INVESTIGATION OF PLASMA HOMOCYSTEINE CONCENTRATIONS AND THEIR RELATIONSHIP WITH SOME CLINICAL AND LABORATORY CHARACTERISTICS OF PATIENTS TREATED WITH MAINTENANCE HEMODIALYSIS

Nguyen Van Tuyen[1,2], Bui My Hanh[3], Le Viet Thang[4]

SUMMARY

***Objectives**: To investigate plasma homocysteine (Hcy) concentrations and their relationship with some clinical and laboratory characteristics of patients treated with maintenance hemodialysis (MHD). **Subjects and methods**: A cross-sectional descriptive study, compared with control groups on 199 subjects, including 111 patients diagnosed with end-stage chronic kidney disease treated with MHD and 88 healthy people of the same age and sex. The plasma Hcy levels were quantified by turbidimetric immunoassay in all subjects. **Results:** The mean plasma Hcy concentration of the patients was 38.49 ± 11.26 26 µmol/L, higher than that of the control group, p < 0.001. There were 71.2% patients with increased concentration compared to the control group. The mean plasma Hcy concentration in male patients; duration of hemodialysis ≥ 5 years; loss of residual renal function; diabetes mellitus; smoking was higher than the patient group without the above characteristics, p < 0.05. Hcy concentration was negatively correlated with HDL-C concentration, r = -0,301, p < 0.01. **Conclusion**: Increased plasma Hcy levels are common and associated with some MHD patients' clinical and laboratory characteristics.*

** Keywords: Maintenance hemodialysis; Plasma homocysteine; Duration of hemodialysis; Residual renal function.*

INTRODUCTION

Atherosclerosis is the thickening of the artery walls as a result of the accumulation of fats such as cholesterol and other substances including triglycerides and calcium. Atherosclerosis affects the arterial lining, forming a chronic inflammatory response in the arterial walls [2, 3]. Atherosclerosis has many risk factors such as lipid metabolism disorders, hypertension, diabetes, obesity, smoking, inflammation... The consequences of atherosclerosis process are the hardening of the arteries, in which arterial walls become thickened and hardened by plaque, causing loss of elasticity of medium and large arteries. Atherosclerotic plaques can restrict blood flow in the artery, and if the plaque ruptures, the flow can partially or completely obstruct the blood vessels [3, 4].

[1]*Vietnam Military Medical University*

[2]*Duc Giang General Hospital, Hanoi*

[3]*Hanoi Medical University*

[4]*Military Hospital 103,* ***Vietnam Military Medical University***

Corresponding author: Nguyen Van Tuyen (tuyenbvdkdg@gmail.com)

Date received: 20/5/2021

Date accepted: 04/6/2021

In recent years, Hcy has emerged as a non-traditional factor involved in the process of atherosclerosis. For all patients of end-stage renal disease on maintenance hemodialysis (MHD), Hcy is often elevated in the blood, which is associated with arterial stiffness and related factors. In Vietnam, there have not been many studies on Hcy concentrations in MHD patients, for the above reasons, we carried out the study: *To investigate plasma Hcy concentrations and their relationship with some clinical and paraclinical characteristics of MHD patients.*

SUBJECTS AND METHODS

1. Subjects

The study subjects included 199 people divided into 2 groups:

- The disease group: 111 patients with end-stage chronic kidney disease, treated with MHD.

- The control group: 88 healthy people of the same age and sex.

* *Selection criteria:*

- Patients with end-stage chronic kidney disease treated with MHD.

- Duration of hemodialysis ≥ 3 months.

- Dose of hemodialysis, treatment of anemia and hypertension according to the guidance of the Ministry of Health, individualized for each patient.

- Do not take drugs that affect Hcy levels.

- Agree to participate in the study

* *Exclusion criteria:*

- Patients are suffering from acute illnesses such as viral fever, pneumonia, bronchitis...

- Suspected surgical disease.

- Not enough research contents

2. Methods

* *Study design:* Cross-sectional study combined with description, comparison with control groups.

- Take personal and family history.

- Ask the patient and examine the patient to detect clinical signs and symptoms.

- 24-hour urine measurement, assess the status of urine left or lost.

- Take venous blood to test blood count, blood biochemistry.

- Quantification of plasma Hcy (Hcy) concentration: Take venipuncture of fasting subjects. Anticoagulation followed by plasma separation. Quantification of plasma Hcy by enzyme colorimetric method. Unit: µmol/L. Determine increase or reduction of plasma Hcy concentrations basing on values of healthy controls. The patient group values that were considered normal were within $\overline{X}$ ± 2SD of the control group. When the patient has a Hcy concentration value < $\overline{X}$-2SD value, the concentration is determined to decrease, and if the value is > $\overline{X}$+2SD, the concentration is determined to increase.

