Tikrit Journal of Pure Science (2024) 29 (1): 58-66
Doi: https://doi.org/10.25130/tjps.v29i1.1285

TJPS

IASJ
المجلات الاكاديمية العراقية

Tikrit Journal of Pure Science
ISSN: 1813 – 1662 (Print) --- E-ISSN: 2415 – 1726 (Online)

Journal Homepage: http://tjps.tu.edu.iq/index.php/j

The Impact of G6PD Deficiency on Liver Function in Children Under 15

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Keywords: Glucose-6-phosphate dehydrogenase (G6PD) deficiency, pentose phosphate pathway (PPP), hyperbilirubinemia, liver functions.

ARTICLEINFO.

Article history:

-Received:	**15 April 2023**
-Received in revised form:	**31 May 2023**
-Accepted:	**01 June 2023**
-Final Proofreading:	**24 Feb. 2024**
-Available online:	**25 Feb. 2024**

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ABSTRACT

Glucose-6-phosphate dehydrogenase (G6PD) is involved in the first phase of the pentose phosphate pathway (PPP), entailing the reduction of nicotinamide adenine dinucleotide phosphate ($NADP^+$) and the oxidation of glucose-6-phosphate to 6-phosphogluconolactone. A deficiency in the G6PD activity can result in numerous illnesses, including liver diseases. The first three enzymes in PPP are responsible for the oxidative phase. They make NADPH, which is needed to deal with oxidative stress, especially in people who do not have enough G6PD. The study included (90) samples from people with hemolytic anemia (Favzim) and control to determine the levels of G6PD, albumin, total protein, liver function, alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin. The results showed that there was a significant decrease ($P<0.05$) in the serum levels of G6PD in the patients group when compared with the control group. Also, there was a significant increase ($P<0.05$) in the serum levels of albumin, total protein, ALP, ALT, AST and total bilirubin in patients with the G6PD deficiency when compared with the control group. In addition, this study revealed a strong positive correlation between some biochemical parameters studied, such as: Total protein with TSB ($r^2 = 0.897$), TSB with ALT ($r^2 = 0.893$), AST with ALT ($r^2 = 0.623$) and total protein with ALT ($r^2 = 0.804$).

Introduction

The cytoplasmic enzyme glucose-6-phosphate dehydrogenase (G6PD) catalyzes the conversion of glucose-6-phosphate to 6-phosphogluconolactone. Nicotinamide adenine dinucleotide phosphate (NADP) is a coenzyme that is transformed to its reduced form, nicotinamide adenine dinucleotide phosphate, and is present in all cells of NADPH. NADPH is required for glutathione reductase to convert oxidized glutathione (GSSG) to its reduced form (GSH), which protects red blood cells from peroxide and other reactive oxygen species [1]. Exogenous and endogenous oxidants can damage red blood cells; hence, impairments occur in the hexose monophosphate pathway or glutathione metabolism as a result of glucose-6-phosphate dehydrogenase (G6PD) deficiency or decreased function, reducing the red blood cells' ability to defend against oxidative attacks. This causes different levels of hemolytic anemia and infant hyperbilirubinemia, which is one of the main causes of illness and death in newborns [1], [2].

Glucose-6-phosphite dehydrogenase (Oxidoreductase, EC 1.1.1.49) (G6PD) is one of the cytoplasmic enzymes that circulate throughout the body, especially in red blood cells. This enzyme plays the role of a key switch in the pentose phosphate pathway (PPP) [3]. Glucose-6-phosphate dehydrogenase (G6PD) catalyzes the oxidation of glucose-6-phosphate (G6P) to 6-phosophogluconolactone while decreasing ($NADP^+$) to reduced (NADPH) [4]. Glucose-6-phosphate (G6P) enters multiple metabolic pathways to provide energy and/or precursors for biomolecule synthesis required to maintain these activities and regulate cell metabolism and proliferation. First, there are differences in glucose levels between fed and fasting states. The ability of the liver to produce glucose while fasting is essential for maintaining blood glucose levels. In the event of starvation, more G6P is converted to fatty acids in the liver through lipogenesis [5]. Second, during periods of fasting, glucose must be stored in order to provide precursors for biomass maintenance, namely cell renewal. The majority of tissues then derive the majority of their energy from ketone bodies. Through G6P metabolism, the liver plays a crucial role in coordinating glucose storage and production, as well as nutrient redistribution [6].

