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Evaluation of calcium, magnesium, vitamin D and some biomarker parameters levels in children with favsim disease in the Basrah Governorate-Iraq

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Abstract Essential minerals are important for human health because they support biochemical reactions in metabolism and may play a role in the development of glucose-6-phosphate dehydrogenase deficiency (G6PD). We investigated the relationship between calcium, magnesium, urea, creatinine, total protein, glucose and vitamin D levels in G6PD deficiency in this study. The control group consisted of 40 people (23 females and 17 males) and the patient group consisted of 50 people (20 females and 30 males), all of whom were between the ages of (1-12 years). The findings revealed that the calcium level in patients, depending on sex factor, has a highly significant increase (p <0.0001) when compared to the control group, especially in children who are females rather than males who are affected by G6PD deficiency. In addition, the level of magnesium was found to be significantly different (p <0.0001) in children male patients when compared to the control group. On the other side, the level of total protein was found to be significantly high in children patients (p <0.01) when comparing with control group, and the levels of urea, creatinine and glucose were found to be highly significant increase (p <0.001) in patients when comparing to healthy groups, vitamin D levels were significantly lower (p <0.0001) with G6PD deficiency comparing to control group. In conclusion, the low and high significant associations between vitamin D, calcium, magnesium, urea, creatinine, and glucose indicate that more research is needed to better understand their roles in G6PD development.

Keywords Creatinine · Essential minerals · G6PD deficiency · Total protein · Vitamin D · Urea

Introduction

G6PD is an enzyme that is required for the pentose phosphate pathway, which is a metabolic pathway that provides energy to cells. G6PD is particularly important for red blood cell survival and ability to respond to oxidative stress. G6PD deficiency is a hereditary condition caused by a structural defect in the G6PD enzyme. G6PD variants are classified according to the severity of the G6PD deficiency they cause. The severity of G6PD deficiency is expressed as a percentage of normal [1,2].

Each cell in humans contains 23 pairs of chromosomes (46 chromosomes). The sex chromosomes are the 23 pairs; males have one X and one Y chromosome (XY), while females have two X chromosomes (XX). The G6PD enzyme gene is found on the X chromosome [3]. Males have only one X chromosome and thus one copy of the G6PD gene, so if the G6PD gene is mutated, they will have G6PD deficiency. G6PD activity in the blood will be decreased. G6PD deficiency is carried by a female who has a mutant G6PD gene on one of her X chromosomes. On the other X chromosome, she usually has a normal copy of the G6PD gene, which produces enough G6PD to protect the red blood cells. As a result, she may not exhibit any symptoms of G6PD deficiency. Female carriers of G6PD deficiency have a 50% chance of passing on the mutated gene to their offspring. Each son has a 50% chance of inheriting the mutant gene and thus developing G6PD deficiency. Each daughter has a 50% chance of inheriting the mutant gene, making her a carrier of G6PD deficiency [4,5].

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Vitamin D deficiency is common in infancy due to a variety of factors including decreased dietary intake, not taking the child out, an increase in pigmentation, an increase in exclusive breastfeeding, and low maternal vitamin D. As a result, the mother became infected with some diseases, which had a negative impact on the children. GSH is also required for the upkeep of VD-metabolism genes and circulating levels of 25-hydroxyvitamin D (25(OH)VD) [6]. To prevent the exhaustion and depletion of cellular GSH, glucose-6-phosphate dehydrogenase is required. G6PD deficiency is an X-linked genetic condition that primarily affects men [7]. A higher risk of 25(OH)VD inadequacy or deficiency, leaving the body unable to protect its oxidative immune-metabolic physiological functions from COVID-19 insults. An association between disease and 25(OH)VD deficiencies, as well as GSH and G6PD deficiencies, has previously been reported [8]. Overproduction of ROS and excess oxidative damage are to blame for compromised immunity, cytokine storm secretion, and the onset of pulmonary dysfunction in response to COVID-19 infection. Co-optimization of impaired glutathione redox status and excess 25(OH)VD deficiencies has the potential to reduce oxidative stress, boost immunity, and mitigate the negative clinical effects of COVID-19 [9]. The proposed effects of the inherited glucose-6-phosphate-dehydrogenase (G6PD) gene variant and vitamin D deficiency on the increased morbidity and mortality associated with coronavirus (COVID-19) disease, as well as the potential benefits of GSH plus vitamin D supplementation in lowering inflammation and boosting immunity, and thus protection from COVID-19 [10]. In this study, we examined the relationship between mineral elements (calcium and magnesium), vitamin D, urea, creatinine, and glucose in G6PD patients in order to better understand the variant levels, which will aid in the development of future prospective studies of other parameters in G6PD patient.

