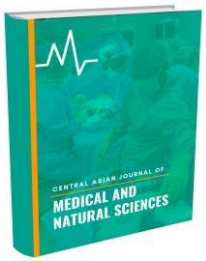

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Article

Assessment of Vitamin D Levels and Some Biochemicals in Chronic Kidney Disease Patients in Al-Hawija District, Kirkuk, Iraq

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Abstract: Chronic kidney disease (CKD) is a major health problem that affects the balance of vitamins and minerals, especially vitamin D and iron. Patients suffer from vitamin D deficiency and iron disturbances, which affect their general health and liver function. In Al-Hawija district, Kirkuk Governorate, Iraq, local research lacks information on this relationship. This study aims to evaluate vitamin D levels, iron forms, and liver function in kidney failure patients in this area, and explore the association between vitamin D and different forms of iron. A case-control study included 90 male and female cases (60 patients and 30 controls) of patients with end-stage renal disease (ESRD). The study was conducted from January to March 2023 at the dialysis center in Hawija. Ferritin and vitamin D were measured using an Ichroma device, UIBC was measured using a Cobas device, and TIBC was measured using a relationship between UIBC and serum iron. Iron, kidney function and liver enzymes were also measured using the spectrophotometric method. The results show a significant increase in indicators of kidney and liver Functions as well as vitamin D and iron deficiencies in patients compared to healthy individuals. These changes reflect the effects of kidney failure on various body systems. This studied also suggests that vitamin D does not directly interact with iron markers (iron serum, ferritin, UIBC and TIBC) in patients with kidney failure.

Keywords: CKD, Dialysis, Vitamin D, Iron forms, Liver Function

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1. Introduction

Kidney failure is a medical condition that occurs when the kidneys fail to perform their functions properly, leading to the accumulation of toxins and waste in the blood, fluid balance, and salt regulation. This condition is divided into two main types: acute kidney failure and chronic kidney failure. Chronic kidney failure is a condition that develops over years and eventually leads to a continuous deterioration in kidney function, while acute kidney failure occurs suddenly and is usually the result of an acute injury or severe medical problem [1]. Patients with kidney failure suffer from multiple disorders including chemical balance, affected hormone levels, and increased risk of cardiovascular disease [2]. Dialysis is a common treatment for advanced kidney failure.

Hemodialysis removes waste products and water from the body by pumping blood through a dialyzer, an external conduit with a semipermeable covering. The dialysate streams in the opposite direction from the blood, which flows in a single direction. The

counter-current progression of the blood and dialysate enhances the fixation angle of solutes between the blood and dialysate, which helps with eliminating more urea and creatinine from the blood. The solutes that are frequently found in urine, such as potassium, phosphorus, and urea, have high concentrations in the blood but low concentrations or none at all in the dialysis setup. Constant dialysate replacement ensures that the concentration of unwanted solutes is prevented from building up on this side of the layer. Mineral concentrations in the dialysis setup, such as those of potassium and calcium, are comparable to those seen in healthy blood. To assist the dispersion of bicarbonate into the blood and act as a pH cushion to kill the metabolic acidosis that is frequently present in these patients, the dialysis arrangement level for another solute, bicarbonate, is set slightly higher than in normal blood. Based on the specific needs of each patient, a nephrologist periodically recommends the dialysate component amounts. During peritoneal dialysis, waste products and water are removed from circulation inside the body through the peritoneum, which is a typical semipermeable layer. Waste products and excess water exit the bloodstream through the peritoneal membrane and enter dialysate, a special dialysis solution located in the stomach cavity [3]. As shown in Figure 1.

