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Evaluation of GRHPR Enzyme and some Biochemical Variables in Patients with Calcium Oxalate Kidney Stone Disease

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Abstract

Kidney stone disease is a highly significant clinical issue that poses a serious threat to both global health and the economy. For the sustainability of good health and well-being of kidney stone patients, this study aimed to elucidate the role of the enzyme glyoxylate reductase and hydroxypyruvate reductase (GRHPR) in formation of the kidney stones and their development. There are many different types of kidney stones, but calcium oxalate kidney stones are the most prevalent in Iraq. In this study, 50 Iraqi patients with calcium oxalate stones (25 males and 25 females) were compared to 30 healthy control groups (17 males and 13 females). The biochemical tests for kidney function, such as serum urea, creatinine, and uric acid, were detected in the sera of both study groups. In addition to fasting blood sugar (FBS), Ca++, Na+, complete blood count (CBC) and blood group tests, Urine was collected for General Urine Examination (GUE), to visualize oxalate crystals in the patient's urine. Also, the relationship between the concentration of the enzymes glyoxylate reductase and hydroxypyruvate reductase (GRHPR) and the development of calcium oxalate kidney stones in Iraqi patients and the control group was examined. It was found that the enzyme concentrations in the control group were (4.78 ± 1.06 mg/dl) significantly higher than the enzyme concentration in the patients group (0.411 ± 0.02 mg/dl). Also, it was found , that the uric acid concentrations were within the normal range for both groups, but urea and creatinine were significantly higher in kidney stone patients, while they were at the normal ranges in the control group.

Keywords: Kidney stone, Nephrolithiasis**,** calcium oxalate, glyoxylate reductase, hydroxypyruvate reductase.

1. Introduction

Nephrolithiasis, often known as kidney stones, is a serious condition that is common around the world [1]. It can affect anyone of any gender, age, or race [2]. Humans have experienced urinary stone illnesses since 4000 BC [3]. It has surpassed 14% in the general population [4], since the prevalence has increased worldwide, particularly in the recent three decades [5], and the prevalence has varied significantly between different countries [6]. It has a high financial cost, a high incidence rate, and a high rate of recurrence that can affect half of the patients in 10 years and virtually all patients in 30 years [7], the recurrence rate varies widely amongst patients, some

sufferers only once, whereas others have repeated recurrences [8] thus increasing the number of individuals who need proper treatment and recurrence prevention [9].

There are many types of kidney stones, including calcium oxalate (CaOx) which is the most common type around the world [10], followed by uric acid, calcium phosphate (CaP), struvite, apatite, and cysteine [11]. Kidney stones, mostly CaOx, have increased continuously in Iraq [12]. They have spread greatly as they have many etiologies [13], for example, diabetes [14], hypertension [15], gout and metabolic syndrome [16], obesity, depending on an animal protein diet [17], and eating a high amount of food containing calcium, potassium, magnesium, and sodium [18]. Also, environmental factors such as high temperatures [19] and some microorganisms that cause kidney stones, such as *Pseudomonas*, *Klebsiella* [20–21], and toxoplasma, may alter kidney function. [22] The genetic factor is very important as it plays a significant role in kidney stone formation [23]. For example, the three types of primary hyperoxaluria, referred to as PH1, PH2, and PH3, were caused by mutations in the *AGXT*, *GRHPR*, and *HOGA1* genes, respectively [24]. These conditions are characterized by an accumulation of oxalate salts in the kidneys, which eventually leads to the development of calcium oxalate stones.

