copper by gender, the results showed that the level of copper increased significantly among male patients compared to healthy males (141.19 ± 22.60 vs 97.00 ± 11.26 mg/dl respectively).

Also, the results showed that there were no significant differences in the levels of copper in female patients as compared to healthy females (107.37 ± 25.05 vs 104.63 ± 17.21 mg/dL respectively). Moreover, the results showed a rise in the level of copper in males compared to females, as shown in figure (7).

Table (1): Parameters of the variables studied in the research

Parameters	Mean ±S.D		P≤
	Control	Patients	
G.6.P.D IU/g	2324 ± 51	397 ± 38	0.008
Testosterone(ng/ml)	7.55 ± 1.37	1.09 ± 0.21	0.006
D H E A (ng/ml)	267.5 ± 28.1	149.0 ± 20.0	0.003
Vit D ng/ml	51.0 ± 11.20	21.68 ± 4.95	0.007
Vit K nmol/L	4.481 ± 0.557	3.790 ± 0.82	0.002
Ca mmol/dl	8.829 ± 0.513	8.974 ± 0.758	N.S
Zn mg/dl	81.4 ± 12.7	29.9 ± 7.3	0.006
Cu mg/dl	100.2± 14.4	127.7 ± 28.8	0.003



**P<0.05

Figure (1): G-6-P-D enzyme activity in patients with G-6-P-D enzyme deficiency compared to healthy controls by gender.

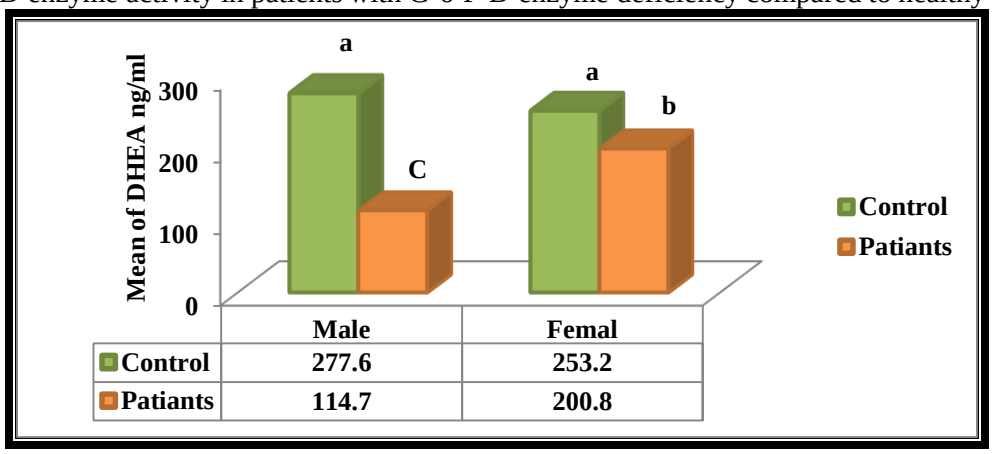


Figure (2): EDHA level in patients with G-6-P-D enzyme deficiency compared to healthy controls by gender

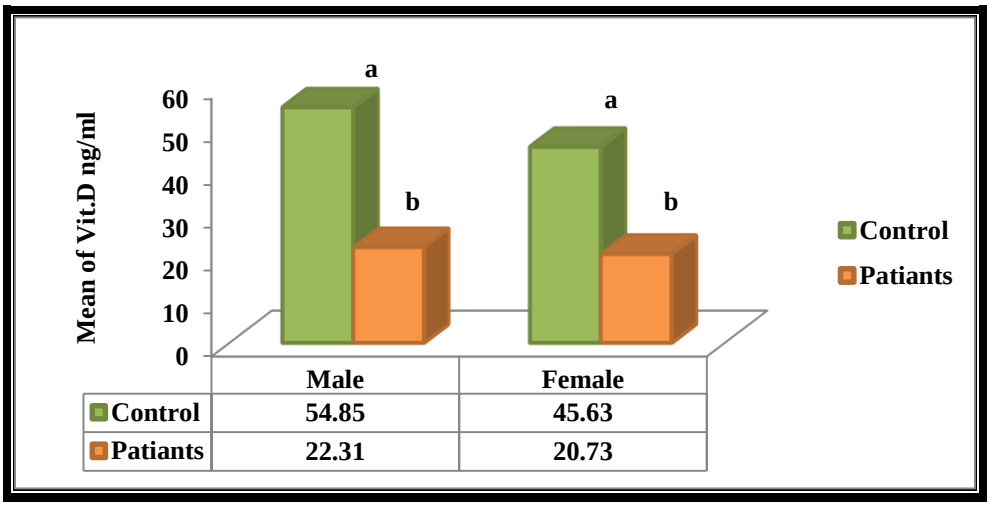


Figure (3): Vitamin D level in patients with G-6-P-D enzyme deficiency compared to healthy controls, by gender