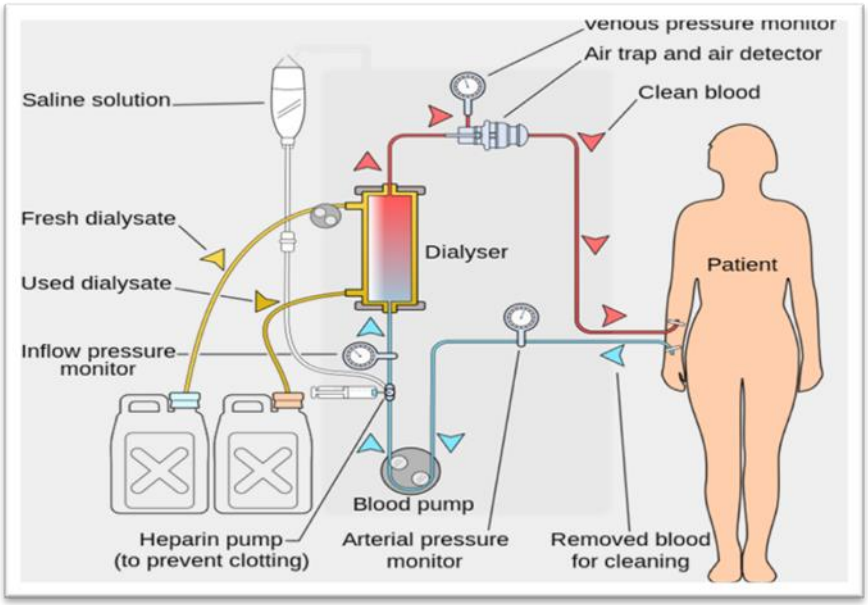

Figure 1. Dialysis process in kidneys

Vitamin D and its relationship to kidney failure:

Vitamin D is a vital nutrient that greatly affects bone health and the immune system by regulating the absorption of calcium and phosphorus in the body. In patients with kidney failure, the ability to convert vitamin D to its active form (1,25-dihydroxyvitamin D) is impaired due to deteriorating kidney function [4]. This deficiency leads to health problems such as weak bones, increased risk of fractures, and worsening heart disease [5]. Studies have shown that vitamin D deficiency is common among patients with kidney failure and may have a significant impact on the development and progression of the disease [6].

Iron forms and their relationship to renal failure:

Iron forms include several important biometrics, such as serum iron, ferritin, unsaturated iron binding capacity (UIBC), and total iron binding capacity (TIBC). Serum iron reflects the level of available iron in the blood, while ferritin is a protein that stores iron in the body. UIBC and TIBC reflect the body's ability to carry iron and its availability [7]. In renal failure, there are significant changes in the levels of these indicators. There is often a

decrease in iron levels due to repeated blood loss or malabsorption, in addition to an increase in ferritin due to chronic infections [8]. An imbalance in these images can lead to anemia and worsening renal failure [9].

Liver functions and their relationship to kidney failure:

Liver functions are evaluated by measuring liver enzymes such as AST (Aspartate Aminotransferase), ALT (Alanine Aminotransferase), and ALP (Alkaline Phosphatase). These enzymes play a role in determining liver health and metabolic functions [10]. In cases of kidney failure, there may be negative effects on the liver, leading to changes in the levels of these enzymes. Damaged kidneys lead to increased pressure on the liver, which may cause increased levels of AST, ALT, and ALP, and may indicate the presence of hepatitis or cirrhosis [11].

Vitamin D and its relationship with different forms of iron:

Vitamin D plays an indirect role in regulating iron levels in the body. Studies have shown that vitamin D deficiency can affect iron absorption and distribution, which may lead to iron deficiency and anemia [12]. Furthermore, vitamin D deficiency may lead to increased levels of ferritin as a marker of chronic inflammation, which in turn is associated with iron disorders [13]. A better understanding of these relationships may help improve treatment and prevention strategies for patients with kidney failure.

Previous studies:

Table 1, shows previous studies that provide insights into how kidney failure affects vitamin D, iron levels, and liver function, as well as the relationship between vitamin D and different forms of iron.