Materials and Methods

Each patient informed consent was obtained at the Al-Zubair hospital (Governorate hospital). Blood samples were collected from both favism patients and healthy individuals; the number of controls was 40 (23 females and 17 males), and the number of patients was 50 (20 females and 30 males). The ages of all patients and the control group ranged from 1 to 12 years, and all biochemical markers were measured based on the type of sex and age. G6DP was diagnosed based on symptoms and a laboratory enzyme test performed on an automated chemistry analyzer chemwell-t, FL34991, USA. This research was conducted from November 2020 to January 2021.

Determination of Magnesium and Calcium level

Creating calcium and magnesium concentrations in accordance with Biolabo's protocol. The magnesium was prepared in three tubes: a blank tube (1 mL of the reagents + 10 µL of distilled water), a standard tube (1 mL of the reagents + 10 µL of standard), and a sample tube (1 mL of the reagents + 10 µL of sample). The three tubes were mixed thoroughly and allowed to stand for 5 minutes at room temperature. The atomic absorption spectrometry read absorbance at 530 nm was recorded, and the magnesium concentration in (mg/dL) was calculated using the equation [11]:

$$\text{Magnesium concentration}\left(\frac{\text{mg}}{\text{dL}}\right)=\frac{\text{Abs(Assay)}}{\text{Abs(Standard)}}\times\text{standard concentration}$$

The calcium was prepared in three tubes, blank tube (1 mL of the reagents + 25 µL of d.w), standard tube (1 mL of the reagents + 25 µL of standard), sample tube (1 mL of the reagents + 25 µL of the sample), and all tubes were thoroughly mixed before being incubated at room temperature for 5 min. The read absorbance at 570 nm was measured using atomic absorption spectrometry, and the calcium concentration in (mg/dL) was calculated using the equation [12]:

$$\text{Calcium concentration}\left(\frac{\text{mg}}{\text{dL}}\right)=\frac{\text{Abs(Assay)}}{\text{Abs(Standard)}}\times\text{standard concentration}$$

Determination of vitamin D

The I-Chroma technique was used to determine vitamin D by adding 50 µL serum + 50 µL solution one yellow color in a cup, mixing 10 times, and waiting 5 minutes in an incubator at 35 °C temperature. To observe the color, we added 100 µL of solution and mixed thoroughly before placing it in the incubator for 15 min. 75 µL of the mixture was placed in the strep and kept in the incubator at 35 °C for 5 min. The device, and instrument for I-Chroma tests, automatically calculates the test result and displays the total 25(OH)D2/D3 concentration of the test sample in terms of (ng/mL) [13].

Determination of Urea, Creatinine, Total protein and Glucose levels

Urea, Creatinine and Total protein levels in accordance with Randox protocol. The urea was prepared in three tubes: a blank tube (1 mL of working solution + 200 µL of reagent 4), a standard tube (10 µL of standard + 1 mL of working solution + 200 µL of reagent 4) and a sample tube (10 µL of sample + 1 mL of working solution + 200 µL of reagent 4). The three tubes were mixed thoroughly and allowed to stand for 5 min at (37 °C). The atomic absorption spectrometry read absorbance at 580 nm was recorded, and the urea level in (m mol/l) was calculated using the equation [14].

$$\text{Urea level}\left(\frac{\text{mmol}}{\text{L}}\right)=\frac{\text{Abs(Assay)}}{\text{Abs(Standard)}}\times\text{standard concentration}$$