* *Data processing:* Using SPSS 22.0 software. Figures were drawn automatically on the computer.

RESULTS AND DISCUSSION

The mean age was 55.22 ± 13.74 years, male accounted for 49.5%, the median duration of hemodialysis was 30 months (13 - 74).

Table 1: Comparison of plasma Hcy concentrations in the disease and the control groups.

Index	Control group (n = 88)	Patient group (n = 111)	p
Mean ($\overline{X}$ ± SD), (µmol/L)	17.23 ± 6.16	38.49 ± 11.26	< 0.001
Min	4.18	19.47	
Max	36.6	61.05	

The mean value of plasma Hcy concentration in the patient group was higher than that in the control group, p < 0.001. The minimum and maximum plasma Hcy values were also higher in the disease group than in the control group.

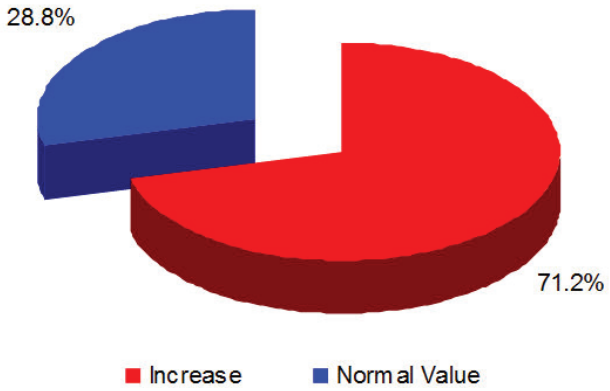

Figure 1: The rate of the increased plasma Hcy concentration (n = 111).

Increased plasma Hcy concentration was common, accounting for 71.2% of patients with MHD. Compared to the research results of domestic and foreign authors, we found that there were many similarities. Diem Thi Van's study (2016) [4] on 112 patients with MHD, compared to 56 healthy people, also showed that the Hcy concentration of the disease group was higher than that of the control group, p < 0.001. In patients with chronic kidney disease (CKD) from stage 1 to 5, Chaitanya V. et al. (2018) [5] also showed that Hcy concentration in the disease group was higher than in the control group and especially, the Hcy concentration was reduced in GFR patients. In patients with CKD with and without MHD, Chen C.H. et al. (2017) [6] also confirmed that the concentration of Hcy in the group of MHD patients was higher than in the group with CKD stage 2 and 3, p < 0.01. To understand why Hcy is higher in CKD patients than in normal people, it is first necessary to understand the metabolism of Hcy in the human body. Hcy is a non-essential, sulfur-containing, protein-free amino acid. It is synthesized by metabolizing the essential amino acid methionine, derived from the diet, and synthesized

only in humans. In patients with end-stage renal failure, there are many factors involved in the increasing blood Hcy. Possible causes include decreased renal clearance (particularly altered tubular function), altered Hcy metabolism (increased peroxidation), genetic abnormalities undiagnosed (cystathionine-β- synthetase deficiency) and/or vitamin deficiency (B6, B12 or folic acid). Folic acid's ability to reduce serum Hcy is due to the stimulation of Hcy remethylation in methionine. These observations suggest that dialysis patients who are resistant to folic acid therapy are also responsible for the elevation of Hcy.

Table 2: Relationship of plasma Hcy concentration with age and sex.

Index		Mean ($\overline{X}$ ± SD), (µmol/L)	Ratio of increase (n, %)
Age	< 40 (n = 15)	33.61 ± 11.27	8 (53.3)
	40 - < 60 (n = 55)	38.72 ± 10.19	40 (72.7)
	≥ 60 (n = 41)	39.96 ± 12.35	31 (75.6)
	pANOVA	> 0.05	> 0.05
Sex	Male (n = 55)	40.73 ± 10.81	43 (78.2)
	Female (n = 56)	36.29 ± 11.35	36 (64.3)
	p	< 0.05	> 0.05

There was no relationship between plasma Hcy concentrations and age groups. However, male patients had a significantly higher mean Hcy concentration than female patients ($p < 0.05$). Elevated plasma Hcy levels are common in many different diseases, including cardiovascular and renal diseases [7]. Hcy has been recognized as an independent risk factor for cardiovascular diseases. It has been estimated that a 2.5 µM increase in plasma, Hcy concentration is associated with a 10% increased risk of cardiovascular disease [7]. It is possible that the male gender is a cardiovascular risk factor, and therefore, Hcy concentration is higher than female.

Table 3: Relationship between hemodialysis time and residual kidney function.