Table 1. Previous studies on variables under study for kidney patients

Study	Objective	Result	Ref. No.
Wolf M, et al.(2007)	Vitamin D and mortality in chronic kidney disease	The study found that vitamin D deficiency is associated with increased mortality rates among patients with kidney failure. The data showed that patients with low levels of vitamin D were more likely to die from cardiovascular disease, in addition to worsening their overall health.	[5]
Kovesdy CP, et al.(2008)	Vitamin D deficiency and mortality in chronic kidney disease	The study showed that vitamin D supplementation can improve survival rates in patients with kidney failure. It also showed positive results in improving kidney function and reducing markers of inflammation, suggesting that boosting vitamin D levels may have beneficial effects on the health of these patients.	[6]
López V, et al.(2018)	Iron metabolism and its role in anemia of chronic disease	The study showed that patients with renal failure often suffer from anemia associated with disorders of iron metabolism.	[7]
Goodnough LT, et al. (2002)	Anemia of chronic disease: mechanisms and management	The study examined the role of different iron profiles, including UIBC and TIBC, in the management of anemia in patients with renal failure. It found that iron balance is significantly affected by the presence of renal failure.	[9]
Montagnino	Chronic kidney disease and	The study confirmed that kidney failure can negatively affect	[11]

G, et al. (2012)	liver dysfunction: interrelationships and implications	liver function, leading to changes in the levels of liver enzymes such as AST, ALT, and ALP. There was a close relationship between deterioration of kidney function and increased levels of these enzymes.	
Liao X, et al. (2020)	The impact of vitamin D on iron metabolism: a review.	The study found that vitamin D plays an indirect role in regulating iron levels in the body.	[12]
Scholl TO, et al. (2013)	Vitamin D and iron metabolism.	The study indicated that vitamin D supplements may improve iron levels in the body and reduce anemia.	[13]

2. Materials and Methods

Study population:

This study was a cross-sectional study conducted on 90 people (females: 38, males: 52). the age group between 35-50 years, consisting of 30 healthy people who are not taking nutritional supplements and vitamins, and 60 people with chronic renal failure who are on dialysis Figure (2). Samples were collected from the Dialysis Center in 2023 in Hawija District, Kirkuk, Iraq, for both patients and healthy people. Blood samples of 5 ml were drawn from the patient intravenously using a plastic syringe and collected in a regular tube. The serum was then separated using a centrifuge at 3000 rpm for 10 minutes and stored at -20 °C until testing.

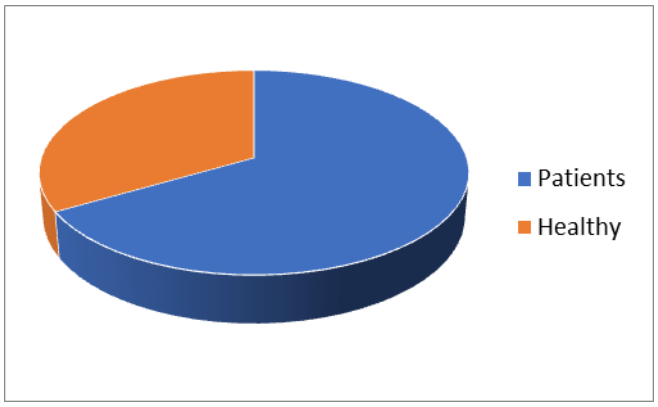

Figure 2. Ratios between healthy individuals and patient models in this study

Data collection and laboratory:

Ferritin

The test uses a sandwich immunodetection method; the detector antibodies in buffer bind to antigen in the sample, forming antigen-antibody complexes, and migrate onto a nitrocellulose matrix to be captured by the other immobilized antibodies on the test strip. More antigens in the sample will form more antigen-antibody complexes, which lead to a stronger fluorescence signal from detector antibodies, which are processed by Instrument for ichromaTM tests to show ferritin concentration in the sample. as a (ng/mL) [14].