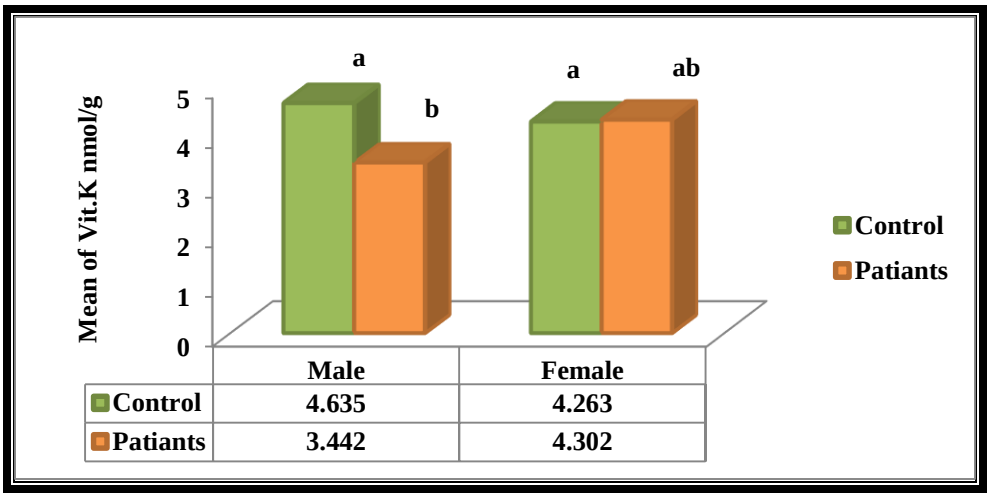


Figure (4): Vitamin K level in patients with G-6-P-D enzyme deficiency compared to healthy subjects by gender.

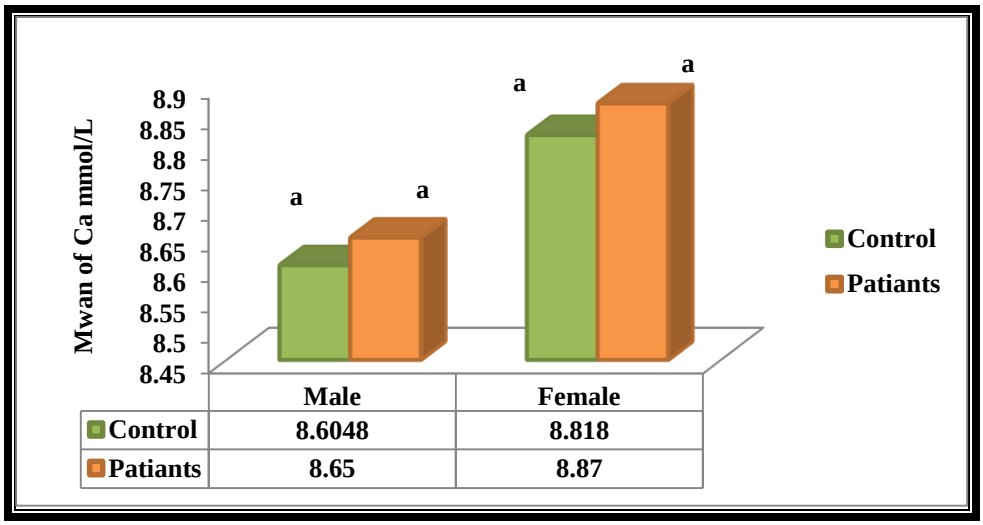


Figure (5): Calcium level in patients with G-6-P-D enzyme deficiency compared to healthy subjects by gender

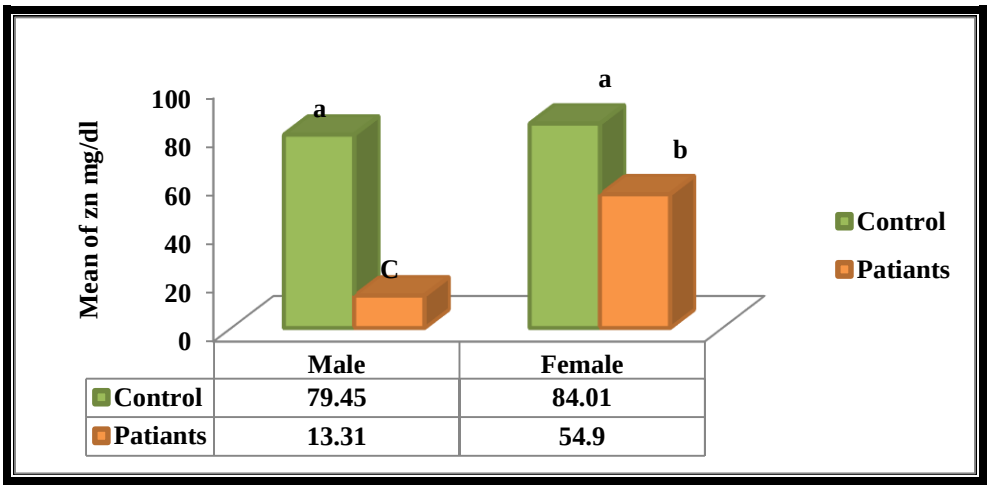


Figure (6): Zinc level in patients with G-6-P-D enzyme deficiency compared to healthy controls by gender.

