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Study of homocysteine and its correlation with biochemical parameters in patients with chronic renal failure

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***Abstract*---**Homocysteine is an amino acid produced when proteins are broken down. A high homocysteine level, also called hyperhomocysteinemia, can contribute to arterial damage and blood clots in your blood vessels. Hyperhomocysteinemia (Hhcy) occurs in chronic kidney disease (CKD) patients because of impaired renal metabolism and reduced renal excretion Chronic renal failure is a condition involving a decrease in the kidneys' ability to filter waste and fluid from the blood. It is chronic, meaning that the condition develops over a long period of time and is not reversible. The condition is also commonly known as chronic kidney disease (CKD). The aim of the study is to measure homocysteine levels for patients with chronic renal failure before dialysis and to find the relationship between homocysteine and biochemical parameters such as B_{12}, folic acid, ferritin and albumin. 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 campared. Results The results showed a significant increase in the levels of homocysteine and ferritin in chronic renal failure patients compared to the control group ($P \leq 0.05$), and a significant decrease in vitamins B_{12}, folic acid, Albumin, in chronic renal failure patients compared to the control group ($P \leq 0.05$). The results of the present study show that there is a negative correlation between homocysteine and (vitamin B_{12}, folic acid, Albumin and a positive correlation between homocysteine and Ferritin.Conclusion Hyperhomocysteinemia is a risk factor for chronic renal failure patients. Serum homocysteine was significantly higher in chronic renal failure patients compared to controls. Homocysteine was positively correlated with ferritin, and negative with vitamin B_{12} and folic acid.

***Keywords*---**chronic renal failure, homocysteine, vitamin B_{12}, folic acid.

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Introduction

Homocysteine (Hcy) is a thiol group containing the amino acid, which naturally occurs in all humans. Hcy is degraded in the body through two metabolic pathways, while a minor part is excreted through kidneys. The chemical reactions that are necessary for degradation of Hcy require the presence of folic acid, vitamins B_6 and B_{12}. Consequently, the level of the total Hcy in the serum is influenced by the presence or absence of these vitamins. An elevated level of the Hcy, hyperhomocysteinemia (HHcy) and homocystinuria is connected with occlusive artery disease, especially in the brain, the heart, and the kidney, in addition to venous thrombosis, chronic renal failure, megaloblastic anemia, osteoporosis, depression, Alzheimer's disease, pregnancy problems, and others [1].

Chronic kidney disease

Chronic kidney disease (CKD) is defined as abnormalities of kidney structure or function, present for >3 months, with implications for health. Therapeutic interventions at earlier stages can prevent or ameliorate most of the complications of decreased kidney function, as well as slow the progression to kidney failure. Coronary artery disease (CAD) is the most common cause of death in CKD patients. Low serum folic acid, low serum vitamin B_{12} and high serum homocysteine are commonly associated with CKD[2].

Homocysteine and the kidney

Hyper homocysteine is present in individuals with declining kidney function, so that this alteration can be considered a major factor in the progression of kidney diseases, mainly CKD [3]. Homocysteine is known to undergo transsulphuration in the kidney. Therefore, significant alterations in enzymes and mechanisms involved in transsulphuration strongly contribute to increase Hcy levels in plasma; at the same time, there is damage to renal and cardiovascular function [3].

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 (March, 2021) to (March, 2022).The study included (120) subjects, (60) control and (60) patients. 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 eppendrof tubes and stored at -20 ° C in a deep freezer for later use. For required measurements:

Determination of homocysteine in serum Hcy ,vitamin B_{12} and folic acid : using ELISA technology by spectrophotometer. Determination of ferritin: 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 ($P \leq 0.05$) were considered statistically significant.

