THE EFFECT OF HOMOCYSTEINE AND SOME BIOLOGICAL PARAMETERS AMONG HEMODIALYSIS PATIENTS IN THI-QAR GOVERNORATE / IRAQ

Riyam Shaker¹, Dr. Mohammed A. Auda²
¹,²University of Thi-Qar – College of Science, Iraq.
riyamsha73@gmail.com

ABSTRACT:

Hemodialysis is one of the leading cause of death among the Iraq. Hyperhomocysteinemia, is total homocysteine concentrations elevated above 15μmol / L. Plasma homocysteine concentration exhibits a strong relationship with (indices of) renal function. Hyperhomocysteinemia has been implicated in patients with CKD (chronic kidney disease). There are several indications that whole body homocysteine metabolism is altered in renal insufficiency. Deficiencies of the vitamins folic acid (B9), pyridoxine (B6), or B12 (cyanocobalamin) can lead to hyperhomocysteinemia. The aim of this study is to evaluate the homocysteine compound levels and some biological parameters (urea, vitamin B6, creatinine, hemoglobin) on hemodialysis patients found that homocysteine compound as a risk factor for hemodialysis patients.

Study design: Blood samples were obtained from (130) men and women. It was divided into (68) hemodialysis patients, aged from (20-80) years and (62) control, aged from (20-80) years, and they were compared.

Results: The results showed a significant increase in the level of homocysteine in the group patients compared to the control group (P≤0.05) and the level of vitamin B6 and level hemoglobin significantly decreased compared to the control group, and a significant increase in creatinine and urea in patients group compared to the control group (P≤0.05) and a significant decrease in the level of vitamin B6 and hemoglobin compared to the control group.

In addition, the results showed an inverse relationship between the level of homocysteine and vitamin B6.

Conclusion: Hyperhomocysteinemia is a risk factor for hemodialysis patients. Serum homocysteine was significantly higher in hemodialysis patients compared to controls. Homocysteine was positively correlated with urea, creatinine and negative with vitamin B6 and hemoglobin.

Keywords: Hemodialysis - Homocysteine - Vitamin B6 - Urea

I. INTRODUCTION

Hemodialysis

Hemodialysis is a method that is used to achieve the extracorporeal removal of waste products such as creatinine and urea and free water from the blood by an artificial kidney machine when the kidneys are in a state of renal failure. The basic principle of the artificial kidney is to pass blood through minute blood channels bounded by a thin membrane. On the other side of the membrane is a dialyzing fluid into which unwanted substances in the blood pass by diffusion (1). Hemodialysis is found in two variants: conventional hemodialysis, where patients receive hemodialysis in a clinic three times a week for 4 hours/session, and nocturnal hemodialysis, where patients are trained to do their own hemodialysis while they sleep, 5–6 nights/week. Hemodialysis is a relatively safe procedure, but there are several complications that can occur including hypotension, cardiac arrhythmia, muscle cramps, anaphylaxis, and restless leg syndrome.
However, with proper monitoring and prompt treatment, many of these complications can be avoided. Of note, better glycemic control (HbA1c < 7.5 %) has been shown to predict better survival of diabetic ESRD patients starting hemodialysis treatment (3).

Subject with homocystinuria were found to have large amounts homocystin (the oxidised form of homocysteine) in the urine. In renal disease, homocysteine levels are elevated. There are two theories on this association. The first is that elevated homocysteine results in impaired renal function. The second is that renal disease causes increased levels of homocysteine. Although glomerular filtration rate (GFR) is correlated with homocysteine, only a small fraction is filtered and excreted in the urine, usually less than 1 %. Another possibility is that homocysteine would be taken up by the renal tubules and metabolised through the transsulphuration pathway to cysteine. However, this has not been found in kidneys with normal function (4,5).

**Homocysteine (Hcy):**

Homocysteine is a sulfur-containing amino acid related to methionine metabolism (6) . Homocysteine is generated metabolically by the Sadenosylmethionine (SAM)-dependent transmethylation pathway (7). Sadenosylmethionine is series of reaction occurs in most cells and tissues; however, the liver is the most prominent site for SAM-dependent transmethylation and the subsequent production of homocysteine (8) .

