PLASMA HOMOCYSTEINE CONCENTRATION IN PATIENTS WITH TYPE 2 DIABETES

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Abstract
Objective: To investigate the total plasma homocysteine concentration and prevalence of hyperhomocysteinemia in type 2 diabetic patients. **Research design and methods**: A descriptive and cross-sectional study was done in 40 type 2 diabetic patients and in 40 healthy subjects as control group. Selected 40 normoglycemic healthy subjects and 40 patients diagnosed type 2 diabetes. Patients were matched for age, gender. Statistical analyses of data were performed on SPSS, Excel 2003. **Result**: Levels of plasma homocysteine in type 2 diabetic patients and normal subjects were 14.03±7.13µmol/l; 10.35±2.52µmol/l, respectively. The prevalence of hyperhomocysteinemia in type 2 diabetic patients was 25%. **Conclusion**: The plasma homocysteine concentration and prevalence of hyperhomocysteinemia were statistically significantly higher in type 2 diabetic patients than normoglycemic healthy subjects.

Keywords: *type 2 diabetes, hyperhomocysteinemia, plasma glucose*

1. INTRODUCTION

Diabetes is the most common metabolic endocrine disease in which type 2 diabetes accounts for 85% to 90% of patients with diabetes. The cardiovascular complications are the most common and cause of death in diabetic patients. Early detection of complications and prevention of their progression help to prolong life and reduce mortality in patients with diabetes.

In recent years, researchers have focused on risk factors for early onset of complications in patients with type 2 diabetes, including blood levels of homocysteine. Homocysteine (HCY) is emerging as an new independent risk factor, comparable with hypertension, diabetes, obesity, dyslipidemia, inflammatory factors… for the development of the cardiovascular disease for more than the recent 3 decades. Homocysteine is biosynthesized from methionine by the removal of its terminal C^{ε} methyl group**.** [15], [18]

Hyperhomocysteinemia in patients with type 2 diabetes will create a dual risk factor of atherosclerosis, kidney disease, neuropathy, stroke, cardiovascular disease and mortality in patients with type 2 diabetes mellitus. Therefore, early detection of hyperhomocysteinemia will prevent the development of complications and reduce the incidence of motarlity in patients with type 2 diabetes. On the other hand, treatment with folic acid in combination with vitamin B6, B12 can reduce the blood HCY easily, inexpensively. [5], [7], [14], [17]

Because of those reasons, objectives of this research are to:

1. Evaluate blood homocysteine concentration in patients with type 2 diabetes.

2. Determine prevalence of hyperhomocysteinemia in patients with type 2 diabetes.

2. RESEARCH DESIGN AND METHODS

2.1. Study population

This study was carried out at Hue Center Hospital included 40 patients (11 men, 29 women, age 40–81 yrs) with type 2 diabetes mellitus, the control group consisted of 40 healthy subjects (12 men, 28 women, age 40–80 yr). The subjects were all informed about the protocol and their written consent was obtained.

2.2. Exclusion criteria

We exclude all cases who had comorbidities affecting homocysteinemia levels as followings [15], [18]:

- The deficiency of vitamins: folic, B6, B12
- The diseases: hypothyroidism, the carcinoma, chronic kidney disease, systemic lupus erythematosus, psoriasis, malabsorption syndrome, organ transplant cases.
- Current treatment: anticonvulsant, bronchodilators, niacin, fibrates, L-dopa, methotrexate, bile acid resin...

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2.3. Research methodology: descriptive, cross-sectional, case-control study

2.4. Diagnostic criteria for diabetes

The diagnosis of diabetes mellitus was based on a previous history of diabetes or on American Diabetes Association (ADA) criteria [1]:

In the absence of unequivocal hyperglycemia, diagnosis should be confirmed by repeat testing.

• Fasting plasma glucose ≥ 126 mg/dl (7 mmol/l).

• Random plasma glucose ≥ 200 mg/dl (11.1 mmol/l) and classic symptoms of hyperglycemia such as frequent urination, increased thirst, weight loss.

• Two-hour plasma glucose ≥ 200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test.

2.5. Classification of diabetes

Based on WHO criteria apply to Vietnamese as followings [16]:

+ Age onset is usually late, over 40 years of age.

+ Often accompanied with obesity.

+ Occult onset, the clinical symptoms of diabetes appear slowly, not frantic.

+ Insulinemia level may be increased, normal or slightly decreased due to the peripheral insulin resistance.

+ Response to dietary, exercise and hypoglycemia therapy with pills.

2.6. Quantification of homocysteinemia concentrations

HCY blood test is a fluorescence polarization immunoassay which determine HCY levels in plasma or serum on AxSYM machine system of Abbot firm.