Results and Discussion

Homocysteine

Table (1) and Figure (1) show a significant increase in the concentration of serum homocysteine patients group in comparison with control group ($p < 0.05$). There were also previous study reporting an increase in the homocysteine concentration in hemodialysis patients [2], [4], [5]. Homocysteine is degraded in the body through two metabolic pathways, while a minor part is excreted through kidneys. The chemical reactions that are necessary for degradation of Hcy require the presence of folic acid, vitamins B_6 and B_{12}. Consequently, the level of the total Hcy in the serum is influenced by the presence or absence of these vitamins. An elevated level of the Hcy, hyperhomocysteinemia (HHcy) and homocystinuria is connected with occlusive artery disease, especially in the brain, the heart, and the kidney, in addition to venous thrombosis, chronic renal failure, megaloblastic anemia, osteoporosis, depression, Alzheimer's disease, pregnancy problems, and others. Elevated Hcy levels are connected with various pathologies both in adult and child population. Causes of HHcy include genetic mutations and enzyme deficiencies in 5, 10-methylenetetrahydrofolate reductase (MTHFR) methionine synthase (MS), and cystathionine β-synthase (CβS). HHcy can be caused by deficiencies in the folate, vitamin B12 and to a lesser extent, deficiency in B6 vitamin what influences methionine metabolism. Additionally, HHcy can be caused by the rich diet and renal impairment [1].

Table (1): Homocysteine levels of control and patients group

Groups	NO.	Homocysteine Mean ± SD
Patients	68	22.95±4.79
Controls	62	8.57±0.98
P. value		0.000

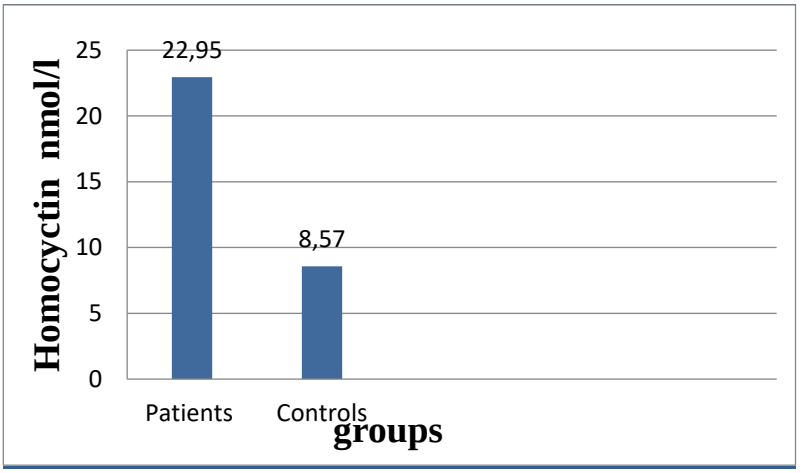

Figure (**1**): Serum homocysteine levels of control and patients group

Serum Vitamin B_{12} Concentration

Table **(**2**)** and figure **(**2**)** show that there was a significant decrease in the concentration of serum B_{12} patients group in comparison with control group ($p < 0.05$). The results were consistent with previous studies [6], [7]. Table (2) shows a decrease in vitamin B_{12} in hemodialysis patients Because Patients with end-stage renal disease (ESRD) are at higher risk for nutritional deficiencies due to medication interactions, dietary restrictions and malnutrition. Furthermore, the dialysis procedure itself may lead to vitamin B deficiencies, especially in the case of folate where its molecular size renders it capable of being cleared during hemodialysis (HD) [8].Vitamin deficiency, mainly folic acid and vitamin B_{12} (cobalamin), is considered to be a major contributor to the hyper-homocysteinemia found in patients with chronic kidney disease (CKD). Most of the currently available evidence suggests that defective remethylation of Hcy to methionine is the main biochemical defect underlying the genesis of the hyperhomocysteinemia in these patients. In the remethylation process of Hcy, 5-methyl-tetrahydrofolate (5-MTHF), the active form of folic acid, is a cosubstrate, and vitamin B_{12} acts as a coenzyme that helps to overcome the partial inhibition of methionine synthase that is found in uremic patients [6].