Index		Mean ($\overline{X}$ ± SD), (µmol/L)	Ratio of increase (n, %)
Duration of hemodialysis	< 5 years (n = 75)	36.06 ± 10.45	50 (66.7)
	≥ 5 years (n = 36)	43.55 ± 11.33	29 (80.6)
	p	< 0.005	> 0.05
Residual kidney function	No (n = 88)	39.75 ± 11.02	67 (76.1)
	Yes (n = 23)	33.66 ± 11.1	12 (52.2)
	OR, p	< 0.05	p < 0.05 OR = 2.925

The group of patients with hemodialysis duration ≥ 5 years had a higher mean plasma Hcy concentration than the group of patients with a duration of hemodialysi < 5 years

(p < 0.01). The patients without residual renal function had a 2.925 times higher rate of increased Hcy concentration than the patients with residual renal function (p < 0.05).

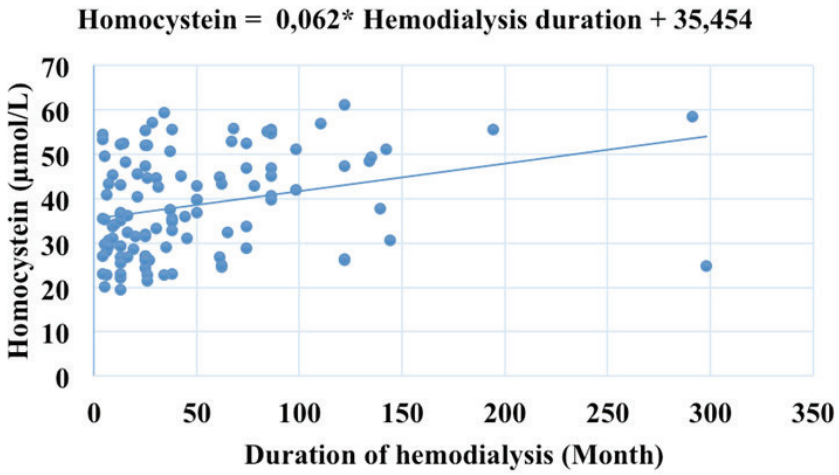

Figure 2: Correlation of plasma Hcy concentration with hemodialysis time in the disease group (n = 111).

The plasma Hcy concentration had a positive correlation with hemodialysis duration, r = 0.287 (p < 0.005). Prolonged time of hemodialysis and loss of residual renal function are two characteristics that always go together. In patients on long-term hemodialysis, especially after 5 years, systemic disorders affected by dialysis are more likely to occur, including inflammation, malnutrition, and atherosclerosis. Furthermore, cardiovascular events are more likely to occur in patients with prolonged hemodialysis and loss of residual renal function [8]. The increasing concentration of Hcy directly affects the process of atherosclerosis, which forms a vicious cycle. That makes the disease progress more seriously, cardiovascular events such as stroke, myocardial infarction or aortic dissection, and peripheral artery disease more severe.

Table 4: The association between plasma Hcy concentration and diabetes and smoking.

Index		Mean ($\overline{X}$ ± SD), (µmol/L)	Ratio of increase (n, %)
Diabetic mellitus	Yes (n = 21)	45.5 ± 10.33	18 (85.7)
	No (n = 90)	36.85 ± 10.88	61 (67.8)
	OR, p	< 0.005	p > 0.05 OR = 2.852
Smoke	Yes (n = 21)	43.88 ± 9.68	18 (85.7)
	No (n = 90)	37.23 ± 11.28	61 (67.8)
	OR, p	< 0.05	p > 0.05 OR = 2.852

The mean value of Hcy concentration in the patients with diabetes or smoking was higher than the group without the above features, $p < 0.05$. Diabetic mellitus (DM) and smoking are two risk factors for atherosclerosis. Hcy is a sulfur-containing amino acid formed by the demethylation of methionine. Plasma Hcy levels are elevated in DM patients, especially those in type 2 DM, as well as in insulin-resistant prediabetes [9]. Changes in plasma Hcy concentrations in DM patients are often related to vitamin B level and vitamin absorption. Vitamin B is an important factor in Hcy metabolism. Elevated levels of Hcy and low levels of folic acid have been observed in DM patients, especially in patients with diabetic retinopathy and diabetic nephropathy. Long-term metformin use increases the risk of vitamin B12 and folate deficiencies, thereby affecting Hcy metabolism and contributing to the progression of diabetic retinopathy as well as diabetic nephropathy. Wang H. et al. [3] investigated the relationship of total plasma Hcy with albuminuria and its clinical applicability in predicting renal function impairment in type 2 DM patients. Study results showed that Hcy levels were significantly increased in patients with proteinuria and microalbuminuria. Elevated plasma concentrations of Hcy may be due to the impaired Hcy clearance in patients with decreased glomerular filtration rate (GFR).