Physiologic Hcy levels are determined primarily by dietary intake and vitamin status. Elevated plasma levels of Hcy can be caused by deficiency of either vitamin B₁₂ or folate (9).

Homocysteine lies at a metabolic branch-point; it may be further metabolized to cystathionine and then cysteine (transsulphuration) which requires vitamin B₆ as a cofactor, or be remethylated to methionine, either by the vitamin B₁₂ dependent enzyme methionine synthase, or by betaine-homocysteine methyltransferase. The structures of homocysteine, cysteine (10).

II. MATERIALS AND METHOD

This study is conducted at the center of dialysis in Thi-Qar at Al-hussien hospital, Biochemistry Laboratory in the College of Science (University of Thi-Qar) at the period between (October, 2020) to (August, 2021). The study included (130) subjects, (62) control their ages from 20 years to 80 years and (68) patients their ages from 20 years to 80 years. In formed oral consent was taken for patients.

**Excluded cases from this study:** Dialysis patients have viral hepatitis And patients whose ages are less than 20 years and over 80 years . A questionnaire was taken for the patients that included (age – gender – number of hemodialysis per week)

**Blood Sample collection**

The blood was collected from a 5 mL vein and placed in a tube gel, then the serum was separated by centrifugation (10 min at 4000 rpm) and the serum was divided into four fractions which were kept in a clean eppendorf tubes and stored at -20 ° C in a deep freezer for later use. For required measurements:

- Determination of homocysteine in serum Hcy and vitamin B₆ : using ELISA technology by spectrophotometer
- Determination of creatinine and urea : using cobas e411 technique electroluminescence (ECL)

**The statistical analysis**

The statistical analysis was performed by using the software of Statistical Package for the Social Sciences (SPSS). Version 23. The results were expressed as mean ± standard deviations (mean ± SD ). With LSD test . The T test was used to compare parameters in different studied groups. Pearson's correlation (r) was applied to determine the relationship among the present study parameters. P-values

(P≤0.05 ) were considered statistically significant.
III. RESULTS AND DISCUSSION HOMOCYSSTEINE:

Table(1) and figure(1) show a significant increase in the concentration of serum homocystiene patients group in comparison with control group (p < 0.05 ). There were also previous study reporting an increase in the homocystiene concentration in hemodialysis patients (2,11)

Patients with ESRD (end stage renal disease ) have moderately elevated plasma HCY concentrations ranging from 20 to 80 µmol/l. The etiology of hyperhomocysteinemia in patients with chronic renal disease is not well understood. Recent data suggest that the remethylation of HCY to methionine in the kidneys plays an important role in the HCY clearance (12). Renal uptake and metabolism could account for approximately 70% of daily HCY elimination from plasma (13)

The authors believe that the apparent cause of hyperhomocysteinemia in HD may be exactly the alteration of the remethylation pathway due to some factors such as deficiency of folate and vitamin B₁₂, disorders of folate metabolism and decreased activity of the enzymes involved in this pathway resulting from specific genetic mutations (14).

The metabolism of homocysteine can be divided into three distinct pathways: the remethylation of homocysteine to methionine by the vitamin B₁₂ dependent methionine synthase; the transsulfuration pathway, converting homocysteine to cystathionine and then cysteine via vitamin B₆ dependent cystathionine β-synthase enzyme; in the liver and kidneys, homocysteine can be remethylated back to methionine by betaine-homocysteine methyltransferase (13).

Several factors may be involved in the reduced remethylation of homocysteine. Patients with ESRD have an increased risk of developing vitamin deficiency. The most obvious reason is a low folate and/or vitamin B₁₂ status. Folate supplementation (12) has been shown to decrease plasma HCY concentrations significantly in patients with ESRD. Dierkes et al. investigated the effect of vitamin B₁₂ supplementation in ESRD patients with low serum cobalamin concentrations (14). All patients had elevated levels of HCY and methylmalonic acid (MMA) as a marker of intracellular vitamin B₁₂ deficiency (15).