Serum Iron

Tested using a kit (from the French company Bio Labo) using a spectrophotometer to determine the serum's iron content. The principle of this method is that ascorbic acid converts Fe3+ iron into Fe2+ iron following the dissociation of iron-transferrin bound in an acidic medium. Fe2+ iron then combines with 3-(2-Pyridyl) -5, -6-difuryl-1, -2, -4-triazine-disulfonate (Ferene) to generate a colored complex. As a result, the absorbance measured at 600 nm (580–620) is directly correlated with the specimen's iron content. To stop the copper interference, thiourea is added to the reagent [15].

Vitamin D

Estimated vitamin D using the I-chroma Vitamin D Kit is a fluorescence immunoassay (FIA) for the quantitative determination of the total vitamin D level in human serum or plasma.

Unsaturated Iron-Binding Capacity (UIBC)

A UIBC test was performed on the serum using the Roche Cobas 6000, Switzerland. The principle was analysis; the material is treated with excess Fe^{2+} in the first step of the measuring procedure. It attaches to the endogenous transferrin that is unbound in an alkaline environment and is transformed into Fe^{3+}. A colorful compound is created when the reagent's unbound Fe^{2+} combines with the ErroZine reagent. This is the opposite result: higher color development denotes more iron occupancy (low UIBC) in the transferrin. Reduced color development suggests a higher binding capacity (high UIBC) for transferrin. All findings are multiplied by a factor (-1) in order to get a positive result because of this characteristic and the calibration model. The measurement of this two-point, endpoint reaction takes place at 546 nm (secondary wavelength: 700 nm). Application Code for Cobas 6000: 779.

The total iron-binding capacity (TIBC)

By calculated method was found by the formula: TIBC (calculated) = serum iron + serum UIBC [16].

Urea and Creatinine

The method used to estimate urea is an enzymatic method (Urease-Modified Berthelot Reaction). The method includes the use of a diagnostic kit from the French company (Biomerieux). While the concentration of creatine in the serum was also estimated using the diagnostic kit prepared by the French company Biolabo using the colorimetric reaction method (Jaffe reaction), which is done by reacting the creatine with the designated reagent.

AST, ALT and ALP

The concentrations of AST, ALT, and ALP were estimated using a diagnostic kit prepared by the French company Biolabo using the spectrophotometric method.

Statistical analysis

Statistical analysis of the data obtained from the study was performed using GraphPad Prism9 software and the data were statistically analyzed using T-test.

3. Results

The results of this study can be presented in Tables 2 and Table 3.

Table 2. The level of biochemical variables in the blood serum of both patients group and control group

Parameters	Unit	Control (N=30) Mean ± SD	Patients (N=60) Mean ± SD	P value
Urea	mg/dL	38.38 ± 8.653	147.3 ± 39.36	<0.0001 ****
Creatinine	mg/dL	0.804 ± 0.1118	7.231 ± 2.118	<0.0001 ****
Vitamin D	ng/ml	25.82 ± 3.507	18.11± 6.552	<0.0001 ****
Iron	umol/L	18.61 ± 4.898	12.68 ± 2.865	<0.0001 ****
Ferritin	ng/ml	214.5 ± 68.64	74.25 ± 53.89	<0.0001 ****
UIBC	µg/dL	186.4 ± 43.65	262.5 ± 136.8	0.0041 ***
TIBC	µg/dL	203.8 ± 42.52	273.2 ± 137.2	0.0083 **
AST	U/L	13.53± 0.325	41.74 ±2.461	<0.0001 ****
ALT	U/L	12.673 ± 0.645	31.645 ± 0.6524	<0.0001 ****
ALP	U/L	111.34 ± 1.51	514.645 ± 1.36	<0.0001 ****

* (P ≤0.05)

Table 3. The level of biochemical variables in the blood serum of both males and females patients and their comparison with the control group