The glyoxylate reductase hydroxypyruvate reductase (GRHPR) is an enzyme encoded by the *GRHPR* gene that may be related to its deficiency, which is found in the pericentromeric region of chromosome 9 [25]. It has nine exons and eight introns, and it encodes for 328 amino acids that are included in a 36 kDa protein [26]. The enzyme performs the functions of glyoxylate reductase, hydroxypyruvate reductase, and D-glycerate dehydrogenase [27]. It uses the coenzyme NADPH to catalyze the reduction of glyoxylate and hydroxypyruvate; glyoxylate is typically eliminated through the conversion to glycolate in the liver cytosol and mitochondria, and hydroxypyruvate is normally reduced to D-glycerate. If this enzyme is deficient, it results in the conversion of glyoxylate to oxalate by the action of L-lactate dehydrogenase, resulting in the accumulation of oxalate in the kidney, and in the conversion of hydroxypyruvate to L-glycerate also by the same cytoplasmic LD enzyme [28]. Despite the widespread tissue expression where this enzyme is mostly found in the liver, it can also be found in the kidneys, brain, cardiac, skin, and skeletal muscles [29]. This gene has multiple reported polymorphisms and mutations that have been found in PH2 patients, which is evidence that the disease has a genetic basis. Low GRHPR expression has been linked to a high degree of risk of hepatocellular carcinoma (HCC), as it functions as a tumor suppressor gene [30]. It was recently reported that it has a low expression level in the cancerous tissue from it in the neighboring normal tissues, and its expression was controlled by microRNA miR-138-5p [31]. Also, because the metabolic pathways were impacted, it was discovered that the CpG regions (cytosine nucleotides followed by guanine nucleotides) of the *GRHPR* gene could be methylated [32]. The research was intended to investigate the connection between enzyme concentration and stone development in Iraqi patients, since the relationship between this enzyme and stone formation in Iraqi society has not been investigated.

2. Materials and Methods

2.1. Studied Subjects

Peripheral whole blood samples were collected from 80 individuals during the period from February 2022 to August 2022. Both groups' samples were collected from Al Karma Teaching

Hospital in Baghdad Governorate. The patients were diagnosed (by the specialists) with oxalate kidney stones according to an abdomen ultrasound, kidney stone analysis, biochemical tests, and the presence of oxalate in the urine. They visited the lithotripsy unit at Al Karma Teaching Hospital for Fragmenting and Treating Kidney Stones. The patients were 50 (25 males and 25 females); their ages ranged from 19 to 60 years; the control group included 30 individuals (17 males and 13 females), whose ages ranged from 15 to 53 years. The members of the two groups were nonsmokers, didn't have hypertension, and did not suffer from diabetes or obesity. A questionnaire was prepared and filled out by all the participants. The oral agreement was obtained from patients and controls, and the ethical approval was registered with the Iraqi Ministry of Health.

2.2.Biochemical parameters and laboratory investigations

The laboratory investigation was performed in the hospital laboratories for both groups (patients and control); it included biochemical tests for kidney function like serum uric acid, urea, and creatinine. In addition to fasting blood sugar (FBS), complete blood count (CBC), and blood group tests, Urine was collected for General Urine Examination (GUE), to be examined for urinary tract infection and to visualize oxalate crystals in the patient's urine, in addition to measuring GRHPR enzyme concentration in the sera of both groups by Enzyme Linked Immunosorbent Assay (ELISA) The technique was made using the sandwich enzyme immunoassay in the serum of both groups according to the manufacturer's company instructions (YL Biont) at Al Shameem scientific office.

2.3. Statistical analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of different factors on study parameters. T-test and least significant difference (LSD) test (Analysis of Variation, ANOVA) were used to significantly compare between means. A chi-square test was used to significantly compare between percentages (0.05 and 0.01 probability) in this study.

3. Results and Discussion

The distribution of samples according to gender was studied in the control and patient groups. The patient group registered in this study includes 25 (50%) males and 25 (50%) females, thus the results were non-significant for this group. This may be due to the differences in sampling methods and may be due to the fact that males present more CaOx stones because of the testosterone hormone [33], while the Estrogen hormone in women prevents stone formation [34]. The control group registered in this study was 17 (57% male) and 13 (43% female); these results were also non-significant, as shown in **Table 1**.

Table 1. The distribution of samples according to gender

Group	No	Male No. (%)	Female No. (%)	P-value
Control	30	17(57%)	13(43%)	0.071 NS
Patients	50	25(50%)	25(50%)	1.00 NS
P-value	--	0.773 NS	0.427 NS	---

NS: Non-Significant.

The differences in samples according to age in the patients were significant, as 10 (20%) patients were <30 years old, 20 (40%) patients were from 30 to 40 years old, and 20 (40%) patients were >40 years old. The distributions of samples for the control were highly significant, as 16 (53%)

control were <30 years, 11 (37% control) were from 30 to 40 years, and 3 control were >40 (10%) years. The comparisons of distributions between control and patients that are less than 30 years were significant; the distribution of samples for control and patients that are between 30 and 40 years was non-significant, while the distribution for those who are older than 40 years for both groups was highly significant, as shown in Table 2. Therefore, people over 30 years old make up more than half of the patients in the study, and this agrees with the results of [30], but it does not agree with the results of the Iraqi study of Fadhil [35] that indicated a high incidence of stones in elderly patients between the ages of 50 and 57 years old. This difference may be due to the difference in regions and populations in the two studies.