Normal fasting HCY concentrations is 5-15 µmol/L. Hyperhomocysteinemia is defined as HCY concentrations > 15 µmol/L [18].

2.7. Statistical method: using SPSS software, Microsoft Excel 2003.

3. RESEARCH RESULTS AND DISCUSSIONS

3.1. The average concentration of homocysteine of the diabetic group and the control group

Table 3.1. The average concentration of homocysteine of the diabetic group and the control group

Group / Hcy	Diabetic group	Control group
n	40	40
Average Hcy concentration (µmol/l)	14.03±7.13	10.35±2.52
p	p < 0.01	

The average concentrations of blood HCY in patients with type 2 diabetes was 14.03 ± 7.13 µmol/l, in the control group was 10.35 ± 2.52 µmol/l. This difference was statistically significant (p <0.05).

Hyperhomocysteinemia has long been proven to be an new independent risk factor for the development of cardiovascular disease, a common complication and cause of death in diabetic patients. Hyperhomocysteinemia increases frequency of complications of diabetes. Hyperhomocysteinemia in patients with diabetes also increases the risk of motarlity more 2-4 times than hyperhomocysteinemia in patients without diabetes. Hyperhomocysteinemia contributes to develop the atherosclerosis process in cerebral, coronary and peripheral vascular. [5], [7], [14]

The results of our study are similar to the findings of other researchers.

Erkan cobalt (Turkish) et al studied HCY levels in 40 patients with type 2 diabetes; 40 patients with impaired fasting blood glucose (IFG) and 40 healthy subjects with results were 14.8 ± 2 µmol/l; 12.4 ± 2.1µmol/l; 11.1 ± 1.4µmol/l espectively, (p <0.001)[8].

Ndrepepa G, Kastrati A (Germany) et al studied 507 patients with type 2 diabetes and 1614 healthy subjects with results 12.4µmol/l (9.9-15.9µmol/l); 11.7µmol/l (9.6 to 14.5 µmol/l) espectively, (p = 0.011) [12].

Masanori Emoto (Japan) et al studied 75 patients with type 2 diabetes and 54 healthy subjects with results 12.0 ± 0.7µmol/l; 8.7 ± 0.3µmol/l, espectively (p <0.0001) [11].

The mechanism for explaining hyperhomocysteinemia in patients with type 2 diabetes are still controversial. However, it can be explained as follows: [14], [15], [17], [18]

- The insulin resistance is a hypothetical explanation for hyperinsulinemica in type 2 diabetes patients. The hyperinsulinemic euglycemic clamp technique demonstrated that there was a significant increase HCY levels in the blood of diabetic patients with insulin resistance.

- Hyperhomocysteinemia in patients with diabetes also be explained by diabetic renal disease:

decreased metabolism and clearance of HCY.

- In addition, several other studies have shown that insulin can affect the activity of some enzymes related to metabolism HCY as cystathionine β synthase (CBS); 5.10- methylenetetrahydrofolate reductase (MTHFR).

- Patients with type 2 diabetes were treated with metformine group can cause a decrease in the absorption of vitamin B12, a vitamin plays an important role in the process of re-methylation of HCY

Blood HCY concentration depends on many factors, including the difficiency of some important vitamins affecting HCY metabolism as folic acid, vitamin B6, B12. Because we did not quantify the concentration of these vitamins in this study, so we could not eliminate this deficiency as other studies, this is a limitation of our research. [17]

Hyperhomocysteinemia in patients with type 2 diabetes would create a dual independent risk factor effecting of increasing the risk of complications such as atherosclerosis, kidney disease, neurology, stroke, cardiovascular disease and mortality in patients with type 2 diabetes.

On the research of Martin Buysschaert, surveying on 122 patients with type 2 diabetes, the prevalence of macrovascular disease in the hyperhomocysteinemia group (20.8 ± 5.1μmol/l) was more than the normal homocysteinemia group (10.2 ± 2.0μmol/l) respectively 70% and 42% (p <0.01); the prevalence of coronary artery disease in the hyperhomocysteinemia group was higher than normal hyperhomocysteinemia group respectively 46% and 21% (p <0.02); the prevalence of diabetic kidney disease in the hyperhomocysteinemia was higher than normal homocysteinemia group respectively 32% and 10% (p <0.005). However, the prevalence of retinopathy in the hyperhomocysteinemia group was higher than normal homocysteinemia group respectively 45% and 42% but p>0.05; the prevalence of neuropathy in the hyperhomocysteinemia group was higher than normal homocysteinemia levels respectively 70% and 59% (p>0.05). [10]

In another study of Alev Eroglu Altinova on 116 subjects with type 2 diabetes have also noted the difference in HCY levels in diabetic kidney disease group compared to the group without diabetic kidney disease was 14.6 ± 8.8μmol/l compared with 9.6 ± 3.5μmol/l (p<0.05); Diabetes group with coronary artery disease compared with diabetes group without coronary artery disease was 13.6 ± 5.5μmol/l compared with 9.6 ± 3.5μmol/l (p <0.05) [3].