In the Mediterranean region, fava beans represent a common cultigen that may exacerbate the illness in G6PD-deficient individuals and lead to hemolytic anemia. Repeated mild or severe hemolysis can cause problems that get worse over time, especially in the liver and kidney, which are the main organs involved in this process [7]. So, a portion of the hemolytic process can occur extravascularly, and these elevated liver enzymes can be produced by recurrent hepatic hemolytic events. As the means of the liver functions tests from its biochemical parameters such as, albumin, total protein, ALT, AST, ALP and TSB, G6PD deficiency in the liver may also be a cause of hepatic problems in these people. Proteins are among the vital components in all cells and tissues. They are substances made up of collections of smaller units called amino acids. Human blood serum contains more than 125 different types of proteins, the liver and immune system are the primary sites of plasma protein synthesis, and the ratio of synthesis to degradation determines the level of total proteins in the blood [8]. In addition, one form of blood protein is albumin. It is a single protein chain that is mostly made in the liver and is quickly expelled into the cell's outer environment at a rate of 9 to 14 grams per day. Albumin has a half-life that is typically between 12 and 19 days. The human albumin chain has 585 amino acids and a molecular weight of 66.5 kDa, and it is arranged in the shape of a heart [9]. ALT is a transaminase (EC 2.6.1.2) and also known as serum glutamic pyruvic transaminase (SGPT) or alanine transaminase. ALT is present in serum and a variety of human tissues, but is most often seen in the liver. Also, blood AST levels are often used as an indicator of liver function. AST, on the other hand, is found in more tissues than ALT, and illness or injury can change the AST levels in many tissues, including the skeletal and cardiac. Alkaline phosphatase (ALP) is an enzyme that is made in the liver, bones, and other tissues. It releases phosphate when the pH is alkaline. Alkaline phosphatase (ALP) levels have different reference ranges based on the patient's age, gender, and medical history. ALP is assessed in normal blood tests, with high levels in the serum believed to be indicators of bone disease, liver illness, or bile duct obstruction, and it appears to be a substantial independent cancer prognostic biomarker [10]. G6PD deficiency, which is the most common enzyme deficiency in humans, is one of the biggest causes of hyperbilirubinemia in newborns. In the liver, uridine diphosphate glucuronosyltransferase (UGT), encoded by UGT1A1, binds non-conjugated bilirubin to glucuronic acid to create conjugated bilirubin, which is then eliminated in vivo via a variety of complex processes [11].

Material and Methods

Blood samples were taken from (90) people, divided into two groups (60) people with hemolytic anemia (Favzim) whose ages ranged between (1 – 15) years old. There were (29) females and (31) males in this group. The samples were taken from Khanaqin General Hospital and Al-Batool Hospital in Diyala Governorate. For the control group, 30 samples were collected from healthy people of the same age, consisting of fifteen females and fifteen males, and they were collected from external labs. The level of enzyme activity in red blood cells was estimated using the diagnostic kit supplied by (BIOLABO-France), where the principle of action depended on the estimation of the increase in the concentration of NADPH at a wavelength (340 nm), representing the activity ratio of the enzyme G6PD in the sample [12].

Other tests were performed clinically as part of a diagnostic test of liver function to assess liver health. Serum albumin was determined by binding method of Bromocresol green (BCG) where a neutral solution was used at pH (4.2), by using (Albumin-Kit BIOLABO-France). These dyes were combined with albumin and its color changed from yellow to green. The complex BCG Albumin was formed, and its absorbance was measured by spectrophotometer at a wavelength of (630 nm), representing the concentration of albumin in the sample [13]. The total protein was measured by using total Protein-Kit BIOLABO-France, where total protein concentration was based on the colorimetric method described by (*Gornall et al.*). The peptide bonds in proteins reacted with copper (Cu) in alkaline solution to form a colored complex with absorbance. The total protein concentration was measured at the wavelength (550 nm) [14]. For estimating the ALT level, it was commonly measured in units per liter (U/L) for diagnostic purposes, using (ALT) Activity Assay – Kit GENWAY BIOTECH-USA. ALT catalyzed the transfer of an amino group from alanine to ketoglutarate, with pyruvate and glutamate being the products of this reversible transamination process. ALT was detected as described in the manufacturer's instructions, the kit provided a quick, easy, sensitive, and reliable test that was good for high throughput ALT activity assays [15]. The AST Activity Assay Kit SIGMA-ALDRICH-USA offered a straightforward method for evaluating AST activity in several samples. After that, AST was detected as described in the manufacturer's instructions. The ALP level was determined by using the ALP Activity Assay – Kit Linear Chemicals- Spain, depending on the acceptor of the phosphate group of alkaline buffer [16]. Finally, by using the Total bilirubin Assay - Kit Linear Chemicals- Spain and diazotizing sulfanilic acid, bilirubin was transformed into colored azobilirubin, which was then quantified photometrically [17].