Measurement of Creatinine in serum also was prepared in three

tubes, blank tube (0.5 mL of distilled water + 0.5 mL of TCA + 1 mL of the reagents), standard tube (0.5 mL of standard + 0.5 mL of TCA + 1 mL of the reagent), sample tube (1 mL of the sample + 1 mL of the reagent), and all tubes were thoroughly mixed before being incubated at room temperature for 5 min. The read absorbance at 520 nm was measured using atomic absorption spectrometry, and the Creatinine level in (μ mol/l) was calculated using the below equation [15].

$$\text{Creatinine level}\left(\frac{\mu\text{mol}}{\text{L}}\right)=\frac{\text{Abs(Assay)}}{\text{Abs(Standard)}}\times\text{standard concentration}$$

The total protein level was prepared in three tubes, blank tube (0.02 mL of d.w + 1 mL of R1), standard tube (0.02 mL of standard + 1 mL of R1), sample tube (0.02 mL of the sample + 1 mL of the R1), and all tubes were thoroughly mixed before being incubated at (20-25) temperature for 30 min. The read absorbance at 546 nm was measured using atomic absorption spectrometry, and the total protein level in (mg/dL) was calculated using the below equation [16].

$$\text{The protein level}\left(\frac{\text{g}}{\text{dL}}\right)=\frac{\text{Abs(Assay)}}{\text{Abs(Standard)}}\times\text{standard concentration}$$

The glucose level in accordance with Biolabo protocol. was prepared in three tubes, blank tube (10 μL of d.w + 1 mL of Reagent), standard tube (10 μL of standard + 1 mL of Reagent), sample tube (10 μL of the sample + 1 mL of the Reagent), and all tubes were thoroughly mixed before being incubated at (37 °C) for 10 min. The read absorbance at 500 nm was measured using atomic absorption spectrometry, and the Glucose level in (mg/dL) was calculated using the below equation [17].

$$\text{Glucose level}\left(\frac{\text{mg}}{\text{dL}}\right)=\frac{\text{Abs(Assay)}}{\text{Abs(Standard)}}\times\text{standard concentration}$$

Statistical analysis

The SPSS, V23 software measure of mean standard deviation (SD) by method ANOVA, was used to calculate the significant differences between different groups of children patients and healthy children and according age, sex .The coefficient of correlation regression (r) was used to differentiate between the means of the children's patient groups. The p-value was less than 0.05, which corresponded to the lowest significant limit.

Results

The findings for G6PD deficiency disease in children from a lack of this biochemical, so any biological and chemical disorder in this enzyme will lead to various complications in the physiological role of red blood cells, were presented in this study.

Vitamin D, magnesium, and calcium levels in children with favism disease

Table 1 shows the vitamin D, magnesium, and calcium levels measured in children with favism and healthy groups based on sex factor (male and female). The levels of this biochemical in favism patients were determined to be in males and females, respectively. In addition, in healthy individuals (control group), males and females. Significant increases in magnesium levels in children male patients when compared to males in the control group. While significant increases in calcium levels in children female patients when compared to females in the healthy group. Furthermore, higher mineral levels in serum in G6PD deficient children's patients may play some roles in red blood cell resistance to Plasmodium falciparum.

Urea, creatinine, total protein, and glucose levels in children with favism disease

Table 2 shows the serum levels of urea, creatinine, total protein and glucose in children with favism disease and control groups on sex factor (male and female), the levels of urea, creatinine and glucose in patients were determined to be in males and females, respectively when comparing healthy and sick males and females, it was found Significant increases in urea, creatinine and glucose levels in favism patients (male and female) as control group (male and female) while the level of total protein in favism patients (male and female) show significant also increases when comparing with (male and female) control groups.

Vitamin D, magnesium, and calcium levels in children with G6PD deficiency

Table 3 shows the serum levels of vitamin D, magnesium, and

Table 1 The levels of Mg, Ca, and vitamin D in favism patients and the control group are shown (female and male)

Sex	Groups	Ca (mg/dL)	Mg (mg/dL)	Vit. D (ng/mL)
Male	Control (No.=17)	9.047±0.571	1.79±0.108	41.34±9.81
	Patients (No.=30)	9.552±0.49**	2.49±0.24***	22.73±2.20***
Female	Control (No.=23)	9.182±0.55	1.60±0.542	41.76±9.23
	Patients (No.=20)	12.23±0.92***	1.87±0.109*	24.98±1.96***