<table>
<thead>
<tr>
<th>Groups</th>
<th>NO.</th>
<th>Homocystiene Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>68</td>
<td>22.95±4.79</td>
</tr>
<tr>
<td>Control</td>
<td>62</td>
<td>8.57±0.98</td>
</tr>
<tr>
<td>P. value</td>
<td></td>
<td>0.000</td>
</tr>
</tbody>
</table>

No: Number of subjects.
SD : Standard deviation.

Hcy ; Homocystiene
Figure 1: Serum Homocystiene levels of control and patients group

**Vitamin B6**

Table (2) and figure (2) show a significant decrease in the concentration of serum B₆ patients group in comparison with control group (p < 0.05). The result was consistent with previous studies (11,16).

Vitamin B₆ decreases in hemodialysis patients. Because vitamin B₆ is water soluble, some of this vitamin is probably removed during peritoneal dialysis and hemodialysis. In renal failure, there may also be inhibitors of vitamin B that could interfere with the vitamin B₆ assays. Indeed, it is theoretically possible that such inhibitors may alter the tests for vitamin B₆ deficiency without causing a true metabolic abnormality. But, because evidence for vitamin B₆ deficiency has been obtained by microbiologic assay, kidney may be necessary for normal metabolism of vitamin B₆ and that even in mild renal failure the parenchymal damage may impair this function. Medicines that impair vitamin B₆ action could also contribute to vitamin B₆ deficiency in these patients (17).

<table>
<thead>
<tr>
<th>Groups</th>
<th>NO.</th>
<th>B₆</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>68</td>
<td>98.50±29.13</td>
<td>0.000</td>
</tr>
<tr>
<td>Control</td>
<td>62</td>
<td>159.45±14.77</td>
<td></td>
</tr>
</tbody>
</table>

Table (2) B₆ levels of control and patients group
Figure 2: Serum Vitamin B\textsubscript{6} levels of control and patients group

Table (3) and figures (3) shows the negative correlation between homocysteine and B\textsubscript{6} in hemodialysis patients with correlation coefficient (r = -0.390, P.value=0.001). The result was consistent with previous studies (11,18,19).

Homocysteine is metabolized through two pathways. Folate and vitamin B\textsubscript{12} are both involved in the remethylation of homocysteine metabolism, while pyridoxal 5'-phosphate (PLP, the physiological coenzyme form of vitamin B\textsubscript{6}) acts as a coenzyme during transsulfuration in the homocysteine metabolism. Since B-vitamins (folate, vitamin B\textsubscript{6} and B\textsubscript{12}) are required for homocysteine metabolism, the loss of B-vitamins during HD (hemodialysis) treatment in these patients may lead to higher raise homocysteine levels, resulting in an increased oxidative stress and higher risks of cardiovascular disease in HD patients (18).

Table (3) Correlation coefficient of Homocysteine and B\textsubscript{6}

<table>
<thead>
<tr>
<th>Hcy with</th>
<th>r</th>
<th>p. value</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>B\textsubscript{6}</td>
<td>-0.390</td>
<td>0.001</td>
<td>Negative correlation</td>
</tr>
</tbody>
</table>

r: Pearson correlation coefficient

P. value: Probability

Creatinine

Table (4) and figure (4) show a significant increase in the concentration of serum Creatinine patients group in comparison with control group (p < 0.05). The result was consistent with previous studies (20,21).

Increases creatinine because creatinine in the blood is then removed by filtration through the glomeruli of the kidney for excretion in the urine.