In Vietnam, many studies on different subjects also showed significantly higher HCY in patients with coronary atherosclerosis and cerebral. The study of Dang Van Phuoc et al (HCM) showed significantly higher HCY in patients with coronary artery disease. Cao Phi Phong (HCM), Nguyen Duc Hoang (Hue) study showed significantly higher HCY in patients with stroke. [3], [4], [12]

3.2. The prevalence of hyperhomocysteinemia in patients with type 2 diabetes

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Cut off value	Diabetic group		Control group		p
	n	%	n	%	
> ($\overline{X}$ +1SD) = 12.87μmol/l	15	37.50	6	15.00	> 0.05
> 15μmol/l	12	30.00	1	2,50	> 0.05
> ($\overline{X}$ +2SD) = 15.39μmol/l	10	25.00	1	2.50	< 0.05

If the cut off value was selected as the average value of the control group plus one standard deviation ($\overline{X}$ + 1 SD), the cut-off value is 12.87μmol/l (10.35 + 2.52). The percentage of hyperhomocysteinemia in patients with type 2 diabetes was 37.50% and in the control group was 15.00%. However, this difference was not statistically significant (p> 0.05).

If the cut off value was selected as the average value of the control group plus two standard deviation ($\overline{X}$ + 2 SD), the cut-off value was 15.39 μmol/l (10.35 + 5.04). The percentage of hyperhomocysteinemia in patients with type 2 diabetes was 25.00% and in the control group was 2.50%. This difference was statistically significant (p <0.05).

If the cut off point limit was selected as >15μmol/l, the percentage of hyperhomocysteinemia in patients with type 2 diabetes was 30.00% and in the control group was 2.50%. This difference was not statistically significant (p> 0.05).

According to a study of Buysschaert M. et al on 122 patients with type 2 diabetes, the prevalence of hyperhomocysteinemia in patients with type 2

diabetes with cut off value as ($\overline{X}$ + 1 SD) = (10.2 + 2.0) was 31% (p <0.05). [10]

Study of Ellen K. Hoogeveen et al on patients with type 2 diabetes, the prevalence of hyperhomocysteinemia (> 14μmol/l) was 25.8%. [6], [7]

The study of Ilhan Tarkun, Berrin C. Arslan et al on 38 type 2 diabetes patients without cardiovascular complications, the prevalence of hyperhomocysteinemia was 10.5% and on control group (n = 25) was 4%. [9]

Satyendra Kumar Sonka et al (2014) demonstrated epidemiologically and clinically that hyperhomocysteinemia was an independent risk factor of coronary atherosclerotic disease, stroke, peripheral arterial disease and venous thrombosis. [14]

Blood HCY concentration depends on many factors such as age, gender, vitamins, drugs ... It can lower blood levels of HCY by providing folic acid in combination with oral vitamin B group, which is prevention strategies of cardiovascular disease and other complications when having hyperhomocysteinemia. Especially in patients with type 2 diabetes, it is a risk factor of cardiovascular disease, good blood glucose control via HbA1c control may also limit increase of blood HCY. [17]

In our study, we found a significant prevalence of hyperhomocysteinemia in patients with type 2 diabetes. So, it is necessary to control blood HCY concentration in patients with type 2 diabetes to reduce cardiovascular risk and mortality, reduce the frequency of vascular complications, kidney disease, neuropathy, retinopathy... in patients with type 2 diabetes.

According to a study of Touceda Luis A. (2001) on 12 cases of hemodialysis while be infusing high-dose folic acid 40 mg/day, followed up for 64 days and measured the blood HCY concentration in the moment of 0 th, 21st, 42nd, 64th days showed that blood HCY concentrations statistically significantly decreased, the first day was 33.2 ± 15.8μmol/l; day 21st was 21.6 ± 8,2μmol/l, p <0.02; Day 42nd was 15.8 ± 13.2μmol/l, p <0.02; Day 64th was 10.6 ± 7.5μmol/l, p<0,001. Authors concluded that the use of high-dose folic acid was an effective method to reduce blood HCY.