Values were expressed as mean ± SD
*The level of significance between Patients and control groups
**p <0.01, ***p <0.0001, *p <0.05

Table 2 The levels of urea, creatinine and total protein in favism patients and the control group are shown (female and male)

Sex	Groups	Urea (mmol/l)	Creatinine (µmol/l)	Total Protein (g/dL)	Glucose (mg/dL)
Male	Control (No.=17)	4.823±1.032	72.581±5.745	7.182±0.341	72.581±7.045
	Patients (No.=30)	9.463±0.423***	109.812±6.361***	8.987±0.367***	122.135±10.176***
Female	Control (No.=23)	4.869±1.012	63.478±8.025	7.169±0.352	76.468±4.803
	Patients (No.=20)	8.429±0.522***	96.411±2.181***	8.641±0.458**	117.535±9.305***

Values were expressed as mean ± SD
*The level of significance between Patients and control groups
**p <0.01, ***p <0.001, *p <0.05

Table 3 The levels of vitamin D, magnesium, and calcium in G6PD deficient patients and the control group are shown as a function of age

Age (Years)	Groups	Ca (mg/dL)	Mg (mg/dL)	Vit. D (ng/mL)
1-5	Control (No.=17)	9.227±0.607	1.881±0.12	40.90±7.11
	patients (No.=18)	10.112±1.53**	2.261±0.58***	23.28±2.32***
6-12	Control (No.=23)	9.041±0.51	1.78±0.083	42.27±11.9
	patients (No.=32)	10.84±1.34**	2.24±0.47**	23.82±2.21***

Values were expressed as mean ± SD
*The level of significance between patients and control groups
*p <0.05, **p <0.01, ***p <0.0001

Table 4 The levels of urea, creatinine and total protein in G6PD deficient patients and the control group are shown as a function of age

Age (Years)	Groups	Urea (m mol/l)	Creatinine (µmol/l)	Total Protein (g/dL)	Glucose (mg/dL)
1-5	Control (No.=17)	4.931±0.943	66.318±8.571	7.1045±0.394	72.186±4.215
	patients (No.=18)	8.805±0.764***	104.121±9.137***	8.813±0.348**	122.832±8.410***
6-12	Control (No.=23)	4.750±1.086	68.611±8.240	7.134±0.293	78.053±6.204
	patients (No.=32)	9.284±0.542***	106.110±7.938***	8.728±0.430**	119.465±10.691***

Values were expressed as mean ± SD
*The level of significance between patients and control groups
*p <0.05, **p <0.01, ***p <0.001

calcium in children with G6PD deficiency disease and in the control group at various ages. Significant decreases in vitamin D levels in patients of various ages, while significant increases in magnesium levels in patients due to oxidative stress in patients.

Urea, creatinine, total protein, and glucose levels in children with G6PD deficiency

Table 4 shows the serum levels of urea, creatinine, total protein and glucose in children with G6PD deficiency disease and in the control group at various ages. Significant increases in total protein levels in patients of various ages, as well as significant increases in urea, creatinine and glucose levels in patients due to oxidative stress in patients

Statically analysis using pearson's correlation coefficient

The correlation was explored through linear regression analysis. In Fig. 1, pearson's correlation coefficient revealed a positive relationship between vitamin D and calcium in patients, while it revealed a negative relationship between magnesium, urea, creatinine and vitamin D in patients, Fig. 2. Also, there revealed a positive relationship between urea and creatinine in patients, as shown in Fig. 3.

Discussion

G6PD deficiency was carried by approximately 400 million people worldwide, with a high prevalence in people of African, Asian, and Mediterranean descent. In Iraq, the G6PD population in the northern part is approximately 10.9 percent, and data from the middle and southern parts of Iraq prior to 2003 ranged from 14 to 16 percent. The data is no longer available after 2003.