Results and Discussion

The measurement of glucose-6-phosphate dehydrogenase activity

As indicated in table (1), the value of G6PD activity in patients with hemolytic anemia (Favzim) was (4.869±0.815) IU/g Hb, which was significantly lower at the probability level ($p < 0.05$) than in the sample of control group whose value was (9.017±1.346) IU/g Hb.

Table 1: The G6PD activity in the blood of patients with hemolytic anemia compared to control group

Parameters	Mean ± SD		P - Value
	Patient	Control	
G6PD IU/g Hb	4.869 ± 0.815	9.017 ± 1.346	0.05

When assessing the activity of the G6PD enzyme in patients of various races with hemolytic anemia, no significant differences were identified at the level of probability (P=0.005), as its value was (4.570 ± 0.782) IU/g Hb in females and (5.149 ± 0.755) IU/g Hb in males. As demonstrated in table (2), statistically significant differences were identified at the level of probability ($P < 0.05$) between the age groups. In this regard, (4.545 ± 0.660) IU/g Hb was recorded in the age group of less than 10 years and (5.216 ± 0.833) IU/g Hb was recorded in the age group of more than 10 years. When comparing the results of the activity of the enzyme G6PD between hemolytic anemia patients and the healthy group in both genders and age groups, it was found that there were significant differences at the probability level ($P \leq 0.05$).

Table 2: The activity of G6PD in the blood of patients with hemolytic anemia compared with the control group in different genders and age groups

Groups / Parameters		Mean ± SD			
		Gender Group		Age Group	
		Males	Females	Less than 10 years	More than 10 years
G6PD IU/g Hb	Patient	5.149±0.755	4.570±0.782	4.545±0.660	5.216±0.833
	P-Value	P < 0.05		P < 0.05	
	Control	8.556±1.303	9.479±1.265	8.334±0.998	9.701±1.325
	P-Value	P ≤ 0.05		P < 0.05	

Hemolytic anemia patients have a disorder in the synthesis of red blood cells, and their bone marrow does not produce healthy red blood cells; therefore, cells cannot produce proteins. Since the G6PD enzyme is more prevalent in red blood cells, its level decreases because distorted red blood cells do not generate sufficient quantities of enzyme. Red blood cells are highly exposed to oxidative stress from exogenous oxidants, oxygen radicals, and the deoxygenated and oxygenated hemoglobin cycle [18]. G6PD deficiency has a significant impact on red blood cells, as they cannot generate the reduced form of nicotinamide adenine dinucleotide [19].

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Levels of Albumin and Total Protein Concentration

The results showed a significant increase in the total protein concentration in the blood serum of patients with hemolytic anemia (8.312±1.026) g/dL compared to the control group (6.556±0.643) g/dL at ($P < 0.05$). As for albumin, the results showed a significant increase in the concentration of albumin in hemolytic anemia patients (5.310±0.286) g/dL compared to the control group (3.975±0.333) g/dL at ($P < 0.05$), as shown in table (3).

Table 3: The concentrations of total protein & albumin in the blood of patients with hemolytic anemia compared with control group

Parameters	Mean ± SD		P - Value
	Patient	Control	
Total Protein con. g/dL	8.312±1.026	6.556±0.643	0.05
Albumin con. g/dL	5.310±0.286	3.975±0.333	0.05

There was a significant increase in albumin concentrations between the patients group and the control group. In contrast, previous research demonstrated that the plasma albumin concentration decreases in patients with hemolytic anemia [20]. In addition to poor macronutrient intake, the disease inflammatory process is the primary cause of albumin depletion. Chronic inflammation lowers albumin synthesis and speeds up the breakdown of proteins, which lowers albumin levels in people with G6PD deficiency [21]. Comparing the albumin concentrations of patients based on their age and gender revealed no statistically significant differences (Table 4). Due to its high level in children than in adults [22], thus the albumin level was higher in the children of the control group.