Table (2): B_{12} levels of control and patients group

Groups	NO.	B_{12}(pmol/l) Mean ±SD
Patients	68	168.61±44.74
Control	62	277.48±22.78
P. value		0.001

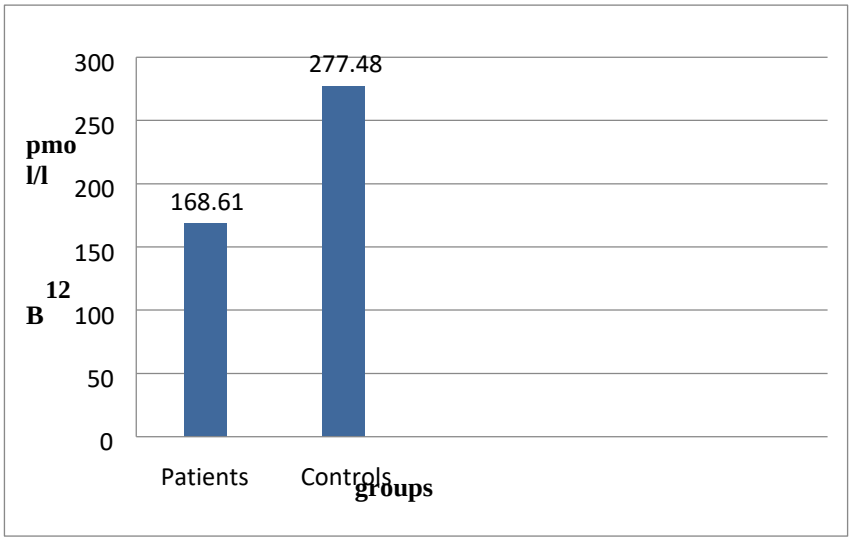

Figure (**2**): Serum B_{12} levels of control and patients group

Table (3) and Figures (3) show the negative correlation between homocysteine and B_{12} in hemodialysis patents with correlation coefficient (r= -0.622, P.value=0.000). The results were consistent with previous studies[9], [10]. prevalence of hyperhomocysteinemia remains a serious problem for these patients. Since folate, vitamin B_6 and B_{12} are involved in either the remethylation or transsulfuration pathway of homocysteine metabolism, the dialytic removal of these B-vitamins during HD treatment might exacerbate the elevation of plasma homocysteine levels after dialysis. In addition to the status of B-vitamins, defects of gene polymorphisms encoding homocysteine metabolism related enzymes (i.e., 5, 10methylenetetrahydrofolate reductase, methionine synthase, cystathionine β-synthase) might be associated with the elevated plasma levels of homocysteine. Vitamin B_{12} (Vit B_{12}) was also found recently to be effective in correcting hyperhomocysteinemia in uremic patients. Pharmacological dose of Vit B_{12}, in combination with folic acid, has been reported to be effective in lowering the plasma Hcy levels [11]Vitamin B_{12} levels decrease in renal failure levels, which impedes the remethylation pathway of homocysteine to methionine, which leads to elevated homocysteine levels[12].

Table (3): Correlation coefficient of Homocysteine and B_{12}

Homocysteine with	r	p. value	Results
B12	-0.622	0.000	Negative correlation

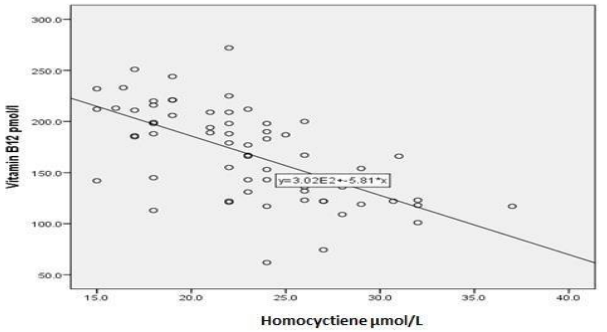

Figure (**3**): Correlation between serum homocysteine and B_{12} patients group

Serum Vitamin Folic Acid Concentration

Table (4) and Figure (4) show a significant decrease in the concentration of serum folic acid patients group in comparison with control group ($p < 0.05$). The results were consistent with previous studies [13], [14]. Folic acid decreases in hemodialysis patients Because Folate is not stored in the body in large amounts, deficiency can develop within a few weeks [8]. Previous studies have shown that serum folate concentrations are low in hemodialysis patients in comparison with that of the general population [15]. From the many, mostly short-term, homocysteine-lowering intervention studies in renal failure patients, one can conclude that folate compounds are the most effective and consistent in lowering plasma homocysteine compared with other therapies. Vitamin B_6 does not seem to have a significant impact on plasma homocysteine levels in dialysis patients, whereas vitamin B_{12} may lower homocysteine somewhat further when added to folic acid, especially in patients with subclinical B_{12} deficiency [16].