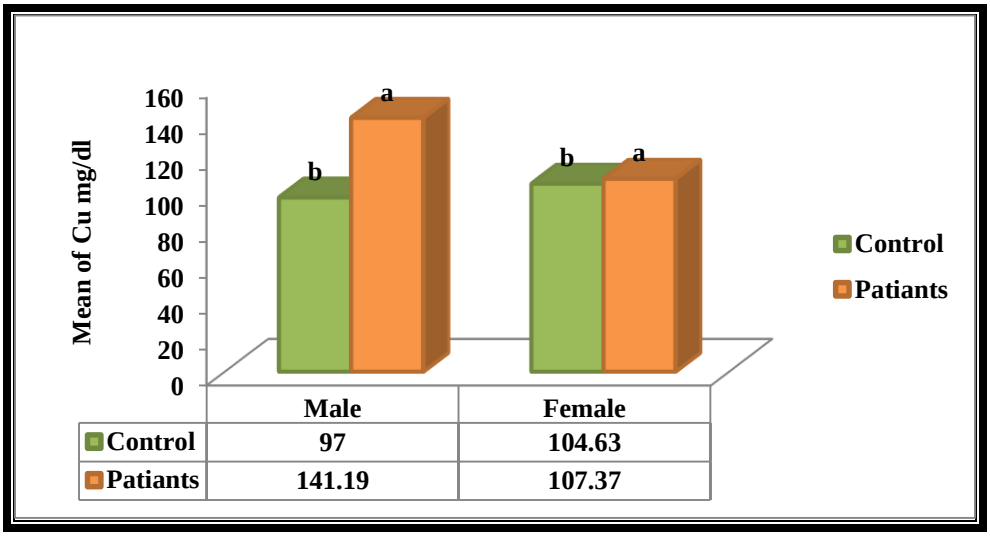


Figure (7): Copper level in patients with G-6-P-D enzyme deficiency compared to healthy subjects by gender.

DISCUSSION

Our results agree with **Amir** [14] and **Wu *et al.*** [15], who indicated in their study that the activity of G.6.P.D enzyme is significantly lower in the hemolytic anemia group compared to the healthy group, as the decrease in the effectiveness of this enzyme affects all cells of the body, including the red blood cells. The reason is that the main action of the enzyme G6PD is to protect the reduced glutathione in the red blood cell through the formation of the reduced energy transporter NADPH, and in the absence of glutathione or a decrease in its concentration in the red blood cell, this leads to an increase in oxidative stress on the globule membrane, and this results in a state of hemolysis [16].

There are many factors, such as some antibiotics, anti-malarial drugs, some types of sedatives and stimulants, and eating uncooked beans, which lead to a state of hemolysis associated with G6PD enzymatic deficiency. The activity of the enzyme affects these sites where the enzyme spreads, but to a lesser extent than the rate of infection of the red blood cells [17]. The results of the current study does not agree with the findings of **Cindy *et al.*** [13] and **Luigi *et al.*** [18], who showed in their study the high level of testosterone by treatment in elderly patients with anemia.

As regards the level of happiness hormone (DHEA) in patients with G-6-PD enzyme deficiency compared to healthy people according to gender, the results of the current study agree with the findings of **Ko *et al.*** [19] and **Yamada *et al.*** [20], who showed in their study a decrease in the secretion of DHEA resulting in inhibition of the activity of erythropoiesis in aging men with hemolytic anemia.

The results of the current study agree with the findings of **Jumaa** [21], who showed in his study a low level of vitamin D in patients with hemolytic anemia compared to healthy group. As hemolytic anemia patients are at risk of a number of complications, including poor

growth, endocrinopathy, and metabolic abnormalities [22-25]. Vitamin D is necessary for calcium homeostasis and skeletal mineralization. Osteopenia Osteoporosis Syndrome [26].

The results of the current study does not agree with the findings of **Michael *et al.*** [27], who showed in their There are no significant differences in vitamin K levels between patients with hemolytic anemia and the healthy group, according to a study. Vitamin K has traditionally been associated with blood clotting, as it is a vitamin that plays various roles, including connective tissue calcification [28]. Vitamin K deficiency in patients with hemolytic anemia may cause oxidative damage in G-6-PD resulting in its deficiency and thus be responsible for the documented low-grade hemolysis in these patients [29]. Although some drugs can cause hemolytic anemia in people with G-6-PD deficiency that may be due to oxidative damage of the enzyme [30].

The results of the current study does not agree with the findings of **Laura *et al.*** [31], who showed in their study a high level of calcium in patients with hemolytic anemia. Calcium levels increased significantly within these cells [32]. The increase in calcium may also be due to the reduction of NMDA and its intracellular blockade in hemolytic anemia patients [33], as well as erythrocyte overload and dull polycytosis causing ATP energy depletion and an increase in active calcium levels, then overall cytoskeletal stability is impaired in RBCs in patients and this impairment may lead to changes in mechanical stability for cells such as Piezo1, which again leads to an increase in intracellular calcium [34] The results of the current study agree with the findings of [35]who showed in their study the high level of copper in patients with hemolytic anemia compared to the control group..

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