Since the excretion of creatinine in healthy individuals is independent of diet and thus relatively constant, the creatinine clearance (CC) test is one of the most sensitive tests to diagnose renal function especially the glomerular filtration rate (GFR) the concentration of creatinine in serum being dependent almost entirely upon its rate of excretion by the kidney. Elevated levels of creatinine in serum are usually associated with renal diseases, especially those related to GFR such as glomerular nephritis. Therefore, the clinical significance of the creatinine level in plasma or serum is usually determined in conjunction with the plasma urea level since there is an increase in both levels in postrenal uremia (20).
Table (4) creatinine levels of control and patients group

<table>
<thead>
<tr>
<th>Groups</th>
<th>NO.</th>
<th>S.creatinine mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>68</td>
<td>8.02±2.09</td>
</tr>
<tr>
<td>Control</td>
<td>62</td>
<td>0.43±0.14</td>
</tr>
<tr>
<td>P. value</td>
<td></td>
<td>0.000</td>
</tr>
</tbody>
</table>

Figure 4: Serum creatinine levels of control and patients group

Table (5) and figures (5) show the positive correlation between homocysteine and creatinine in hemodialysis patients with correlation coefficient (r = 0.189, P.value=0.125). The result was consistent with previous studies (2).

Creatine synthesis and homocysteine (Hcy)-formation are metabolically connected. Hcy is derived from methionine in a multiple step metabolic cycle. Methionine can intracellularly be converted to S-adenosyl methionine, a sulfonium compound with a highly reactive methyl group, which acts as a universal methyl donor in numerous transmethylation reactions in vivo. The demethylated product S-adenosylhomocysteine, a thioether, is readily hydrolyzed to Hcy and adenosine by S-adenosyl-homocysteine hydrolase. Transsulfuration of homocysteine to yield cystathionine is favored in conditions with methionine excess. Remethylation of homocysteine to methionine occurs in conditions of low methionine intake through methyldonation from 5-methyltetrahydrofolate (5-CH$_3$THF) by means of methionine synthase. Creatine is synthesized in humans by two successive metabolic steps. Guanidinoacetate is synthesized from glycine and arginine by arginine: glycine aminidinotransferase (AGAT; EC 2.1.4.1), mainly in the kidney. Second, guanidinoacetate is methylated in the liver to creatine by guanidinoacetate-methyltransferase (GAMT) (EC 2.1.1.2) with S-adenosylmethionine as methyl-dono (22). Dietary intake and endogenous synthesis in liver compensate for a daily loss as creatinine of about 2%. Creatine supplementation represses AGAT biosynthesis. guanidinoacetate and creatine formation. Methylation of guanidinoacetate during creatine biosynthesis has been estimated to account for up to 70% of the transmethylation reactions in the body with formation of Hcy (23,24).

Table (5) Correlation coefficient of Homocysteine and creatinin

<table>
<thead>
<tr>
<th>Hcy with</th>
<th>r</th>
<th>p. value</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>0.189</td>
<td>0.125</td>
<td>positive correlation</td>
</tr>
</tbody>
</table>
Urea

Table (6) and figure (6) show a significant increase in the concentration of serum urea patients group in comparison with control group (p < 0.05). The result was consistent with previous studies (20).

Urea is the chief product of protein metabolism in the body. The importance of the urea concentration in blood lies in its value as an indicator of kidney function. Uremia (an abnormal increase in plasma urea level) is seen mainly in renal disorders, dehydration, increase protein catabolism, high-protein diets, or gastrointestinal hemorrhage.

There are two types of uremia. The first, prerenal uremia, is caused by impaired perfusion of the kidneys due to decreased cardiac output or for any of the former causes. The second, postrenal uremia, is caused by an obstruction in the urine outflow such as nephrolithiasis, prostatism, and tumors of the genitourinary tract. The clinical significance of the urea level in plasma is usually determined in conjugation with the plasma creatinine level. In prerenal uremia, an increase in the plasma urea level is usually associated with a normal plasma creatinine level, where as in postrenal uremia, there is an increase in both the urea and the plasma creatinine levels. A decrease in the urea plasma level may be associated with acute dehydration, malnutrition, and pregnancy (20).