Table 4: The concentration of total protein and albumin in the blood of patients with hemolytic anemia compared to the control group in different genders and age groups

Groups / Parameters		Mean ± SD			
		Gender Group		Age Group	
		Males	Females	Less than 10 years	More than 10 years
Total Protein g/dL	Patient	8.434±0.880	8.181±1.163	8.332±0.955	8.291±1.113
	Control	6.711±0.515	6.401±0.735	6.524±0.806	6.587±0.452
	P-Value	P < 0.05	P < 0.05	P < 0.05	P < 0.05
Albumin g/dL	Patient	5.297±0.261	5.325±0.314	5.244±0.245	5.381±0.312
	Control	3.962±0.330	3.987±0.348	3.823±0.318	4.126±0.283
	P-Value	0.05	0.05	0.05	0.05

Due to decreased availability of binding protein, hypoalbuminemia may also contribute to an increase in urine zinc excretion [23].

The Levels of ALT, AST, ALP, and TSB

The (mean± SD) levels of ALT, AST, ALP and bilirubin in the serum of patients and controls are shown in table (5). There was a significant increase in all levels of ALT, AST, ALP and TSB in the serum of the patient group compared with the control group at ($P < 0.05$).

Table 5: The levels of ALT, AST, ALP and bilirubin in the blood of patients with hemolytic anemia compared with the control group

Parameters	Mean ± SD		P - Value
	Patient	Control	
(ALT) U/L	51.222±8.229	36.503±2.874	0.05
(AST) U/L	50.028±8.093	35.686±10.502	0.05
(ALP) U/L	106.928±14.320	65.169±6.758	0.05
TSB con. mg/dL	1.253±0.246	0.850±0.111	0.05

ALP is an enzyme present in the cells lining the bile ducts of the liver. Serum ALP activity has been used as a marker of hepatocellular and bone disease for a long time, despite the fact that its precise physiological function is uncertain [24]. Blood ALP levels are a sensitive indicator of intrahepatic and extrahepatic biliary obstruction and the presence of liver infiltrative diseases [25].

Table 6: The concentration levels of ALT, AST, ALP and TSB in the blood of patients with hemolytic anemia in different genders and age groups

Groups / Parameters		Mean ± SD			
		Gender Group		Age Group	
		Males	Females	Less than 10 years	More than 10 years
(ALT) U/L	Patient	52.858±7.287	49.473±8.926	52.635±0.080	49.712±8.257
	P-Value	0.05		0.05	
	Control	36.501±3.593	36.504±2.048	36.518±3.245	36.487±2.553
	P-Value	ns		ns	
(AST) U/L	Patient	53.496±4.849	53.218±3.990	52.760±4.386	54.004±4.440
	P-Value	ns		0.05	
	Control	38.607±5.136	38.566±4.579	34.629±10.576	36.744±10.687
	P-Value	ns		0.05	
(ALP) U/L	Patient	112.436±15.250	101.385±11.890	116.423±46.886	104.365±24.241
	P-Value	0.05		0.05	
	Control	77.883±15.615	75.988±15.734	75.391±14.601	78.480±16.584
	P-Value	0.05		0.05	
TSB mg/ dL	Patient	1.287±0.207	1.216±0.280	1.290±0.244	1.213±0.245
	P-Value	ns		ns	
	Control	0.863±0.129	0.837±0.093	0.870±0.123	0.829±0.098
	P-Value	ns		ns	

As for the ALP level, there was a significant difference between males and females at ($P < 0.05$), where its value was (112.436±15.250) U/L in males and (101.385±11.890) U/L in females, as shown in table (6). Also, the ALP levels in the serum of patients were significantly higher that those of the control group at ($p<0.05$). Also, there were no significant changes in the levels of bilirubin in the serum of patients based on their gender and age at ($p<0.05$), although there was a significant increase in the serum of patients compared to the control group.