The child with G6PD deficiency has higher oxidative stress than the control child, which accounts for the higher levels of some elements. On the other hand, this results in a deficiency of vitamins such as vitamin E, C, and D [18]. The increase in magnesium and calcium levels in child patients led to oxidative stress, red blood cell degradation due to G6PD deficiency in child may increase mineral levels also in this study found that level of magnesium high in child male compared to healthy child male, while level of magnesium was little higher in child female compared to healthy child female that may be due to G6PD deficiency in child [19]. The increase in calcium levels in child patient female and male compared to control child was caused by G6PD deficiency, which resulted in a decrease in antioxidants and an increase in oxidative stress, which may have an effect on

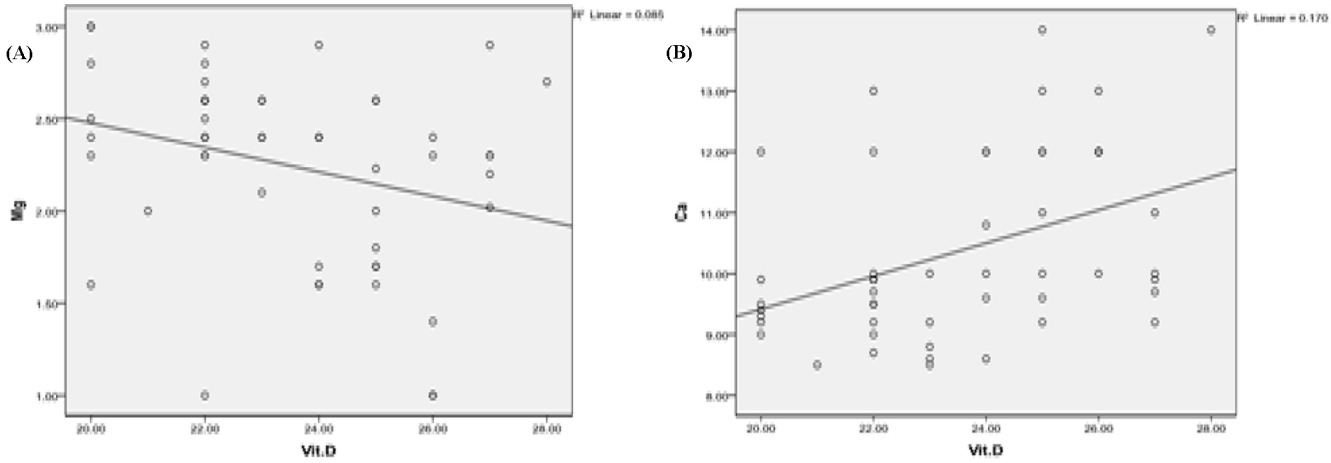

Fig. 1 Showed the correlation between: A- Vitamin D and calcium in patients. B- Vitamin D and magnesium in patients

