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EVALUATION THE LEVELS OF HOMOCYSTEINE AND FERRITIN IN CHRONIC KIDNEY DISEASE (CKD) WITH ANEMIA

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Abstract
Aims: The current study was aimed to estimate the levels of homocysteine, and ferritin in chronic kidney disease (CKD). Materials and methods: In this study, 100 blood samples (100) were taken from chronic renal failure (CRF) patients ranging in age from 35 to 70 years. From April to May 2023, those patients visited Kirkuk General Hospital, and all were exposed to a personal interview utilizing a specially devised questionnaire format with entire history with full details. For patients and healthy subjects, 5ml was taken from each patient and healthy subjects (50) without any disease. Results: for kidney functions, urea and creatinine levels in patients significantly increased ($P<0.05$) than in control. Serum ferritin and homocysteine concentrations Patients' levels were significantly ($P<0.05$) higher than in control. Conclusions: It is concluded from the current study, and based on the results of the study, that there is a strong association between homocysteine levels and chronic renal failure, with changes in kidney function.

Keywords: homocysteine, kidney function, chronic kidney disease.

Introduction

Homocysteine (Hcy) is a sulfur-containing non-proteinogenic amino acid generated from the demethylation of methionine (Met), an amino acid that is required. It is a biosynthetic intermediate amino acid that is capable of transforming Met to cysteine. Met, which is mostly present in the liver, is converted to Hcy via S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH), both of which are released in a variety of reactions called methylation [1]. This molecule is formed through the degradation of proteins or various food sources, and it is metabolized via two primary pathways: remethylation and transsulphuration [2-4]. An enzyme action occurs during the methionine remethylation cycle for methyl donation to this molecule from methyltetrahydrofolate and betaine, with cobalamin being one of the

precursors of this activation that produces Met. In contrast, transsulphuration is an irreversible process in which Hcy condenses with the serine and undergoes degradation by cystathionine--synthase to produce cystathionine. The dietary pyridoxine cofactor (pyridoxal 5-phosfate initiates this action [1]. Additionally to being a predictor of high-risk mortality [9], elevated plasma Hcy correlates with vascular and arterial abnormalities [5-6], CKD [7], brain lesions [8,] and skeletal system alterations. Hyperhomocysteinemia affects more than 80% of dialysis patients [10]. Hyperhomocysteinemia in kidney failure is thought to be caused by deficiencies in folate, and pyridoxal 5′-phosphate (PLP), as well as reduced clearance of homocysteine (tHcy) resulting from defective renal and/or extra kidney metabolism of tHcy [11]. The reasons of hyperhomocysteinemia among individuals with CKD in the early stages have received little consideration [12]. In the general population, hyperhomocysteinemia appears to be related with an elevated risk of cardiovascular disease [13]. So the current study was aimed to evaluation the levels of homocysteine in CKD with anemia and compare the homocysteine levels in patients with healthy persons.

Materials & Methods

Patients

In this study, 100 blood samples (100) were taken from chronic renal failure (CRF) patients ranging in age from 35 to 70 years. From April to May 2023, those patients visited Kirkuk General Hospital, and all were exposed to a personal interview utilizing a specially devised questionnaire format with entire history with detailed information. For patients and healthy participants, 5ml was drawn from each patient and healthy person (50).

Collection of blood

Trained nurses drew blood from each patient. The importance of samples is debatable among experts. As a result, drawn blood is unnecessary. Each subject had five milliliters of venous blood drawn and divided between an EDTA tube (1.0 ml) and serum tube (4.0 ml).

Kidney function

The creatinine and urea levels was measured using commercially available assay kit (BIOLABO – France).

Ferritin

The Ferritin ELISA Kit is a solid phase direct sandwich ELISA method.

Homocysteine Assay

After adding the Fluorogenic Developer Mix, incubate the plate at room temperature for 15 min with constant shaking (to achieve appropriate mixing). In endpoint mode, measure the fluorescence of all the specimen, and standard curve wells at ex = 658 nm/em = 708 nm.

Statistical Analysis

The computer programs SPSS version 21 were used for statistical analysis. To express statistical test results and bar graphs, MeanSE was utilized. The unpaired T-test (Man-Whitney U) test was used to compare variable means between patient and health individuals.

Results & Discussion

Kidney functions

Table 1 show some kidney functions in of CKD patients, where urea levels (157.12 ± 39.53) in patients significantly increased (P<0.05) compared to control group (24.58 ± 5.19). creatinine levels (7.75 ± 1.593) in patients significantly increased (P<0.05) compared to control group (0.891 ± 0.257).

Table 1 kidney functions in studied groups

Groups Parameter	Control (50)	Patients (100)	P-Value
Urea mg/dl	21.58 ± 5.19	194.58 ± 27.831*	0.0001
Creatinine mg/dl	0.891 ± 0.257	7.75 ± 1.593*	0.006