These results contradicted those found by *Kamal and Hassan (2021)* who revealed that gender influences the occurrence of jaundice in newborns, with male infants having a lower risk than female infants [26]. Various degrees of liver disease exist because ALT is restricted to the liver, unlike AST, which is abundant in other organs, such as kidneys, brain, and heart. AST is considered the most reliable indicator of hepatocellular damage [27]. The G6PD deficient male patients had significantly higher AST, ALP, and ALT ratios than the G6PD deficient female patients. This is consistent with a recent study by [28], which discovered that females had an advantage in terms of sickle cell disease's influence on biochemical markers. According to *Airaodion et al.*, an elevated AST/ALT (De Ritis) ratio indicates hepatotoxicity [29]. Consequently, the abnormalities in liver function tests could not be attributable to a single reason, but rather to a combination of variables. In this study, the total protein and albumin concentrations of G6PD-deficient individuals were significantly higher than those of the control group. This is congruent with the previous findings, evaluating the relationship between age and biochemical liver function tests in patients with sickle cell anemia in a steady state [30]. The G6PD deficiency may have increased the functional activity of the liver [31], by interfering with the balance between the rates of protein synthesis and degradation, elimination, or clearance of total protein and albumin. Nonetheless, an increase in total protein may promote dehydration, which is detrimental to cellular balance [32]. This will negatively affect the metabolic activities of the liver and, consequently, the patients' health, where Albumin binds and transports ions of metal, bilirubin, and medications, and its concentration is used to evaluate the liver's metabolic activity. A considerable increase in these markers may indicate that its production in the liver has increased. Liver synthesis regulates serum protein levels, and serum protein levels indicate the liver's ability to synthesize. When the G6PD deficiency patients were compared to the control group, a significant increase ($p < 0.05$) was observed between them [30].

Generally, constant hemolysis in patients with G6PD deficiency increases bilirubin levels and the plasma load of AST, but hepatocyte damage from a variety of sources may account for the comparatively elevated levels of ALT and AST [33]. A new research shows that men have slightly higher levels of bilirubin than women [34]. Bilirubin is the breakdown product of heme, the iron-containing tetrapyrrole portion of hemoglobin, myoglobin, and a number of enzymes. Heme cleavage, performed by microsomal heme oxygenases, generates biliverdin, which is reduced by biliverdin reductase to bilirubin. At low doses, bilirubin provides health benefits as an antioxidant, but at very high amounts, it can cause neurological damage (BIND). Bilirubin's tissue toxicity is prevented by its

binding to plasma albumin, fast absorption by hepatocytes, UGT1A1-mediated conjugation with glucuronide, and ATP-dependent pumping into bile canaliculi [35]. A portion of the bilirubin glucuronides produced by periportal hepatocytes is released into sinusoidal blood and reabsorbed by hepatocytes positioned downstream of sinusoidal blood flow. Hyperbilirubinemia can be caused by inherited diseases that lead to more bilirubin being made, less bilirubin being glucuronidated, less bilirubin leaving the body through the canaliculi, or abnormal reuptake [36].

Correlation of Biochemical Variables

The correlation coefficient (r) was computed to describe the relationship and degree of correlation between the numerous researched measures based on the association between two variables from the same sample. Table (7) demonstrates the relationship between biochemical tests, where "r^2" ($r^2 \leq 0.925$) reveals the highest correlation between parameters.

Table 7: The highest values of (r^2) between biochemical parameters in patients with G6PD deficiency

G6PD deficiency	AST	(ALT)	Total Protein	Albumin
Total Protein	0.450	0.804	1	0.441
Albumin	0.292	0.409	0.441	1
AST	1	0. 623	0.450	0. 292
ALT	0.623	1	0.804	0. 409
ALP	0.261	0.382	0.309	0.568
TSB	0.535	0.893	0.897	0.329

Gray color indicates the highest values of r^2.

Actually, the G6PD deficiency has been linked to decreased oxidative stress metabolism and an increased risk of hemolysis and thrombosis in the G6PD-deficient patients [37], [38]. Consequently, individuals with a G6PD deficit are more susceptible to liver dysfunction. The G6PD deficient individuals may be more susceptible to hemolytic anemia. In this work, the effects of G6PD deficiency on liver functions were studied. Serum biochemical markers, such as total protein, albumin, total bilirubin, ALP, ALT and AST were examined in both of the patients group and the control group. The G6PD-deficient patients had higher levels of TSB, ALP, ALT, AST, albumin and total protein.