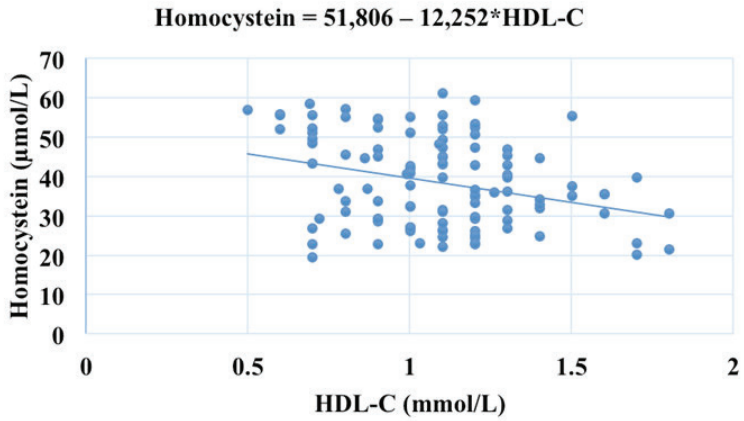

Figure 3: Correlation between plasma Hcy level and HDL-C in the disease group (n = 111).

An inverse, moderate correlation between Hcy concentration and HDL-C was detected in the study, $r = -0,301$, $p < 0.01$. Momin M. et al. [146] reported an association between increased Hcy, which was independently associated with lower HDL-C and higher triglycerides, in a clinical epidemiological study of 4,660 Chinese people, $p < 0.001$. Wang Y. et al. [10] evaluated the association between Hcy levels and apoA-I in normal healthy subjects. The study results showed that plasma Hcy concentration was negatively correlated with HDL-C and apoA-I levels.

Thus, the studies found an inverse correlation between Hcy and HDL-C in both normal people and patients with MHD. In the study of Wang Y. et al. [10], plasma Hcy concentration was negatively correlated with HDL-C and apoAI concentrations in normal healthy subjects, and plasma Hcy concentration was an independent factor affecting apoA-I concentration. Therefore, it can be suggested that increased Hcy is associated with low plasma concentrations of apoA-I and HDL-C in healthy subjects. That inhibition of apoA-I synthesis is a further mechanism by which Hcy may be associated with the development of atherosclerosis in patients with elevated Hcy.

CONCLUSION

Surveying plasma Hcy levels of 111 patients with end-stage chronic kidney disease treated with MHD, compared with 88 healthy people, we have the following comments:

- The mean plasma Hcy concentration in the hemodialysis patients was 38.49 ± 11.26 µmol/L, significantly higher than the control group, $p < 0.001$. The rate of the increasing plasma Hcy concentration was 71.2%.

- Male patient, hemodialysis time ≥ 5 years, loss of residual renal function, diabetes mellitus, smoking has a higher mean plasma Hcy concentration than patients without the above characteristics, $p < 0.05$. Hcy concentration was negatively correlated with HDL-C concentration, $r = -0,301$, $p < 0.01$.

REFERENCES

1. Diem Thi Van, Hoang Trung Vinh. Investigation of plasma Hcy, forlate and vitamin B12 concentrations in patients with regular hemodialysis. Journal of Practical Medicine 2016; 12(1029):71-74.

2. Kobiyama K., Ley K. Atherosclerosis. Circ Res 2018; 123(10):1118-1120.

3. Taleb S. Inflammation in atherosclerosis. Arch Cardiovasc Dis 2016; 109(12):708-715.

4. Wang H., Cui K., Xu K., et al. Association between plasma Hcy and progression of early nephropathy in type 2 diabetic patients. Int J Clin Exp Med 2015; 8(7):11174-11180. eCollection.

5. Chaitanya V., Devi N.H, Suchitra M.M, et al. osteopontin, cardiovascular risk factors and carotid intima-media thickness in chronic kidney disease. Indian J Nephrol 2018; 28(5):358-364.

6. Chen C.H, Yeh E.L, Chen C.C, et al. vitamin b-6, independent of Hcy, is a significant factor in relation to inflammatory responses for chronic kidney disease and hemodialysis patients. Biomed Res Int 2017; 7367831. eCollection.

7. Škovierová H., Vidomanová E., Mahmood S., et al. the molecular and cellular effect of Hcy metabolism imbalance on human health. Int J Mol Sci 2016; 17(10):1733. eCollection.

8. Mathew A.T, Fishbane S., Obi Y., et al. Preservation of residual kidney function in hemodialysis patients: reviving an old concept. Kidney Int 2016; 90(2):262-271.

9. Lei X., Zeng G., Zhang Y., et al. Association between Hcy level and the risk of diabetic etinopathy: A systematic review and meta-analysis. Diabetol Metab Syndr 2018; 10:61. eCollection.

10. Wang Y., Liu J., Jiang Y., et al. HyperHcymia is associated with decreased apolipoprotein AI levels in normal healthy people. BMC Cardiovasc Disord 2016; 16:10. eCollection.