Groups	Male (N=52) Mean ± SD			Female (N=38) Mean ± SD		
Parameters	Control (n=17)	Patients (n=35)	**P value**	Control (n=13)	Patients (n=25)	**P value**
Urea	39.84 ± 9.658	139.8 ± 36.51	<0.0001 **	37.42 ± 6.767	148.7 ± 39.67	<0.0001 ****
Creatinine	0.8824± 0.14	7.583 ± 2.322	<0.0001 **	0.7531 0.083	6.850 ± 1.953	<0.0001 ****
Vitamin D	26.78±2.777	17.03±6.051	<0.0001 **	24.58±4.058	19.63±7.042	0.0255*
Iron	20.24 ± 4.480	12.31± 2.862	<0.0001 **	16.95± 4.958	13.20±2.845	0.0052 *
Ferritin	260.5± 49.10	84.31 ± 56.4	<0.0001 **	168.5 ±34.29	76.14±64.17	0.0003 *
UIBC	165.6±27.71	247.0±142.2	0.0241 *	210.2±48.59	279.4±129.2	0.0720 NS
TIBC	185.9 ± 27.59	259.3 ± 142.5	0.0412 *	227.2± 48.04	292.6 ±129.7	0.0889 NS
AST	9.62 ± 0.342	34.944 ±1.254	<0.0001 **	17.44 ± 0.752	48.536± 1.976	<0.0001 ****
ALT	10.1 ± 0.64	24.4 ± 0.536	<0.0001 **	15.24 ± 0.56	38.945± 0.742	<0.0001 ****
ALP	113.5 ±1.42	501.4 ±1.23	<0.0001 **	109.2±1.62	528.28±1.46	<0.0001 ****

* (P ≤0.05), NS: Non-Significant

4. Discussion

Urea and creatinine

These are two basic indicators of kidney function in patients with kidney failure, as nitrogen accumulates in the blood due to the reduced ability of the kidneys to excrete it, leading to an increase in urea levels (BUN). Particularly due to the breakdown of proteins and amines in the body [17]. While creatinine levels increase due to the breakdown of creatinine. Muscles, and this increase indicates reduced kidney function [18]. Table 2 and Table 3 show a significant increase in urea and creatinine levels in patients compared to healthy people, with urea in patients 147.3 ± 39.36 mg/dl and in healthy people 38.38 ±

8.653 mg/dl reached. This reflects the accumulation of nitrogen resulting from the breakdown of proteins and amines in the body due to reduced kidney function. Creatinine also recorded a significant increase in patients where it was 7.231 ± 2.118 mg/dL, while in healthy people it was 0.804 ± 0.1118 mg/dL. This increase indicates worsening kidney function because creatinine is filtered from the blood by the kidneys and a high level indicates accumulation in the blood.

Vitamin D

The results in Table (2) and Table (3) showed a significant decrease in vitamin D levels in patients compared to healthy people, as the vitamin D concentration in patients was 18.11 ± 6.552 ng/ml and was measured at 25.82 ±3.507 ng/ml in healthy people. In patients with kidney failure, vitamin D may be reduced because the kidneys are unable to adequately convert active vitamin D (calcitriol) [19].

Iron and Ferritin

From the results shown, it was found that there was a decrease in iron and ferritin levels in patients, as the iron level in patients was 12.68 ± 2.865 umol/L while in healthy people it was 18.61 ± 4.898 umol/L. L. There was a significant decrease in ferritin as its concentration was 74.25 ± 53.89 ng/ml in patients and 214.5 ± 68.64 ng/ml in healthy people. This may be due to a deficiency in the production of the hormone erythropoietin by the kidneys, which affects hemoglobin formation and iron metabolism [20].

UIBC and TIBC

The results showed that there was an increase in UIBC and TIBC in patients compared to healthy people, as the concentration of unsaturated bound iron (UIBC) in patients was 262.5 ± 136.8 μg/dL and at 186.4 ± 43.65 μg/dl in healthy people. Total iron concentration (TIBC) also recorded a significant increase in patients compared to healthy people, as its concentration was 273.2 ± 137.2 μg/dl in patients and 203.8 ± 42.52 μg/dl in healthy people. High levels of unsaturated bound iron (UIBC) and saturated bound iron (TIBC) may indicate iron deficiency in patients as the body attempts to compensate for this deficiency by increasing its ability to bind available iron [21].

