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Study of the Effect of the Activity Level of the Enzyme Glucose-6-Phosphate Dehydrogenase with Some Electrolytes in Female Patients Suffering From Hemolytic Anemia in the City of Hilla

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Annotation: This study aims to show the relationship between the level of the enzyme glucose-6-phosphate dehydrogenase and its relationship to electrolyte levels, which include (calcium, zinc, copper) in addition to vitamins (D, K). This study included 35 blood samples taken from patients suffering from anemia. Patients aged between (40-60) years and 35 samples as a control group (healthy people) for the period from (February to April 2024, when samples were collected from the chronic diseases unit of Al-Hilla Teaching Hospital in Babylon Governorate. The results of the current research showed a significant increase in each of the levels (calcium, copper) in the blood serums of patients with hemolytic anemia with a control group (healthy subjects), and the results also showed a significant decrease in each of the levels (G.6.P.D., zinc, vitamin D). (In the blood serums of patients with hemolytic anemia with a control group (healthy people) with no significant differences in the level of (vitamin K), and at its level, the potential for P ≤ 0.001.

Keywords: Glucose 6-phosphate dehydrogenase, electrolytes, vitamin D and vitamin K.

Introduction

Anemia is a public health concern worldwide and is a clinical condition characterized by low hemoglobin concentration, which leads to the loss of the blood's ability to carry oxygen and the supply of oxygen to tissues becomes insufficient to meet physiological needs, especially in high-demand conditions such as exercise and pregnancy [1]. It affects Anemia affects a third of the world's population and contributes to increased morbidity and mortality rates, decreased work productivity in adults, and poor neurological and cognitive development in children [2].

The main cause of anemia is iron deficiency, which is believed to be a primary cause in 50% of all cases [3]. Therefore, the body's physiological need for oxygen varies according to the sex and age of the person, height above sea level, and smoking [4].

The effectiveness of the G6PD enzyme was discovered in humans in the middle of the twentieth century in 1931 [5], in black Americans and African Americans [6].This was after observing several cases of lysis of red blood cells in these people after taking antimalarial drugs such as Pamaquine and Primaquine, which encouraged the study of the impairment of this enzyme in detail [7,8], as it is the main enzyme in the pentasaccharide pathway, so its deficiency causes NADPH depletion in erythrocytes [9],

Therefore, a deficiency of this enzyme may cause death, in addition to its deficiency can cause death as a result of acute kidney failure as a result of the obstruction of the renal tubules mediated by hemoglobin released from the breakdown of red blood cells [10]. A deficiency of this enzyme also leads to a state of hemolytic anemia resulting from the breakdown of red blood cells, and this rate of breakdown increases in individuals suffering from a deficiency of the enzyme when they are exposed to any oxidizing substance, whether external, such as fava beans, as a result of it containing two compounds: Convieine and Vieine or taking some oxidizing drugs, or endogenous oxidizing substances formed inside the human body as a result of metabolic processes or due to some infections, including viral infections such as acute and chronic viral hepatitis [11]. There are many diseases associated with G6PD deficiency, including sickle cell anemia [12], thalassemia [13], neonatal jaundice, and glaucoma in the eyes [14].

The G6PD enzyme is of great importance in the pentose phosphate pathway, or what is called the pathway for converting hexose monophosphate sugars. The G6PD enzyme is called the speed-determining enzyme for this pathway. The P-pentose phosphate pathway leads to the production of NADPH, the enzymatic coenzyme for oxidation and reduction reactions, and covers approximately % 60% of the human output of NADPH, there is no difference between NADH and NADPH, but NADPH inserts its hydrogen into the biosynthesis of fatty acids and to defend against oxidative damage, while NADH inserts its hydrogen into the respiratory chain to produce ATP [15].

Calcium is a necessary mineral for muscle contraction, egg activation, building strong bones and teeth, blood clotting, transmitting nerve impulses, regulating heartbeats, and balancing fluids within cells. Requirements are greater during developmental periods such as childhood, pregnancy, and breastfeeding, and long-term deficiency can lead to osteoporosis [16]. Foods rich in calcium include milk, dairy products, broccoli, calcium-rich citrus juices, mineral water, fish, and soy products [17].

Zinc is an essential antioxidant in the body. The adult human body contains approximately (2-3)gm distributed throughout all tissues and in high concentration in the eyes, bones, liver, pancreas, muscles and heart. It is necessary for many types of hormones such as the hormone insulin, It is found in the form of crystals in insulin. Zinc deficiency during pregnancy leads to genetic imbalances, as it changes the spread and concentration of insulin, the concentration of fats and lipoproteins, weak sugar metabolism in the liver, weak functions of beta cells that secrete insulin, and many diseases such as delayed growth. Hair loss, liver and heart diseases, anemia, and diabetes [18].