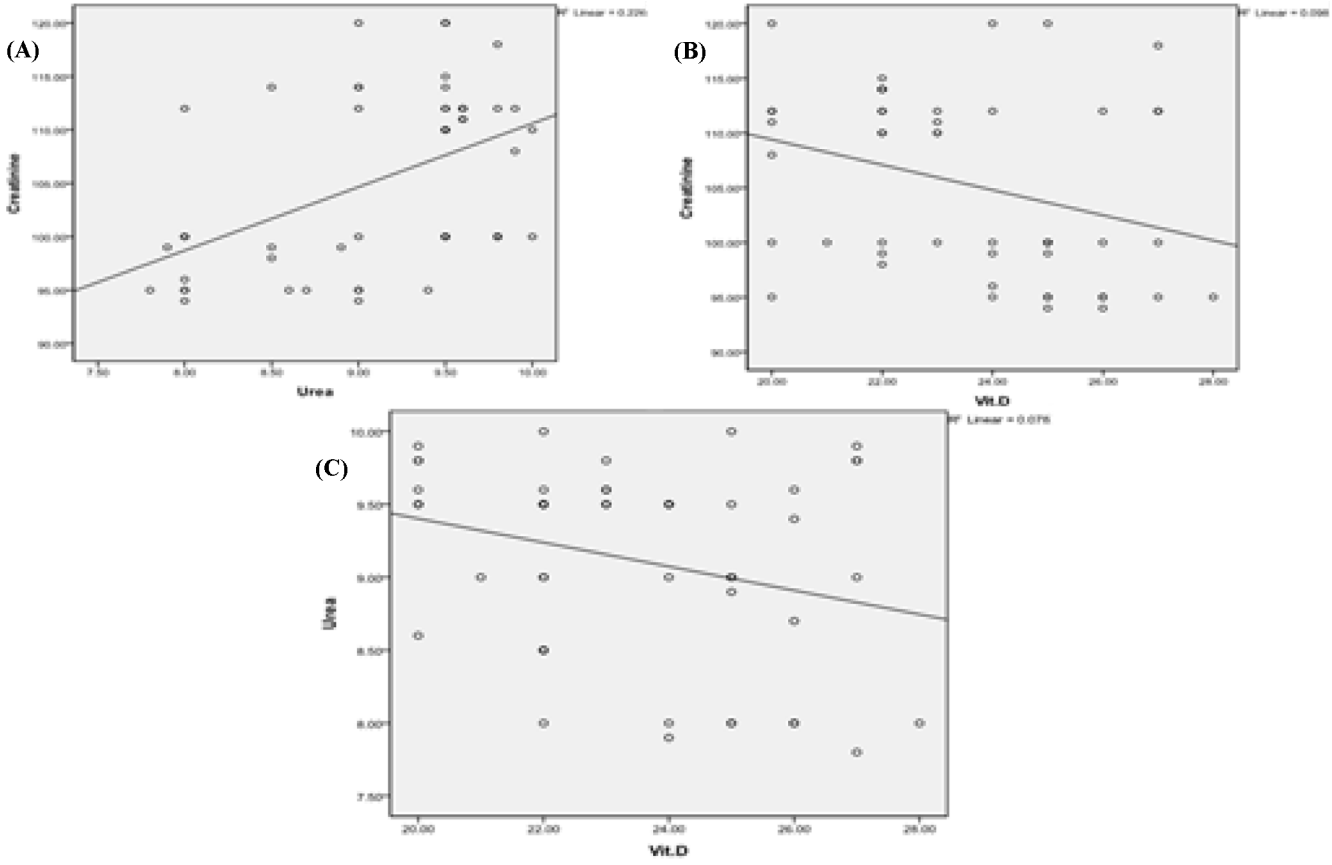

Fig. 2 Showed the correlation between: A- Urea and creatinine. B- Vitamin D and creatinine .C- Vitamin D and urea in patients

calcium levels in the child. G6PD deficiency is also important in RBC resistance to Plasmodium falciparum [20]. Minerals work as a cofactor with vitamin E, which scavenges free radicals and is involved in the restoration of normal serum trace element concentrations in G6PD-deficient hemolysis [21].

The decrease in vitamin D levels in child patients resulted in a lack of G6PD due to hemolysis of red blood cells, which increased oxidative stress and may have resulted in a lack of vitamin D levels in child patients compared to the healthy group. Furthermore, G6PD enzyme and vitamin D act as antioxidants, as G6PD deficiency has an effect on the level of vitamin D in blood serum. Furthermore, G6PD deficiency causes NADPH depletion, which leads to decreased glutathione GSH recycling, which causes reactive oxygen species ROS [22]. A vitamin D-binding