Table 2. The distribution of samples according to Age

Group	No	<30 yr. No.(%)	30-40 yr. No.(%)	>40 yr. No.(%)	P-value
Control	30	16(53%)	11(37%)	3(10%)	0.0084 **
Patients	50	10(20%)	20(40%)	20(40%)	0.0255 *
P-value	--	0.0319 *	0.385 NS	0.0006 **	---

* (P≤0.05), ** (P≤0.01).

The results of the sample distribution regarding the blood groups in the control group were highly significant, as well as in the patient group. The O blood type was the dominant type in this study, as 18 individuals, or 60% of the controls, had this type, and more than half of the patients, or 28 individuals, or 56%, had this type. These results may be because the O blood type is the most common in Iraq [36]. The comparison of sample distribution between the control group and the patient group in the A and O blood groups was non-significant, while it was significant for the B and AB blood groups, as shown in **Table 3**.

Table 3. The distribution of sample study according to blood group in control and patients

Group	No	A No. (%)	B No. (%)	AB No. (%)	O No. (%)	P-value
Control	30	9(30%)	2(7%)	1(3%)	18(60%)	0.0001 **
Patients	50	7(14%)	11(22%)	4(16%)	28(56%)	0.0008 **
P-value	--	0.183 NS	0.0379 *	0.0498 *	0.702 NS	---

* (P≤0.05), ** (P≤0.01).

3.1. Biochemical tests

3.1.1. General Urine Examination

In patients' urine samples, the presence of high levels of calcium oxalate crystals was observed by a compound microscope, which may be caused by animal proteins like meat, fish, and poultry that have high purines that are metabolized to uric acid and oxalate, and it also reduces urine pH and helps the growth of calcium oxalate stones [37], in addition to reduced fluid intake, which lowers the rates of urine flow and leads to oversaturation and crystallization of salts in the urine [38], or it may be due to coax stones caused by the GRHPR enzyme absence that cause calcium oxalate kidney stones leading to crystal formation in the urine, as is shown in **Figure 1**.

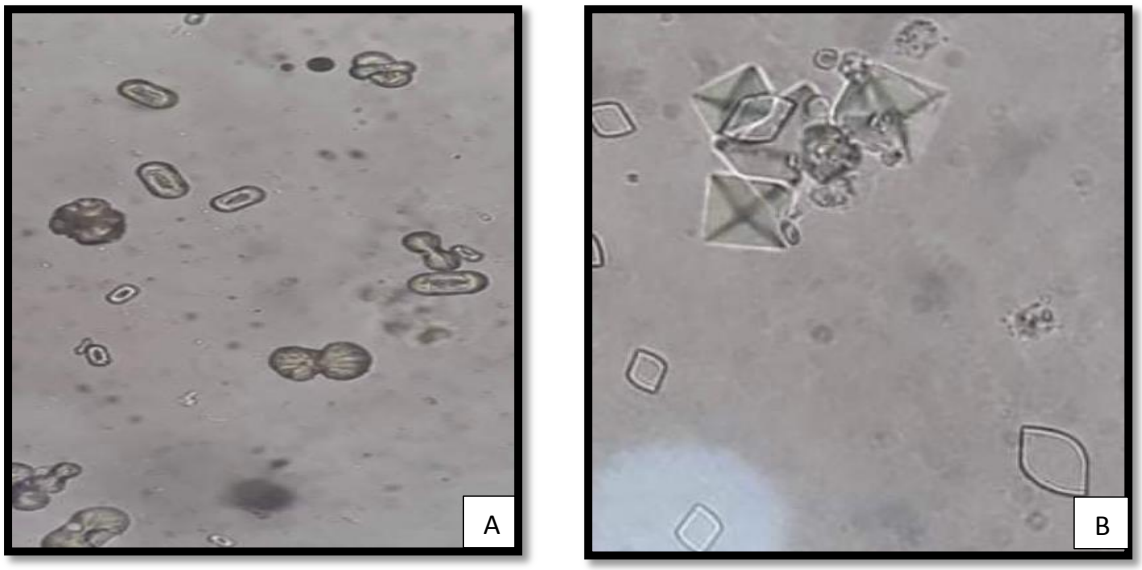

Figure 1. Calcium Oxalate under the electron microscope (40X) in urine samples. A: calcium oxalate monohydrate crystals, B: calcium oxalate dihydrate crystals

