Study the correlation between the folic acid supplements and the levels of each serum homocysteine in patient with chronic kidney disease

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Abstract

This study compared blood homocysteine and other indicators in chronic renal disease patients to healthy individuals. The study also examined how folic acid supplements affect serum homocysteine levels. This study selected 150 blood samples from 35–70-year-old chronic renal failure (CRF) patients. Kirkuk General Hospital patients from September to December 2021 were interviewed using a specially prepared questionnaire to acquire thorough medical history. Diabetes, liver disorders, hypertension, medication dose, and anemia were eliminated from the study. All 50 disease-free patients and healthy individuals gave a 5ml sample. Patients showed a significant increase ($P<0.05$) in urea, creatinine, and homocysteine levels compared to the control group. The new study found a strong association between folate concentration and homocysteine levels after giving folic acid. Chronic renal failure patients' homocysteine levels improved.

***Keywords**: homocysteine, chronic renal failure, folic acid, kidney function, urea.*

1. Introduction

Chronic kidney disease (CKD) is a global epidemic that causes death on a massive scale. Poor renal function not only leads to patient mortality due to uremia, but it also has a significant correlation with cardiovascular disease in individuals with chronic kidney disease (CKD). This correlation, which is also evident in earlier stages of CKD, has heightened the recognition of the need of maintaining a healthy kidney function. While developed nations are prepared to cover the expenses of dialysis, the cost is steadily rising on an annual basis. One possible explanation could be the growing demand for RRT (Renal Replacement Therapy). The prevalence of end-stage renal disease (ESRD) in the United States was 354 cases per million population-years in 2007, which is higher than the rates of 300 cases per million population-years in 1996 and 150 cases per million population-years in 1986 [1-3]. The prevalence is rising at a higher rate among the senior population, with an annual growth of 10.4% for individuals aged 75 years and more, compared to a 5.5% annual increase for those aged 20-44 years. In 2006, the overall incidence in Europe was 125 cases per million population-years, while in 1997 it was 110 cases per million population-years [4-5]. The yearly growth rate was approximated at 4.8% throughout the 1990s [6], but it appears to have reached a steady state of 0.6% in recent times. The stabilization primarily arises from decreased occurrence rates in the elderly [7]. The incidence rate in Sweden was 117 per million population-years in 2006 and 115 per million population-years in 1997 [8]. Homocysteine (Hcy) is a sulfur-containing amino acid that is nonessential for the body and is produced during the metabolic pathway of Hcy-Methionine. It plays a significant role in this metabolic pathway by disrupting the functions of folic acid and B12 [9]. Hcy is observed to induce endothelial damage and enhance the activity and synthesis of coagulation factors [10]. There is abundant data indicating that hyperhomocysteinemia negatively affects the functioning of endothelial cells, promotes the oxidation of low-density lipids, and triggers inflammation and proliferation of smooth muscle cells [11]. Scientific investigations on the spread of diseases have demonstrated that high levels of Hcy (homocysteine) raise the likelihood of cardiovascular incidents in both the general population and individuals with CKD (chronic kidney disease) [12]. A 25% reduction in Hcy levels in the general population leads to an 11% drop in the risk of coronary artery disease (CAD) and a 19% decrease in the risk of stroke. CKD-induced reduction in glomerular filtration leads to elevated Hcy levels in the majority

of patients, with the extent of renal abnormalities determining the severity of this increase. Studies have shown that Hcy levels decrease following kidney transplantation, indicating that renal processes play a significant role in the development of Hcy levels in patients with chronic kidney disease (CKD) [13]. Hence, the primary objective of this study was to assess the serum homocysteine levels and various parameters in individuals with chronic renal disease, and subsequently compare them with those of healthy individuals. Additionally, the study aimed to investigate the relationship between folic acid supplements and the levels of each serum homocysteine.

2. Materials & methods

2.1 Patients

This study chose a total of 150 blood samples from individuals with chronic renal failure (CRF) who were between the ages of 35 and 70. The patients who visited Kirkuk General Hospital between September and December 2021 underwent a personal interview using a specifically designed questionnaire. This questionnaire collected comprehensive information about their medical history, including details about any conditions or medications that could potentially affect the study, such as diabetes mellitus, liver diseases, hypertension, medication dosage, and anemia. Patients with these conditions were excluded from the study. A total of 5ml of blood was collected from each of the 50 patients and healthy volunteers, who were free of any medical conditions.