Table (4): folic acid levels of control and patients group

Groups	NO.	Folic acid (mmole/l) Mean ±SD
Patients	68	1.24±0.35
Control	62	2.94±0.56
P. value		0.032

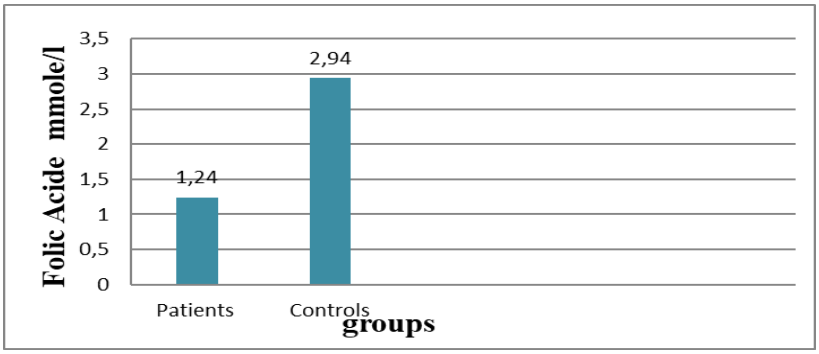

Figure (**4**): Serum folic acid levels of control and patients group

Table (5) and Figures (5) shows the negative correlation between homocysteine and folic acid in hemodialysis patients with correlation coefficient (r= -0.109, P.value=0.380). The results were consistent with previous studies [17], [18]. The study demonstrated that folate treatment in ESRD patients ameliorates DNA hypomethylation and restores altered expression of genes that depend on DNA methylation, again without normalization of plasma homocysteine. Together, these interesting findings suggest that folate therapy may have biological effects in ESRD patients which are not mediated by the lowering of plasma homocysteine , but rather by the improvement of the transmethylation pathway, in which many important methylation reactions take place [19]. Several attempts have been made to reduce tHcy levels in ESRD patients. Folic acid (FA) is considered to be the most important vitamin by far to reduce tHcy levels [16].

Table (5): Correlation coefficient of Homocysteine and folic acid

Homocysteine with	r	p. value	Results
Folic acid	-0.109	0.380	Negative correlation

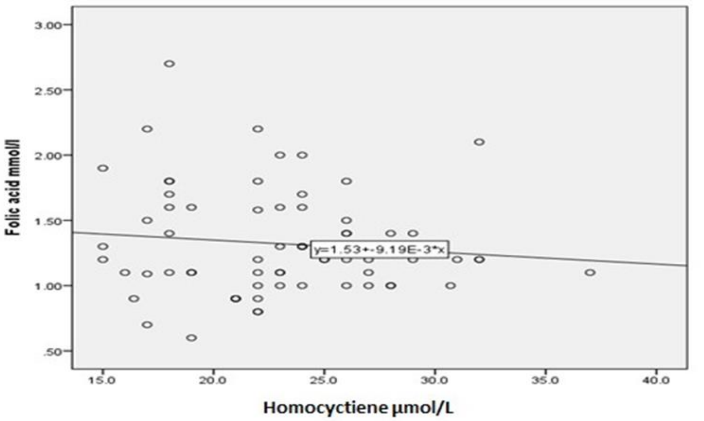

Figure (**5**): Correlation between serum homocysteine and folic acid patients group