The ability of the kidneys to eliminate waste materials is closely tied to blood urea nitrogen levels. Urea accumulates in the because the kidneys are unable to remove it during CKD. This failure to eliminate urea is caused by kidney damage, which produces tubular necrosis and a loss of filtering capacity. Medication-related kidney injury is also a possibility. Dehydration caused by CKD might raise urea levels due to the poor pace of renal elimination [15]. Because creatinine is eliminated via glomerular filtration, it is the most important indication of GFR. GFR decreases due to a limitation in tubular excretion [16]. Creatinine clearance assessment has been shown to have several limitations because it is dependent on the patient's weight and muscular mass and declines with age ([17]. As stated in table (1), the results showed a

considerable rise in the screening or diagnostic tests of kidney function (BUN and SCr). In chronic hemodialysis, serum urea rises according to illness development, which is heavily impacted by a catabolic condition or excessive protein consumption, As a result, many different waste products resulting from protein catabolism are produced at a higher rate [18]. Despite the fact that an increase in creatinine levels in the blood of CRF patients is related with a decrease in the number of working nephrons, which lowers GFR and resulting in a significant drop in renal excretion of water and solutes [19], These findings are consistent with Khalidah [20]. The kidneys remove Cr from the body; when the kidneys malfunction, Cr concentrations in the blood rise because only a little quantity is excreted in the urine, resulting in a large decrease in CrCl [21].

Ferritin

Table 2 shows ferritin levels in individuals with CKD, with ferritin concentrations significantly (P 0.05) higher in CKD individuals (289.05 ± 12.94) compared to control group (157.38 ± 13.63).

Table 2 the concentration of ferritin

Groups Parameter	Control (50)	Patients (100)	P-Value
Ferritin (ng/ml)	157.38 ± 13.63	289.05 ± 12.94*	0.031

Serum ferritin levels are an important measure of iron status [22]. At this point, iron is administered to stimulate ferritin synthesis. Ferritin levels in patient sera were significantly lower for the reasons stated above. Ferritin concentrations increased in CRF patients before and after hemodialysis as compared to control groups, and increased after hemodialysis when compared to before. This finding is supported by [23]. Ferritin levels are normal or high in chronic illness anemia, indicating that iron is retained within cells and ferritin is created as an acute phase reactant, but cells have not produced their iron. Because there is less erythropoiesis (the synthesis of red blood cells and hemoglobin), iron is not consumed. To store the unneeded iron, more ferritin, the protein that links iron, is produced [24]. Within labs analytic imprecision, varying from 2.9 to 8.4% CVA for the techniques used here, introduces additional uncertainty into ferritin assays. Finally, biologic or intraindividual variation in blood ferritin is significant in these stable patients on dialysis throughout the two and six-week study periods, and it may be much greater if assessed quarterly [23]. Ferritin is

an iron status marker as well as an acute phase reactant that is affected by infection, inflammatory processes, and malignancy. Nutritional status [25] and hepatic disease [26] can also influence serum ferritin levels. We chose people who were clinically stable and excluded those who had established malignancy, autoimmune, or hepatic illness, which presumably lowered the intraindividual variability in ferritin that we identified. The mean CVI for ferritin in participants with normal renal function is also significant, which is consistent with our findings in stable hemodialysis patients [27]. The dose of intravenous iron provided, as well as the rate of infusion and kind of intravenous iron product, have an effect on serum ferritin [28].

Homocysteine levels

Table 3 shows homocysteine levels in patients with CKD, with homocysteine concentrations significantly ($P<0.05$) higher in individuals with CKD. (289.05 ± 12.94) compared to control group (157.38 ± 13.63)

Table 3 concentration of homocysteine in studied groups

Groups	Control (50)	Patients (100)	P-Value
Parameter			
homocysteine (umol/L)	4.91 ±0.23	18.57 ± 4.02*	0.02

Hyperhomocysteinemia is widespread in renal patients and has received a lot of attention due to its link to renal failure [29]. Homocysteine (Hcy) metabolism is genetically controlled in non-uremic individuals. Increased total plasma Hcy is frequently induced by genetic abnormalities in metabolic enzymes such 5,10-methylenetetrahydrofolate reductase [30]. Regardless of hereditary abnormalities, total plasma Hcy is considerably higher in renal patients [31]. In fact, hyperhomocysteinemia is frequent in uremic MHD patients [32], with elevated plasma Hcy in more than 90% of dialysis patients. Elevated blood Hcy levels may induce dysfunction of endothelial cells, most likely due to oxidative deactivation of endothelium-derived nitric oxide [31]. Homocysteine additionally promotes oxidative stress in the body by reducing the production or efficiency of antioxidant enzymes such glutathione peroxidase-1 (GPx-1) [33]. A cross-sectional study was conducted to investigate the relationship between Hcy levels and serum creatinine levels in persons aged 18-60 years with creatinine values ranging from 1.5 to 8 mg/dl. Among pre-hemodialysis CKD patients, the authors identified a moderate relationship between age and creatinine clearance and Hcy concentrations.

A one-ml/minute reduced creatinine clearance led in a 0.2 mmol/l increase in Hcy levels; a one-year rise in age resulting in a 0.2 mmol/l increase in Hcy levels [34]. Several previous research investigated the relationship between Hcy and CKD. Hcy is related with tubular interstitial lesions in the early stages of IgA nephropathy, according to Li et al. [35]. Wang et al. [36] discovered that HHcy was more prevalent in IgA nephropathy patients than in persons with other primary glomerular illnesses, especially in the beginning stages of CKD, and that it may be a predictor of a faster deterioration in kidney function and future CKD incidence. The aggregation of data demonstrated a graded relationship between Hcy quartiles and eGFR reduction. Individuals in the highest Hcy quartile were considerably more likely than those in the lowest quartile to experience rapid eGFR reduction [37].

Conclusions

The current study's findings suggest that there is a substantial correlation between homocysteine levels and protracted renal failure, as well as alterations in kidney function.

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