2.2. Complete Blood Counts (CBC)

The complete blood count (CBC) is a battery of tests that assess the cellular components present in the bloodstream. A complete blood count (CBC) can assess your overall well-being and identify a range of illnesses and disorders, including infections, anemia, and leukemia. A volume of 1 milliliter of blood was collected in an EDTA test tube for hematological assays, namely for measuring red blood cell count (RBC), hemoglobin (HB), hematocrit (HCT), and mean corpuscular volume (MCV). The Sysmax device was utilized for these tests.

2.3. Estimation of creatinine and urea levels

The concentration of creatinine was determined using a commercially available assay kit from (BIOLABO – France).

2.4. Homocysteine Assay

This kit utilizes the sandwich enzyme-linked immuno-sorbent assay methodology. An antibody is applied in advance onto a 96-well plate. The wells are supplemented with standards, test samples, and biotin-conjugated reagent, and then incubated. Next, the HRP-conjugated reagent is introduced, and the entire plate is subjected to incubation. Unbound conjugates are eliminated by employing a wash buffer at every step. The TMB substrate is employed to measure the HRP enzymatic reaction. Following the addition of TMB substrate, only wells containing an adequate amount of HCY will generate a blue-hued product, which then transitions to yellow upon the introduction of the acidic stop solution. The intensity of the yellow hue is directly proportional to the quantity of HCY that is bound to the plate. The Optical Density (OD) is quantified using spectrophotometry at a wavelength of 450 nm in a microplate reader, enabling the calculation of the concentration of HCY.

2.5. Folate ELISA kit

The Folate Elisa kit is a dependable technique for accurately measuring the concentration of Folate in Human Serum using a colorimetric microplate Enzyme Immunoassay.

2.6. Statistical Analysis

The data was subjected to statistical analysis using both Minitab, a software for statistical analysis, and Excel, a computer for creating spreadsheets. The data were reported in terms of the mean and standard deviation. The present study employed statistical analysis techniques, namely the Dunkin' multiple test and the ANOVA test, to examine potential significant differences between the experimental groups by comparing their arithmetic means.

3. Results & Discussion

3.1. Kidney functions

Table 1 presents the renal functions of patients diagnosed with chronic kidney disease (CKD), with a particular emphasis on the significant increase ($P<0.05$) in urea levels (165± 52.269) compared to the control group (25.941± 9.15). The creatinine levels of the patients (6.21± 2.4) exhibited a notable increase ($P<0.05$) in comparison to the control group (0.962 ± 0.352).

The findings indicate a notable rise in the utilization of screening and diagnostic tests for renal function (BUN and SCr) ($P > 0.05$). The increase in serum urea levels during chronic hemodialysis is closely correlated with the advancement of the disease. The primary factor contributing to this rise is a catabolic condition or excessive protein intake, resulting in an elevated creation of additional waste chemicals through protein breakdown [14]. Conversely, the increase in blood creatinine levels in individuals with chronic renal failure (CRF) can be linked to a reduction in the quantity of functional nephrons. The decrease in nephrons causes a decrease in glomerular filtration rate (GFR), leading to a significant reduction in the elimination of solutes and water by the kidneys [15]. These findings align with the observations presented by Khalidah [16]. The kidneys are accountable for the elimination of creatinine (Cr) from the body. Reduced kidney function results in increased levels of Creatinine in the bloodstream due to its restricted elimination in urine. This leads to a substantial decrease in the creatinine clearance (CrCl) [17]. The rise in serum creatinine and urea levels in hemodialysis (HD) patients is due to the reduction in the number of functional nephrons. This reduction results in a drop in the glomerular filtration rate (GFR), leading to significant reductions in the renal excretion of solutes and water [15].

3.2. Serum folate

Table 2 displays the blood folate concentrations in patients with chronic kidney disease (CKD). The results indicate a substantial decrease ($P <0.05$) in serum folate levels in patients (7.291±1.261) compared to the control group (15.049±2.39).

The body absorbs serum folate by glomerular filtration and reabsorption in the proximal tubule. Serum folate transporters in the proximal tubule play a role in reabsorbing serum folate and maintaining homeostasis. Research has shown that there is a reduction in the activity of proteins responsible for transporting folate in the blood, as well as a decrease in the level of folate in the blood, in a rat model of acute kidney injury [18]. This finding is significant as it can help us investigate the connection between remaining kidney function and folate levels in patients undergoing Peritoneal dialysis for chronic kidney failure. This study aimed to evaluate the levels of ceratinine, urea, and other indicators as direct or indirect measures of renal function in patients with persistent chronic kidney disease. Subsequently, we analyzed the associations between these indicators and serum folate levels to offer a logical interpretation. The initial serum folate levels in our extremely ill patients with chronic renal failure are comparable to those reported by Lasker et al. [19] and Hampers et al. [20] in individuals with the same condition. The explanation likely involves a protracted period of insufficient consumption of folate in the diet, coupled with the loss of the vitamin through vomiting. Patients with chronic renal failure who were mildly affected or had experienced severe illness for a short duration consistently exhibited normal serum folate levels.