Copper is one of the elements necessary for growth and health. It is found in various tissues of the

body, but it is concentrated in the liver, brain, kidney, heart, and hair. As for glandular tissues such as the thyroid, pituitary, and prostate, they contain low concentrations [19]. Copper is involved in the synthesis of many enzymes as a cofactor, especially in antioxidant enzymes It is a catalyst for oxidation and reduction reactions that include the production of free radicals that are harmful to cells, and therefore it protects membranes from damage. Therefore, copper is found in the blood linked with other compounds such as albumin and the enzyme ceruloplasmin. The body's need for copper increases with age, and its deficiency leads to osteoporosis [20].

There is a relationship between the concentration of copper in the blood and many diseases. High levels of copper have been observed in patients with anemia and diabetes, and copper deficiency is a serious factor in heart disease, in addition to the occurrence of general weakness, anemia, and poor respiratory activity [21].

Vitamin K is a fat-soluble vitamin that affects clotting pathways within the body. It is found in foods and can be a nutritional supplement. It is necessary for the synthesis of clotting proteins. It is a cofactor for vitamin K-dependent carboxylation. The process of adding carboxylation to vitamin K allows clotting factors to bind calcium ions vitamin K plays an important role in the proper development of tissues and bones, and its deficiency impairs the clotting process, which leads to bleeding problems and thus leads to osteoporosis and cystic fibrosis [22, 23]. Through the low level of activity of the enzyme glucose-6-phosphate dehydrogenase, the aim of the current research is to study the effect of the level of activity of the enzyme glucose-6-phosphate dehydrogenase with some electrolytes in patients with hemolytic anemia in the city of Hilla.

Collection of specimens

This study aims to show the relationship between the level of the enzyme glucose-6-phosphate dehydrogenase and its relationship to electrolyte levels, which include (calcium, zinc, and copper) in addition to vitamins (D and K). This study included 35 blood samples taken from patients suffering from anemia. Patients aged between (40-60) years and 35 samples as a control group (healthy people) for the period from (February to April 2024, when samples were collected from the chronic diseases unit of Al-Hilla Teaching Hospital in Babylon Governorate.

- **Group of patients:** It included (35) blood samples from patients suffering from hemolytic anemia.
- **Control group (healthy people):** It included (35) blood samples from healthy women.

After that, blood was collected from a group of patients and healthy people and was separated by a centrifuge. Then, the biochemical variables were measured, which included (calcium, zinc, and copper) in addition to vitamins (D and K).

- **Estimation of the level of glucose-6-phosphate dehydrogenase in blood serum**

The effectiveness of the enzyme in red blood cells was estimated using the diagnostic kit prepared by the French company, as the principle of the test depends on the speed of NADPH release due to the enzyme, and the absorbance of the compound formed is measured at wavelength 340 [24].

- **Estimating the level of calcium in blood serum**

The calcium concentration in the blood serum was measured according to a ready-made analysis kit prepared by the Tunisian company Bio Maghreb [25]. The method is based on the reaction of calcium with the red-colored complex compound, forming a complex in the basic solution, Ocresol phthalein, in which we measure the calcium concentration. Absorbance is measured at a wavelength of 560 nm.

- **Estimating the level of zinc in blood serum**

Zinc was detected with a colorimetric test [26] using a special kit for detecting zinc (Zinc colorimetric test Mark) giesse Rome-Italy, of Italian origin.

➢ Estimating the level of copper in blood serum

The level of copper in the blood was estimated using the diagnostic kit prepared by the Egyptian company Spectrum. This kit uses the colorimetric method, in which a kidney complex is formed when copper reacts with dibromo -2—pyridyl azo)-N-ethyl—sulfopropylaniline -(3,5 4). The increase in the absorbance of this complex can be measured and expressed as the total concentration of copper present in the sample [27].

Statistical analysis

The results of the current study were analyzed using the statistical program SPSS to find the mean and the value of the standard deviation ± SD. The averages were also determined for the patients with hemolytic anemia group compared to the control group (healthy people) using the T-test and at the probability level ($P \leq 0.001$).