Serum Albumin Concentration

Table (6) and Figure (6) show a significant decrease in the concentration of serum Albumin patients group in comparison with controls group ($p < 0.05$). The result was consistent with previous studies [20], [21]. Albumin decreased in hemodialysis patients Because protein energy malnutrition‖ are potent predictors and morbidity in hemodialysis patients. These include the serum concentration of visceral proteins, such as albumin and prealbumin (transthyretin) Hypoalbuminemia is a potent risk factor for morbidity in ESRD and in other populations, including the older patients and individuals undergoing a variety of types of noncardiac surgery. While reduced dietary intake can lead to hypoalbuminemia, these effects are generally mild [22]. Protein malnutrition is common in patients on maintenance dialysis. Malnutrition and hypoalbuminemia are risk factors for increased morbidity and mortality in patients with ESRD [23]. A decreased serum albumin level predicts poor survival in end-stage renal failure. Hypoalbuminemia is multifactorial and related to poor nutrition, inflammation,

and comorbid disease. Over hydration is also common in renal replacement therapy patients, and hemodilution may also contribute to a low serum albumin level [24].

Table (6): Albumin levels of control and patients group

Groups	NO.	Albumin (g/l) Mean ±SD
Patients	68	2.92±0.69
Control	62	3.98±0.51
P. value		0.005

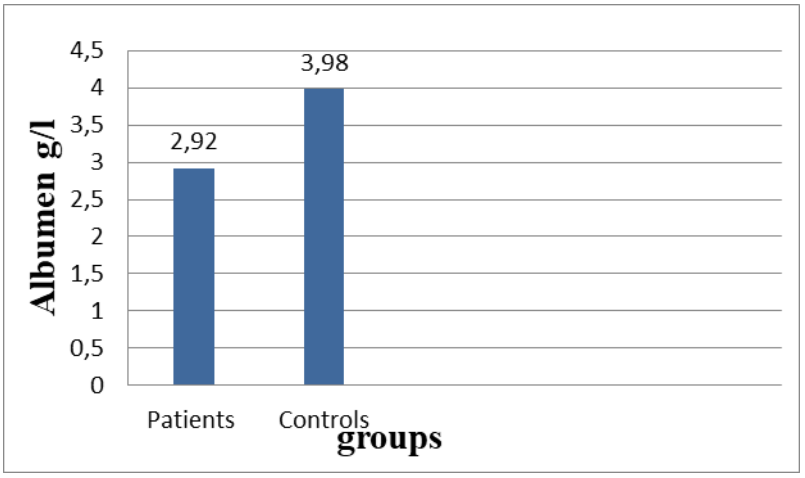

Figure (6): Serum albumin levels of control and patients group

Table (7) and Figure (7) shows a negative correlation between homocysteine and albumin in hemodialysis patents with correlation coefficient (r= -0.107, P.value=0.154). The results were consistent with previous studies [13], [25]. Nutritional status influences plasma tHcy concentrations by the strong protein binding of Hcy to albumin, and by the increased Hcy formation upon methionine uptake from the diet [23].

Table (7): Correlation coefficient of Homocysteine and albumin

Homocysteine with	r	p. value	Results
Albumin	-0.107	0.154	Negative correlation

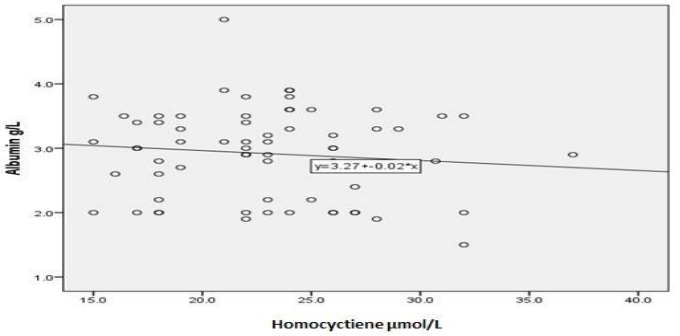

Figure (**7**): Correlation between serum homocysteine and albumin patients group

Serum ferritin Concentration

Table (8) and Figure (8) show a significant increase in the concentration of serum ferritin patients group in comparison with control group ($p < 0.05$). The result was consistent with previous study [26]. Hyperferritinemia is commonly found in hemodialysis (HD) patients regardless of their hemoglobin level and is often considered being related to chronic inflammatory status as well as malnutrition and neoplasias [27]. Hyperferritinemia also is associated with liver dysfunction, probably because liver is the main organ to clear circulating ferritin molecules [26]. High ferritin levels have been reported in patients who had CKD with glomerular disease and proteinuria [28]. Because moderately high levels of serum ferritin do not necessarily indicate Fe overload but most likely inflammation, infection, malnutrition, liver disease, and/or other non–Ferelated factors in CKD [26].