ALT, AST and ALP

The results presented in Table 2 and Table 3 indicate a significant increase in the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in patients with renal failure compared healthy people. Aspartate aminotransferase (AST) was 41.74 ± 2.461 U/L in patients with renal failure and 13.53 ± 0.325 U/L in healthy people. Alanine aminotransferase (ALT) was 31.645 ± 0.6524 U/L in patients with renal failure and 12.673 ± 0.645 U/L in healthy people. As for alkaline phosphatase (ALP), it was also found to be significantly increased in patients with kidney failure compared to healthy people, at 514.645 ± 1.36 U/L in kidney patients and 111.34 ± 1.51 U/L in healthy people. Elevated ALT, AST, and ALP levels indicate damage to the liver or impairment of its proper functioning. This may be due to complications related to kidney failure, such as: High blood pressure and taking medication [22]. ALP levels may also increase in patients with kidney failure due to impaired hormonal interventions or deficiency. In the conversion of phosphate [23].

The relationship between vitamin D and iron profiles in patients with kidney failure:

The relationship between vitamin D and iron levels represents an important issue in the context of chronic kidney disease because kidney failure affects vitamin D metabolism as well as iron levels in the body. I will analyze the results obtained in this study, shown in Table 4 and examine whether there is a direct relationship between vitamin D and forms of iron:

Table 4. Correlation Coefficient between Vitamin D and iron parameters in patients with chronic kidney disease

Parameters	r	R squared	P
Vitamin D Vs. Iron	-0.03069	0.0009416	0.8160
Vitamin D Vs. Ferritin	0.01319	0.0001740	0.9238
Vitamin D Vs. UIBC	-0.1334	0.01780	0.3095
Vitamin D Vs. TIBC	-0.1336	0.01786	0.3087

1. Vitamin D vs. Iron:

Value (r = -0.03069) This number is close Null indicates that there is no statistically significant relationship between vitamin D levels and blood iron levels in patients with kidney failure. This means that changes in vitamin D are not directly related to changes in iron levels.

2. Vitamin D vs. Ferritin:

An r value close to zero (r = -0.01319) indicates that there is no statistically significant relationship between vitamin D and the Ferritin levels in the blood exist. This reflects that vitamin D levels do not change in a way that could affect ferritin levels.

3. Vitamin D vs. UIBC:

The value (r = -0.1334) indicates a weak but statistically insignificant relationship between vitamin D and the UIBC index, which reflects the ability of the plasma to absorb unbound iron. This finding may suggest that the effect of vitamin D on iron metabolism may be indirect or dependent on other factors.

4. Vitamin D vs. TIBC:

The value (r = -0.1336) is similar to that of UIBC, indicating that there is no statistically significant association between vitamin D and the TIBC index, which reflects total iron bound. This supports the assumption that vitamin D levels have no influence on the amount of iron bound in the blood.

There is no statistically significant relationship between vitamin D levels and iron levels in the blood. Additionally, there is no statistically significant relationship between vitamin D levels and ferritin levels. There is a weak but statistically insignificant association between vitamin D and the indicators of unbound iron, UIBC and TIBC. These results suggest that the effect of vitamin D on iron levels in patients with kidney failure may be complex and indirect, and there may be other factors that influence iron metabolism in this patient group. This study shows the importance of future research to understand the precise mechanisms linking vitamin D and iron levels in the context of chronic kidney disease.

5. Conclusion

The results show a significant increase in indicators of kidney and liver damage as well as vitamin D and iron deficiencies in patients compared to healthy individuals. These changes reflect the effects of kidney failure on various body systems and require careful monitoring and treatment of patients to improve their quality of life and prevent possible complications. The results also suggest that vitamin D does not directly interact with iron markers (iron serum, ferritin, UIBC and TIBC) in patients with kidney failure. This may be due to several factors, including the complexity of vitamin D metabolism in the body affected by kidney failure, as well as iron deficiency, which may indirectly affect vitamin D metabolism.

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