A study of Bantwal Suresh Baliga et al on type 2 diabetes patients with hyperhomocysteinemia treated folic acid 15 mg/day, pyridoxine 600 mg/day showed that blood HCY concentration significantly decreased from 2.9 - 12, 3 μmol/l to 1.1 - 9.1 μmol/l (p <0.01). After treatment with methionine, blood HCY concentrations decreased from 11.4 - 39.9 μmol/l to 6.5 - 30.4 μmol/l (p, 0.05). According to a randomized study of Alcyonis on 1114 subjects of 12 study has showed that folic acid supply with dose of 0.5 to 5 mg/day and vitamin B12 with dose of 0.5 mg/day will reduce 1/4 to 1/3 blood HCY levels. Recomendations for treating hyperhomocysteinemia should be considered causes as well as health status of individuals. Supply of folic acid alone or combine with vitamin B6, B12 help to reduce homocysteine concentration but the optimal dose remains unclear, the dose of this vitamin for the prevention and treatment of blood HCY is also controversial. [17]

4.CONCLUSIONS

This study was carried on 40 patients with type 2 diabetes and on 40 healthy subjects, we have some results as followings:

- The average concentration of blood HCY in patients with type 2 diabetes was 14.36 ± 7.13 μmol/l and in 40 healthy subjects was 10.35 ± 2.52 μmol/l (p <0.05).
- The prevalence of hyperhomocysteinemia in patients with type 2 diabetes with cut off value as $\overline{X}$ + 2 SD was 25% (p <0.05).

REFERENCES

1. American Diabetes Association (2014), Standards of Medical Care in Diabetes, Diabetes Care 2014; 37 (S1): S14-80.
2. Alev Eroglu Altinova (2003). Homocysteine Levels in Type 2 Diabetic Patients with Nephropathy and Coronary Artery Disease. Turkish Journal of Endocrinology and Metabolism, (2003) 4 : 159-164.
3. Cao Phi Phong (2004). The correlation between the elevated serum homocysteine levels and cerebral infarction patients. Journal of Ho Chi Minh Neurology.
4. Dang Van Phuoc et al (2003). Homocysteine and Coronary artery disease. Journal of Ho Chi Minh practical medicine.
5. Ebesunun MO. et al (2012). Elevated plasma homocysteine in type 2 diabetes mellitus: a risk factor for cardiovascular diseases. Pan Afr Med J; 12: 48.
6. Ellen K. Hoogeveen et al (1998). Hyperhomocysteine is associated with an increased risk of cardiovascular disease, especially

in non-insulin-dependent diabetes. American heart disease, vol 18, p133-138.
7. Ellen K. Hoogeveen et al (2000). Hyperhomocysteinemia increases risk of death in type 2 diabetes. American heart disease, vol 101, p1508.
8. Erkan coban et al (2005). Levels of homocysteine in Subjects with impaired fasting glucose and diabetes. Turkish journal of endocrinology and metabolism, volume 9, number 1, p5-8.
9. Ilhan Tarkun, Berrin C. Arslan (2003). Homocysteine Concentrations in Type 2 Diabetes Mellitus Patients Without Cardiovascular Disease: Relationship to Metabolic Parameters and Diabetic Complications. Turkish Journal of Endocrinology and Metabolism, (2003) 1 : 11-17.
10. Martin Buysschaert et al (2000). Hyperhomocysteine in type 2 diabetes: Relationship to macroangiopathy, nephropathy and insulin resistence, Diabetes care, vol 23, number 10, p1816-1822.
11. Massanori Emoto et al (2001). Impact of Insulin Resistance and Nephropathy on Homocysteine in Type 2 Diabetes. Diabetes Care 24:533–538, 2001.
12. Ndrepepa G. Kastrati A(2006). Circulating homocysteine levels in patients with type 2 diabetes mellitus. Nutritional metabolic cardiovascular disease. Lazarettstrasse 36, 80636, Munich, Germany.
13. Nguyen Duc Hoang (2007). Research on serum Homocysteine level in cerebral infarction patients, PhD thesis in medicine at Hue College of Medicine and Pharmacy.
14. Satyendra Kumar Sonka et al (2014). Plasma Homocysteine level and its clinical correlation with type 2 diabetes mellitus and its complications. International Journal of Diabetes in Developing countries, March 2014, vol 34, Issue 1, pp 3-6.
15. Tao Huang et al (2013). Association of homocysteine with type 2 diabetes: a meta-analysis implementing Mendelian randomization approach. BMC Genomics 2013, 14: 867.
16. Tran Huu Dang, Nguyen Hai Thuy et al (2007). Endocrinology and diabetes. Book for postgraduate, Hue College of Medicine and Pharmacy.
17. Yiqing Song, Nancy R. Cook, et al (2009), Effect of Homocysteine-lowering Treatment with Folic Acid and B Vitamins on Risk of Type 2 Diabetes in Women: A Randomized, Controlled Trial. Diabetes. 2009;58(8):1921-1928.
18. Wijekoon EP et al (2007). Homocysteine metabolism in diabetes. Biochem Soc Trans 2007 Nov; 35 (Pt5): p 1175-9.