3.1.2. Kidney functions tests

The kidney functions were tested by measuring uric acid, urea, and creatinine in the sera of both patients and control groups. Urea is a small organic molecule made up of a pair of nitrogen atoms (NH_2). It is produced in the liver from protein and nitrogen metabolism. Creatinine is a product made up of creatinine phosphate breakdown from protein metabolism in the muscle [39]. It is the most important indication used for evaluating glomerular filtration rate (GFR) as it is discarded through glomerular filtration [40]. The results of urea showed highly significant differences between the control (15.95±0.19) and the patient group (31.44±2.17); the normal ranges are 7–37 mg/dl. Creatinine is an amino acid that is derived from creatine. It is usually used to evaluate kidney functions [41]. The results of creatinine also showed highly significant differences between the control (0.721±0.04) and the patient group (1.29±0.11) as they were above the normal range; the standard normal ranges are (0.5-1.2 mg/dl).

These results may indicate that the patients have kidney dysfunctions; Arindam and his colleagues found similar results in their study in patients with kidney oxalate stones who have PH2 disease [42]. The high level of urea and creatinine may be associated with a metabolic defect in the kidneys resulting from the destruction of some glomerular filtration units, in addition to a decrease in GFR to precipitate calcium oxalate crystals, which leads to obstruction of the nephrons and thus increased acidity that makes the urine supersaturated, and over time, kidney stones are formed [43]. Thus, the kidneys are unable to purify the blood and excrete waste, which leads to the accumulation of toxic chemicals and other waste products in the blood, which leads to kidney dysfunction [39]. The difference in uric acid levels between the control group and the patient group was not significant (4.45 ±0.19) and (5.16 ±0.48), respectively. The normal range is between 2.4-6 mg/dl, as shown in Table 4. It may be due to the healthy diet that contains mostly vegetables, fruits, and whole grains that the patients avoid the consumption of high-purine foods like red meat and alcoholic beverages. As shown in **Table 4**.

Table 4. Comparison between control and patients' groups in Uric acid, Urea and creatinine

Group	Mean ± SE		
	Uric acid (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Control	4.45 ±0.19	15.95 ±0.91	0.721 ±0.04
Patients	5.16 ±0.48	31.44 ±2.17	1.29 ±0.11
T-test	1.289 NS	5.782 **	0.294 **
P-value	0.278	0.0001	0.0001

** ($P \leq 0.01$).

3.1.3. Fasting blood sugar test

Fasting blood sugar was tested for both patients and control groups because patients with diabetes have a significantly increased risk of developing kidney stone disease, and studies show a significantly increased chance of developing this disease in patients with diabetes [44]. The results showed that both groups were in the normal range, indicating that they didn't have diabetes, as shown in **Table 5**.

Table 5. The comparison between control and patients in F.B.S

Group	Control	Patients	T-test	P-value
Mean ± SE of F.B.S (mg/dl)	84.23 ± 4.34	87.12 ± 4.09	12.485 NS	0.647

NS: Non-Significant.

3.2. The relationship between Gender and the patients' parameters

The comparison between male and female samples for the patients' group in uric acid, urea, F.B.S., and GRGPR concentrations was non-significant, while in creatinine, it was significantly higher in males than females, as shown in **Table 6.** Men have a higher percentage of creatinine in their blood, which may be due to having stronger skeletal muscles than women, or it may be due to eating more meat and protein, which results in the supersaturation of uric acid and calcium oxalate crystals in the urine [45].

Table 6. The relationship between Gender and parameters study of patients

Gender	Mean ± SE				
	F.B.S (mg/dl)	Uric acid (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	GRHPR (mg/dl)
Male	84.08 ±6.25	5.89 ±0.91	34.94 ±4.04	1.554 ±0.18	0.426 ±0.03
Female	90.16 ±5.35	4.43 ±0.31	27.93 ±1.40	1.027 ±0.11	0.396 ±0.01
T-test	13.72 NS	1.955 NS	8.758 NS	0.409 *	0.063 NS
P-value	0.112	0.084	0.187	0.050	0.629

* ($P \leq 0.05$), NS: Non-Significant.