Table (8): Ferritin levels of control and patients group

Groups	NO.	Ferritin (ng/dl) Mean ±SD
Patients	68	502.30±117.18
Control	62	200.43±59.21
P. value		0.001

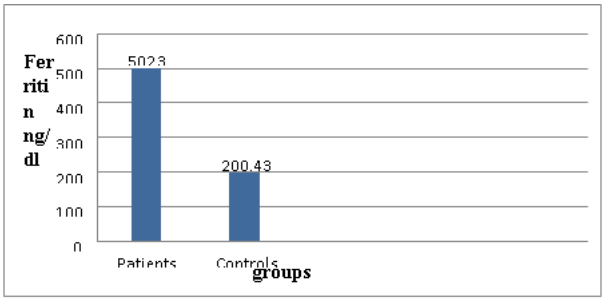

Figure (8): Serum ferritin levels of control and patients group

Table (9) and Figures (9) show the positive correlation between homocysteine and ferritin in hemodialysis patents with correlation coefficient (r= 0.176, P.value=0.154). The results were consistent with previous studies [29], [30]. We found that there is an positive relationship between homocysteine and ferritin in hemodialysis patients compared to healthy people, and this result is consistent with the other studies above.

Table (9): Correlation coefficient of Homocysteine and ferritin

Homocysteine with	r	p. value	Results
Ferritin	0.176	0.154	positive correlation

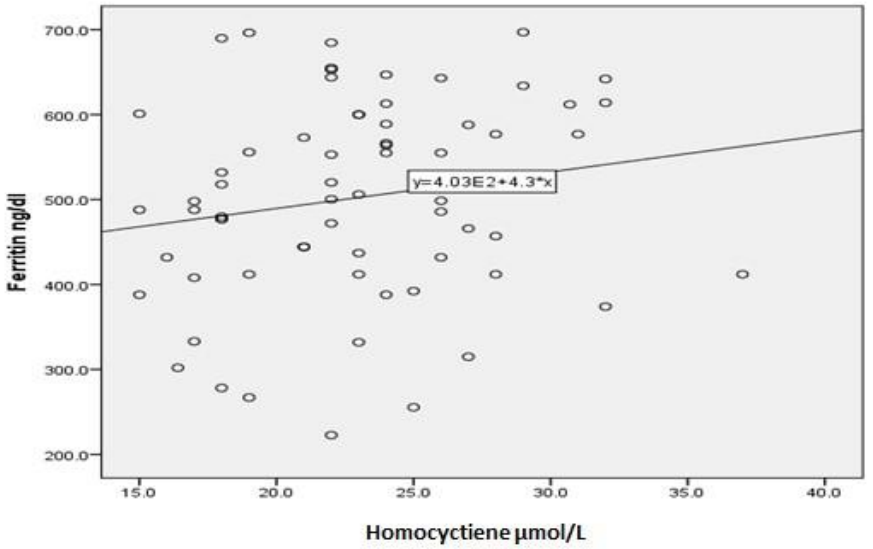

Figure (9): Correlation between serum homocysteine and ferritin patients group

References

1. A. Anurag, “A Study on the Levels of Folic Acid, Vitamin B12 and Plasma Homocysteine in Patients with Chronic Kidney Disease.,” *J. Assoc. Physicians India*, vol. 70, no. 4, pp. 11–12, 2022.
2. A. J. Abutaha, “Homocysteine and hematological indices in hemodialysis patients at Al-Shifa hospital, Gaza Strip,” 2013.
3. A. J. W. Branten, D. W. Swinkels, I. S. Klasen, and J. F. M. Wetzels, “Serum ferritin levels are increased in patients with glomerular diseases and proteinuria,” *Nephrol. Dial. Transplant.*, vol. 19, no. 11, pp. 2754–2760, 2004.
4. A. Kooshki, M. Eftekhari Yazdi, M. Yousefi Moghaddam, and R. A. Akbarzadeh, “Status of antioxidant and homocysteine-lowering vitamins related to cardiovascular diseases in hemodialysis patients,” *Electron. Physician*, vol. 9, no. 7, pp. 4895–4898, 2017, doi: 10.19082/4895.
5. A. Moustapha *et al.*, “Prevalence and determinants of hyperhomocysteinemia in hemodialysis and peritoneal dialysis,” *Kidney Int.*, vol. 55, no. 4, pp. 1470–1475, 1999, doi: 10.1046/j.1523-1755.1999.00378.x.