Bilirubin results from the HbG catabolic processes. After congestion and hepatomegaly, a person with hemolytic illness has problems with the metabolic processes in their liver. ALP, ALT, and AST are the biomarker enzymes that measure liver health, and the elevated AST levels are found to be associated with hemolytic disorders. According to clinical evidence, the levels of ALP, ALT, and AST increase in the G6PD-deficient patients [33] (see Table 7). Figure (1) shows that there is a strong positive correlation between total protein and TSB, where there is a great value of r^2 which means that when the total proteine levels rise, the level of TSB rises. Also, the same thing applies to the correlation between TSB and ALT (see figuer 2), as well as AST and ALT (see figure 3), and the total protein with ALT (figure 4).

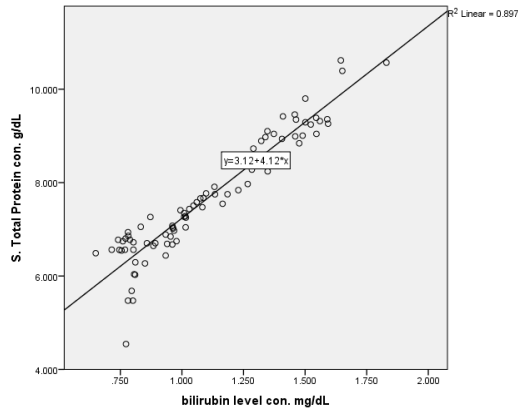

Figure (1): Correlation between total protein and TSB

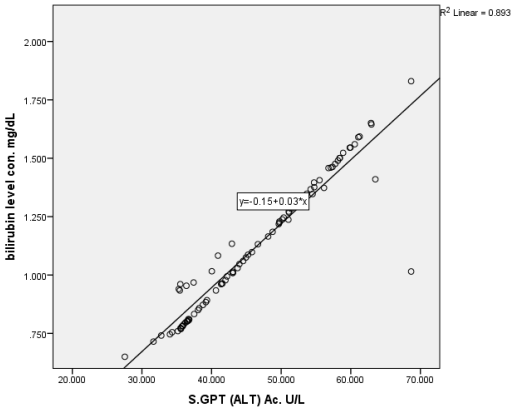

Figure (2): Correlation between TSB and ALT

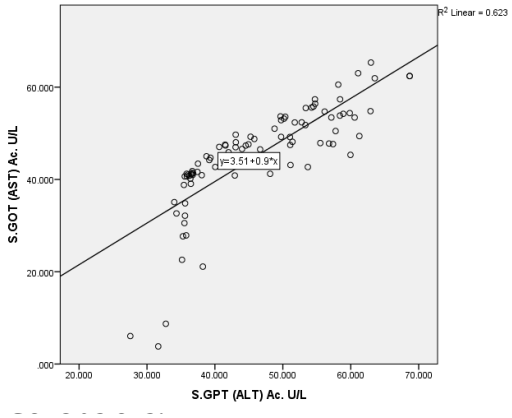

Figure (3): Correlation between AST and ALT

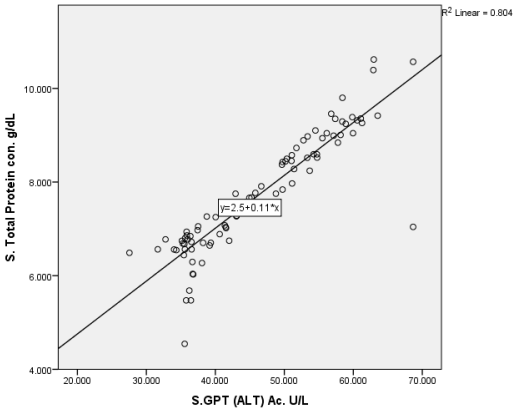

Figure (4): Correlation between total protein and ALT

Conclusions

The activity of glucose-6-phosphite dehydrogenase in serum of patients was lower than that of the control group, which reflected on most other biochemical parameters and led to disruption in their function. In patients with G6PD deficiency, the functional activity of the liver increased by interfering with the balance between the rates of protein synthesis and degradation, or elimination, which is detrimental to cellular balance. This would negatively affect the metabolic activities of the liver and, consequently, the patients' health. The abnormalities in liver function tests could not be attributable to a single reason, but rather to a combination of variables. In this study, the levels of AST, ALP, ALT, bilirubin, total protein and albumin concentrations for the G6PD-deficient individuals were significantly higher than those of the control group. Based on the values of r^2, there was a strong positive correlation between some biochemical parameters, enabling reliance on these parameters in diagnosing patients with G6PD deficiency.

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