3.3. The relationship between age groups and the patient's parameters

The relationship between the age groups of the patients and uric acid, creatinine, and GRHPR concentration was non-significant, meaning that there were no differences in the results of the tests according to age. While it was significant for urea and F.B.S. tests, the F.B.S. test results showed that there were no significant differences between the <30 years and 30-40 years' age groups, but there were significant differences between these two groups and the >40 years' age group as diabetes increased with age [46] as shown in **Table 7**.

Table 7. Relationship between Age Groups and parameters Study of patients

Age groups (year)	Mean ± SE				
	F.B.S (mg/dl)	Uric acid (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	GRHPR (mg/dl)
<30 yr.	79.10 ±5.56 b	4.29 ±0.47	38.54 ±10.02 a	1.388 ±0.27	0.411 ±0.02
30-40 yr.	78.60 ±2.68 b	5.89 ±1.14	28.73 ±1.62 b	1.149 ±0.18	0.388 ±0.02
>40 yr.	99.65 ±8.93 a	4.86 ±0.34	30.59 ±1.55 ab	1.385 ±0.16	0.433 ±0.03
LSD value	18.87 *	2.494 NS	9.081 *	0.533 NS	0.086 NS
P-value	0.0435	0.440	0.0495	0.534	0.475

The means having different letters in the same column differed significantly. * (P≤0.05).

3.4. The relationship between blood group and the patients' parameters

The relationship between the blood groups for the patients in uric acid, urea, creatinine, FBS, and GRGPR concentrations was non-significant because these parameters were not affected by the blood group, as shown in Table 8.

Table 8. The relationship between Blood groups and parameters study of patients

Blood groups	Mean ± SE				
	F.B.S (mg/dl)	Uric acid (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	GRHPR (mg/dl)
A	96.28 ±20.14	4.66 ±0.35	26.29 ±3.07	2.017 ±0.48	0.448 ±0.07
B	82.63 ±3.52	5.19 ±0.43	37.18 ±8.92	1.138 ±0.25	0346 ±0.01
AB	99.00 ±18.76	4.00 ±0.74	25.43 ±4.04	1.175 ±0.48	0.386 ±0.02
O	84.89 ±4.73	5.43 ±0.84	31.33 ±1.44	1.19 ±0.09	0.431 ±0.02
LSD value	NS	NS	NS	NS	NS
P-value	0.601	0.760	0.528	0.113	0.245

NS: Non-Significant.

3.5. Enzyme Linked Immunosorbent Assay for GRHPR

The concentration of GRHPR was tested for both patients and control groups. The comparison between the two groups was highly significant, as shown in Table 9.

Table 9. Comparison between control and patients in Glyoxylate and Hydroxypyruvate reductase GRHPR enzyme

Group	Control	Patients	T-test	P-value
Mean ± SE of GRHPR enzyme (mg/dl)	4.78 ± 1.06	4.78 ± 1.06	1.639 **	0.0001

** (P≤0.01).

The results showed that the concentration mean ± SE of the controls was (4.78 ± 1.06) while the concentration of the patient's group was (0.411 ± 0.02). For that reason, the comparison between the two groups was highly significant, thus the patients lack the activity of this enzyme, which agrees with the results of [47]. The enzyme function is to maintain the concentration levels of glyoxylate and hydroxypyruvate very low to prevent oxalate formation [42]. It also converts glyoxylate to glycolate, and in the case of enzyme deficiency, glyoxylate is oxidized to oxalate by LDH [48]. Oxalate combines with Calcium salts that are greatly insoluble and precipitate in the form of calcium oxalate crystals in normal pH conditions, and eventually forming calcium oxalate stones.

4. Conclusion

Through this study, it was found that creatinine and urea were high in the patients with kidney stones of the type of calcium oxalate compared to the controls, indicating that they had kidney dysfunction. The concentration of the glyoxylate reductase hydroxypyruvate reductase enzyme that is responsible for the conversion of the glyoxylate to glycolate was also low in the patients, which may indicate that calcium oxalate stones were formed as a result of this deficiency, which leads to the accumulation of oxalates and crystals and thus the formation of kidney stones in the kidneys.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Ethical Clearance

Ethics of scientific research were carried out in accordance with the instructions of the Iraqi Ministry of Health and Environment.

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