Results:

➢ Measuring biochemical levels in the two study groups:

Average number (1) shows ± elastic deviation of biochemical levels in the two study groups

Groups / Parameter	Mean ± SD		P-Value
	Control No=35	Patients No=35	
G.6.P.D (IU/g)	2021.2 ± 51.23	397.23 ± 38.12	P ≤ 0.001
Ca (µmol/dl)	4.829± 0.413	8.174± 0.758	P ≤ 0.001
Zn (mg/dl)	75.04± 11.07	30.19± 7.13	P ≤ 0.001
Cu (mg/dl)	99.12± 13.4	130.7± 28.78	P ≤ 0.001
Vit K (nmol/L)	3.790± 1.133	3.691± 1.033	P ≤ 0.001
Vit D (ng/ml)	45.01± 11.20	20.18± 6.61	P ≤ 0.001

The current research showed a significant increase in each level (calcium, copper) in the blood serums of patients with hemolytic anemia with a control group (healthy subjects). The results also showed a significant decrease in each of the levels (G.6.P.D., Zinc, Vitamin D) in the blood serum of patients with hemolytic anemia with a control group (healthy people) with no significant differences in the level of vitamin K, and at its level the probability of $P \leq 0.001$ in the following forms:

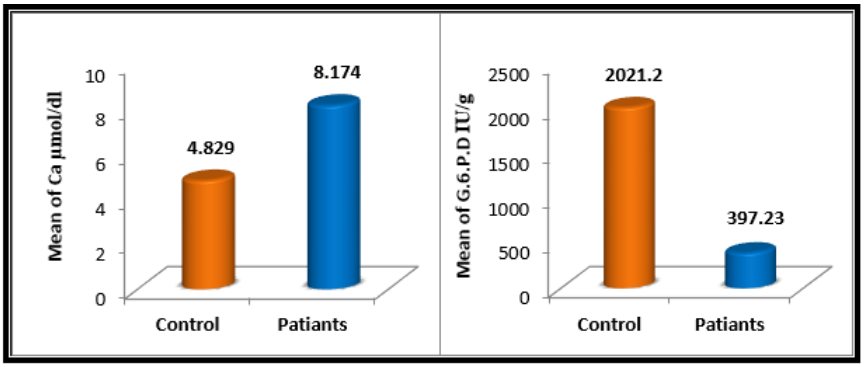

Figure (1): G.6.P.D in the group of patients and healthy people Figure (2): Ca in the group of patients and healthy people

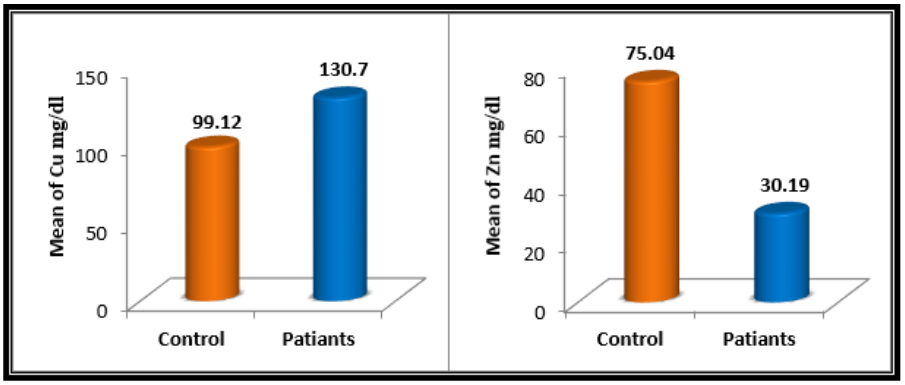

Figure (3): Zn in the group of patients and healthy people Figure (4): Cu in the group of patients and healthy people

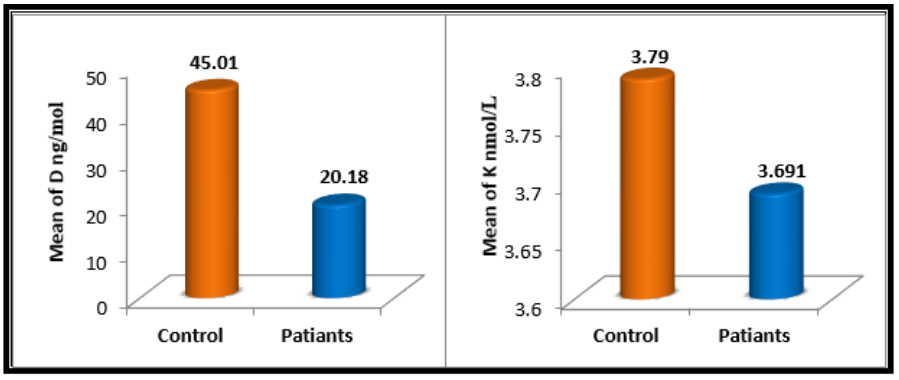

Figure (5): Vit K in the group of patients and healthy people Figure (6): Vit D in the group of patients and healthy people

Discussion:

Interest in studying genetic diseases began after the major revolution that occurred in genetics and DNA science, which explained many unknown phenomena and led to the arrival of many scientific and medical results, which entered into many fields [28]. The results of the current study showed a significant decrease in The level of G.6.P.D in the patient group compared with the control group, as its results agree with the results of Evan M [29] and Avier and his group [30] Who indicated in their study that there was a significant decrease in the effectiveness of the G6PD enzyme in a group of patients with hemolytic anemia compared to a group of healthy people, as the decrease in the effectiveness of this enzyme affects all cells of the body, including the red blood cell, as it is more affected by the deficiency in the enzyme. The main function of the G6PD enzyme is to protect red blood cell glutathione reductase by forming the reductive energy carrier NADPH. In the absence of glutathione or a decrease in its concentration in the red blood cell, this increases oxidative pressure on the blood cell membrane and results in a state of hemolysis [31].

As for the calcium level, its results agreed with the findings of Laura Hertz and his group [32], who showed in their study an increase in the calcium level in patients with hemolytic anemia. The reason for this increase is due to membrane disorder and hereditary erythrocytosis, in addition to hereditary cholangiocytosis in patients with anemia. Hemolytically, calcium levels increased significantly within these cells [33]. The increase in calcium could be due to the reduction of NMDA, such as Memantine and its entrapment within the cells of patients with hemolytic anemia [34], in addition to the overload present in red blood cells and pleocytosis, which causes the depletion of ATP energy and an increase in effective calcium levels in patients with hemolytic anemia [35].

In addition to the genetic mutations found in these proteins in patients with hemolytic anemia, if these mutations lead to the disruption of the complexes that function as ATP accumulators for the Ca 2+ pump, the stability of the overall cytoskeleton is impaired in the patients' RBCs, and this weakness may lead to changes in mechanical stability. cells like Piezo1, again leading to an

increase in intracellular calcium [36].

As for the level of Zinc, the results of his study agree with the findings of Nadhum A. Awad and his group (37) and Abdulkhader [38], who showed in their study a lower level of zinc in patients with hemolytic anemia compared to a group of healthy people, as zinc is one of the most important trace elements in balance. Zinc is a cofactor in heme metabolism as it is part of the zinc finger protein GFi-1B structure. It is a major regulator in erythroid cell growth by modulating erythroid cascade-specific gene expression and regulates transcription during erythropoiesis [39].

As for the level of copper, the results of his study agree with the findings of AL-JANABI [40] and his group, who showed in their study an increase in the level of copper in patients with hemolytic anemia compared to the control group, while another study conducted by researcher Abdulkhader [41] found Low levels of copper in patients with hemolytic anemia compared to the control group. Copper is an essential micronutrient that humans and animals need for the proper function of organs and metabolic processes such as hemoglobin synthesis, as a neurotransmitter, for iron oxidation, cellular respiration, and as a defensive peptide antioxidant. In the formation of pigments and connective tissue. Multiple genetic and acquired factors contribute to the increase in copper deficiency observed clinically over the past decades. Dietary copper is absorbed into intestinal cells via the Ctr1 transporter at the apical membrane side of intestinal cells and in most tissues. Copper deficiency affects physiological systems such as bone marrow hematopoiesis, optic nerve function, and the nervous system in general. It is diagnosed by measuring 24-hour blood copper, ceruloplasmin, and 24-hour urine copper levels [42].

As for vitamin K, its results are consistent with the findings of Michael Kaplana and his group [43], who showed in their study that there are no significant differences in the level of vitamin K in patients with hemolytic anemia compared to a healthy group. Vitamin K is traditionally included in the calcification of connective tissue. Because this process is physiologically essential in bones but pathological in arteries, a significant amount of research has been devoted to finding a possible link between vitamin K and the prevention of osteoporosis, cardiovascular disease, and hemolytic anemia [44]. Vitamin K1 deficiency in patients with hemolytic anemia may cause oxidative damage in G6PD deficiency and thus be responsible for the low-grade hemolysis documented in these patients [45].

As for vitamin D, its results are consistent with the findings of Jumaa and his group [46], who showed in their study a low level of vitamin D in patients with hemolytic anemia compared with the healthy group, as patients with hemolytic anemia are exposed to a variety of complications such as poor growth, endocrinopathy, and deformities. Metabolism. Adequate vitamin D levels are essential for skeletal health and reduce the risk of fractures [47]. Researchers have stated that vitamin D deficiency indicates nutritional deficiency and impaired hydroxylation of vitamin D in the liver due to hemochromatosis. A previous study showed that patients with hemolytic anemia suffer from vitamin D, especially in the winter, due to geographical location, air quality, clothing, and Sunscreen use [48].

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