3.3. Homocysteine levels

Table 3 presents the homocysteine levels in individuals diagnosed with chronic kidney disease (CKD). The concentration of homocysteine in patients (23.921 ± 5.94) was considerably elevated ($P <0.05$) in comparison to the levels in the control group (4.247 ± 0.581). Chronic renal failure patients who were treated with folic acid showed lower levels of homocysteine (10.381±2.17) compared to the chronic renal failure group who were not given folic acid supplementation.

Furthermore, the present investigation demonstrated a significant correlation between folate concentration and homocysteine levels following the administration of folic acid supplementation. Notably, patients with

chronic renal failure exhibited a noteworthy improvement in their homocysteine levels, as depicted in Figure (1).

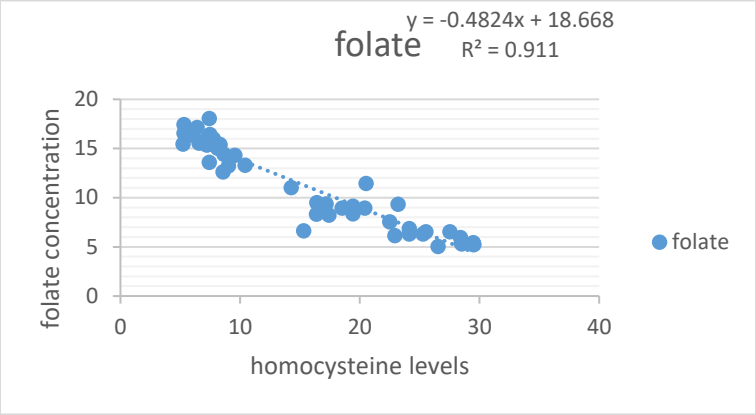

Figure 1 correlation between folate concentration and homocysteine concentrations

In individuals between the ages of 18 and 60, a cross-sectional study was conducted to determine whether there was a relationship between Hcy levels and serum creatinine levels, which ranged from 1.5 to 8 mg/dl. In pre-hemodialysis patients with CKD, the assessment's authors found a moderate correlation between age and creatinine clearance and Hcy levels. A decrease in creatinine clearance by 1 ml/minute led to a proportional rise of 0.2 mmol/l in Hcy levels. Furthermore, each subsequent year of age resulted with a 0.2 mmol/l elevation in Hcy levels[21].

Several prior studies have evaluated the correlation between homocysteine (Hcy) and chronic kidney disease (CKD). In their study, Li et al. [22] found a correlation between Hcy levels and tubular interstitial lesions in the initial phases of IgA nephropathy. Mounting data suggests a proportional relationship between different levels of homocysteine (Hcy) and the decline in estimated glomerular filtration rate (eGFR). Participants with the highest quartile of Hcy levels showed a significantly higher risk for fast eGFR compared to those with the lowest quartile [23].

The present study found that chronic renal failure patients who received folic acid supplementation had reduced levels of homocysteine compared to the chronic renal failure group who did not get folic acid treatment. Supplementing with folic acid (FA), vitamin B12, and vitamin B6 can greatly decrease Hcy levels. Nevertheless, multiple randomized and controlled studies have shown that vitamin supplementation has a limited effect on reducing cardiovascular mortality [24]. The argument remains unresolved: certain research have indicated that supplementation with FA and B vitamins, including cyanocobalamin, has either no benefit or may even be detrimental [25]. However, other investigations have established a connection between the balance of these vitamins, cardiovascular risk, and the progression of chronic kidney disease [26]. The two findings are ultimately attributed to the intricate interplay of HHcy, FA, enzymatic activity/gene variations, and FA fortification schemes present in certain nations [27].

4. Conclusions

The current study revealed a significant increase in urea and creatinine levels, which are crucial indicators for identifying kidney dysfunction. Additionally, the study demonstrated a strong association between homocysteine levels and the consumption of folic acid supplements, suggesting that the use of such supplements enhances homocysteine levels.

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