6. B. L. Zaric, M. Obradovic, V. Bajic, M. A. Haidara, M. Jovanovic, and E. R. Isenovic, “Homocysteine and hyperhomocysteinaemia,” *Curr. Med. Chem.*, vol. 26, no. 16, pp. 2948–2961, 2019.
7. B. M. Martella *et al.*, “Hcy and the kidney HHcy is present in individuals with declining kidney function, so that this alteration can be considered a major factor in the progression of kidney diseases, mainly CKD(17). Hcy is known to undergo transsulphuration in the kidney. Ther,” *J. Bras. Patol. e Med. Lab.*, vol. 54, pp. 170–176, 2018.
8. C. H. Jones, H. Akbani, D. C. Croft, and D. P. Worth, “hydration status in hemodialysis pTdatientshe relationship between serum albumin an,” *J. Ren. Nutr.*, vol. 12, no. 4, pp. 209–212, 2002.
9. C. M. Clase, V. Ki, and R. M. Holden, “Water-soluble vitamins in people with low glomerular filtration rate or on dialysis: A review,” in *Seminars in dialysis*, 2013, vol. 26, no. 5, pp. 546–567.
10. C. van Guldener, “Why is homocysteine elevated in renal failure and what can be expected from homocysteine-lowering?,” *Nephrol. Dial. Transplant.*, vol. 21, no. 5, pp. 1161–1166, 2006.
11. C. van Guldener, F. Stam, and C. D. A. Stehouwer, “Hyperhomocysteinaemia in chronic kidney disease: focus on transmethylation,” *Clin. Chem. Lab. Med.*, vol. 43, no. 10, pp. 1026–1031, 2005.
12. G. A. Kaysen, G. M. Chertow, R. Adhikarla, B. Young, C. Ronco, and N. W. Levin, “Inflammation and dietary protein intake exert competing effects on serum albumin and creatinine in hemodialysis patients,” *Kidney Int.*, vol. 60, no. 1, pp. 333–340, 2001, doi: 10.1046/j.1523-1755.2001.00804.x.
13. H. K. Amin, M.-I. K. El-Sayed, and O. F. Leheta, “Homocysteine as a predictive biomarker in early diagnosis of renal failure susceptibility and prognostic diagnosis for end stages renal disease,” *Ren. Fail.*, vol. 38, no. 8, pp. 1267–1275, 2016.
14. I. Capelli *et al.*, “Folic acid and vitamin B12 administration in CKD, why not?,” *Nutrients*, vol. 11, no. 2, 2019, doi: 10.3390/nu11020383.
15. I. Margalit, E. Cohen, E. Goldberg, and I. Krause, “Vitamin B12 Deficiency and the Role of Gender : A Cross-Sectional Study of a Large Cohort,” no. March, 2018, doi: 10.1159/000488326.
16. K. Kalantar-Zadeh, K. Kalantar-Zadeh, and G. H. Lee, “The fascinating but deceptive ferritin: to measure it or not to measure it in chronic kidney disease?,” *Clin. J. Am. Soc. Nephrol.*, vol. 1 Suppl 1, pp. 9–18, 2006, doi: 10.2215/CJN.01390406.
17. M. BOITAN *et al.*, “RELATION BETWEEN HOMOCYSTEINE LEVEL AND MACROANGIOPATHY IN PATIENTS WITH CHRONIC KIDNEY DISEASE.,” *Acta Medica Transilv.*, vol. 24, no. 1, 2019.
18. M. Ehsani, M. E. Zeynali, M. Abtahi, and A. A. Harati, “linked immunosorbent assay system. The patients were followed up for 1 year to determine the effect of anemia and hyperhomocysteinemia on survival and risk factors of mortality. Normal values for laboratory parameters were considered according to the Kidn,” vol. 18, no. 1, pp. 37–47, 2009.
19. M. K. Van Gelder, A. C. Abrahams, J. A. Joles, G. A. Kaysen, and K. G. F. Gerritsen, “Albumin handling in different hemodialysis modalities,” *Nephrol. Dial. Transplant.*, vol. 33, no. 6, pp. 906–913, 2018, doi: 10.1093/ndt/gfx191.
20. M. Katko *et al.*, “Relationship between serum nickel and homocysteine