Table (6) urea levels of control and patients group

<table>
<thead>
<tr>
<th>Groups</th>
<th>NO.</th>
<th>s.urea Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>68</td>
<td>140.73±35.52</td>
</tr>
<tr>
<td>Control</td>
<td>62</td>
<td>20.17±4.26</td>
</tr>
<tr>
<td>P. value</td>
<td></td>
<td>0.000</td>
</tr>
</tbody>
</table>
Figure 6: Serum urea levels of control and patients group

Table (7) and figures (7) shows the positive correlation between homocysteine and urea in hemodialysis patients with correlation coefficient ( \( r= 0.032 \), \( P\text{-value}=0.794 \) ). The result was consistent with previous studies (16).

The average concentrations of serum urea was found to be markedly elevated in cases compared to controls. It is known that urea and creatinine are marker of the kidney function. Consequently, the present result is logic as the kidney function is almost ceased in hemodialysis patients. Such findings are in agreement with that previously reported in ESRD patients (25,26) and (27).

Person’s correlation test showed significant positive correlations of homocysteine with urea. This implies that hyperhomocysteinemia is linked to kidney function and that homocysteine could be a predictive biomarker of ESRD. The recorded positive correlation of homocysteine with urea was previously reported (28,29) is linked to kidney function and that homocysteine could be a predictive biomarker of ESRD. The recorded positive correlation of homocysteine with urea and creatinine was previously reported (28,29).

**Table (7)** Correlation coefficient of Homocysteine and urea

<table>
<thead>
<tr>
<th>Homocysteine</th>
<th>( r )</th>
<th>( p\text{-value} )</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>0.032</td>
<td>0.794</td>
<td>positive correlation</td>
</tr>
</tbody>
</table>
Figure (7) Correlation between serum Hcy and urea patients group

Hemoglobin

Table (8) and figure (8) show a significant increase in the concentration of serum Hb patients group in comparison with control group (p < 0.05). The result was consistent with previous studies (30).

Hemoglobin (Hb) level was found low in CKD patients due to removal of blood during dialysis. This low Hb level most of the time led to the development of anemia. CKD lead to anemia in most of the patients [19], consistent with our results. Clotting of blood during dialysis is also responsible for low Hb level in CKD patients (30).

Iron deficiency is a commonly encountered reversible cause of CKD related anemia and ESA hyporesponsiveness. In addition to the usual causes of iron deficiency, patients on hemodialysis experience routine iron loss due to the dialysis treatment (retention of blood in dialyzer and blood lines), frequent blood draws for laboratory testing, surgical procedures, accidental blood loss (vascular access), and gastrointestinal blood loss. As a consequence, patients on hemodialysis lose approximately 1000 mg of iron per year (31).

Table (8) hemoglobin levels of control and patients group

<table>
<thead>
<tr>
<th>Groups</th>
<th>NO.</th>
<th>Hb Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>68</td>
<td>9.67±1.87</td>
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<tr>
<td>Control</td>
<td>62</td>
<td>14.22±1.15</td>
</tr>
<tr>
<td>P. value</td>
<td></td>
<td>0.001</td>
</tr>
</tbody>
</table>
Figure 2: Serum hemoglobin levels of control and patient group

Table (9) and figures (9) shows the negative correlation between homocysteine and hemoglobin in hemodialysis patients with correlation coefficient ( $r = -0.028$, $P.value=0.823$ ). The result was consistent with previous studies (32,33).

We found that there is an inverse relationship between homocysteine and hemoglobin in hemodialysis patients compared to healthy subjects, and this result is consistent with other studies (32,34).

**Table (9) Correlation coefficient of Homocysteine and hemoglobin**

<table>
<thead>
<tr>
<th>Hcy with</th>
<th>$r$</th>
<th>p. value</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>0.028</td>
<td>0.823</td>
<td>positive correlation</td>
</tr>
</tbody>
</table>

Figure (9) Correlation between serum Hcy and hemoglobin patients group
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36. AbuTaha AJ. and hematological indices in hemodialysis patients at Al-Shifa hospital, Gaza Strip. 2013;