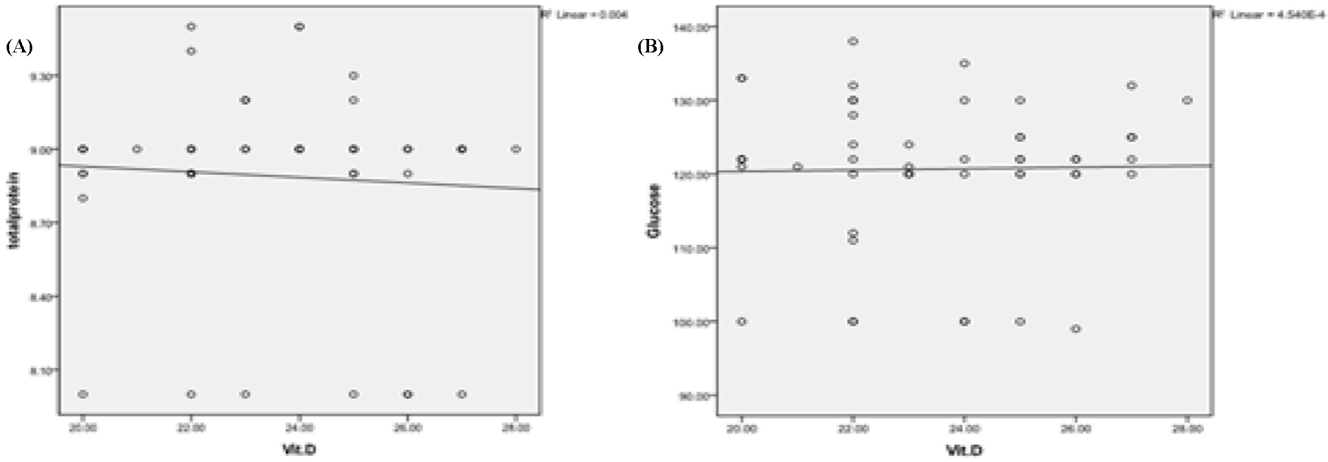

Fig. 3 Showed the correlation between: A- Total protein and vitamin D. B- Glucose and vitamin D in patients

protein transports cholecalciferol-free vitamin D in the blood and liver. Decreased antioxidant activity and excess oxidative stress GSH deficiency reduces vitamin D metabolism genes in the liver 25-hydroxylases CYP2R1, CYP27A1 and the kidneys 1-hydroxylase: CYP27B1, which contributes to lower circulating levels of 25-OH VD major circulating metabolite and a decrease in levels of 1,25-OH D active vitamin D form. GSH deficiency can contribute to metabolic immune-inflammatory dysfunction, which can lead to an increase in viral infection and lung dysfunction. GSH or its precursors, such as N-acetyl cysteine and L-cysteine, are antioxidants that may act as an adjuvant therapeutic target for normalizing VD status in disease-vulnerable populations [23,24]. Natural products containing vitamin D and GSH have the potential to scavenge superoxide and other ROS produced in response to infection, boost immune defensive pathways, and protect against the excessive oxidative damage and pathology associated with respiratory infections and COVID-19 [25]. Improving cellular redox status will help to prevent inflammation, cytokine storms, and viral replication. In the absence of a COVID-19 vaccine, vitamin D and L-cysteine co-supplementation provides an alternative strategy for boosting bodily defenses and accelerating viral clearance [26]. G6PD deficiency may be caused by hemolysis of red blood cells. It interferes with the work of the kidneys, which may affect urea and creatinine levels in patients when compared to healthy groups. The study discovered that there is an increase in the levels of urea and creatinine in the disease when compared to the healthy ones. However, the mean levels of creatinine and urea were significantly higher than in subjects with enzyme deficiency. It is also important to note that diet influences plasma urea and creatinine levels. As a result, additional in-depth investigations are required. These findings are significant given the high prevalence of G6PD deficiency. It will aid in the proper interpretation of glomerular insufficiency, which is common in anemia disease [27,28].

The increase in total protein levels in pediatric patients resulted in a lack of G6PD due to red blood cell hemolysis, which may have an effect on the level of total protein in the blood serum and lead to a decrease in antioxidants and an increase in the oxidative state [29,30]. People who are G6PD deficient are more likely to have impaired glucose tolerance. This finding is consistent with previous reports of hyperglycemia and an increased risk of diabetes in people with G6PD deficiency. The coexistence of these two factors may have serious clinical consequences. There is an increased prevalence of proliferative retinopathy in patients with type 1 diabetes who have G6PD deficiency, implying that G6PD deficiency speeds up the progression of diabetes' microvascular complications. Diabetic acidosis can cause hemolysis in G6PD-deficient patients, which can be exacerbated by hypoglycemic drugs like glibenclamide [31]. According to the findings of this study, patients with a history of diabetes or impaired fasting glucose detected by a rapid glucose test in the primary health care system could be screened systematically for G6PD deficiency, potentially avoiding further clinical complications [32].

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Ethics declarations:
Funding: Self funded by authors.

Conflict of Interest: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval: All procedures in this study that involved human participants were performed in accordance with the ethical standards of the Ministry of Health, General Directorate of Basrah Health, Iraq, with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Written informed consent was obtained from all individual participants included in the study.

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