concentration in hemodialysis patients," *Biol. Trace Elem. Res.*, vol. 124, no. 3, pp. 195–205, 2008, doi: 10.1007/s12011-008-8139-2.
21. M. M. Sirdah, M. M. Yassin, S. El Shekhi, and A. M. Lubbad, "Homocysteine and vitamin B 12 status and iron deficiency anemia in female university students from Gaza Strip, Palestine," *Rev. Bras. Hematol. Hemoter.*, vol. 36, pp. 208–212, 2014.
22. M. Soohoo *et al.*, "Association of serum vitamin B12 and folate with mortality in incident hemodialysis patients," *Nephrol. Dial. Transplant.*, vol. 32, no. 6, pp. 1024–1032, 2017, doi: 10.1093/ndt/gfw090.
23. P. L. Spada *et al.*, "Ferritin iron content in haemodialysis patients: Comparison with septic and hemochromatosis patients," *Clin. Biochem.*, vol. 41, no. 12, pp. 997–1001, 2008, doi: 10.1016/j.clinbiochem.2008.05.003.
24. R. B. Jain and A. Ducatman, "Perfluoroalkyl acids serum concentrations and their relationship to biomarkers of renal failure: Serum and urine albumin, creatinine, and albumin creatinine ratios across the spectrum of glomerular function among US adults," *Environ. Res.*, vol. 174, pp. 143–151, 2019.
25. S. V. Shevchuk *et al.*, "The relationship between homocysteine level and vitamins B12, B9 and B6 status in patients with chronic kidney disease," *Wiad. Lek.*, vol. 72, no. 4, pp. 532–538, 2019, doi: 10.36740/wlek201904105.
26. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, *5*(1), i-v. https://doi.org/10.53730/ijhs.v5n1.2864
27. U. Schwab *et al.*, "Orally administered betaine has an acute and dose-dependent effect on serum betaine and plasma homocysteine concentrations in healthy humans," *J. Nutr.*, vol. 136, no. 1, pp. 34–38, 2006.
28. V. D. Alvares Delfino, A. C. de Andrade Vianna, A. J. Mocelin, D. S. Barbosa, R. A. Mise, and T. Matsuo, "Hoffer LJ. Testing the homocysteine hypothesis in end-stage renal disease: problems and possible solutions. Kidney Int 2006;69:1507–10," *Nutrition*, vol. 23, no. 3, pp. 242–247, 2007, doi: 10.1016/j.nut.2007.01.002.
29. W. F. Keane *et al.*, "Reviews and Original Articles," *Perit. Dial. Int. J. Int. Soc. Perit. Dial.*, vol. 16, no. 6, pp. 557–573, 1996, doi: 10.1177/089686089601600606.
30. Y. E. C. Taes *et al.*, "Creatine supplementation does not decrease total plasma homocysteine in chronic hemodialysis patients.," *Kidney Int.*, vol. 66, no. 6, pp. 2422–2428, 2004, doi: 10.1111/j.1523-1755.2004.66019.x.
31. Y. W. Chiu, J. M. Chang, S. J. Hwang, J. C. Tsai, and H. C. Chen, "Pharmacological dose of vitamin B12 Is as effective as low-dose folinic acid in correcting hyperhomocysteinemia of hemodialysis patients," *Ren. Fail.*, vol. 31, no. 4, pp. 278–283, 2009, doi: 10.1080/08860220902780010.