# SERUM TOTAL HOMOCYSTEINE LEVELS AND METABOLIC CONTROL IN PATIENTS WITH TYPE 2 DIABETES

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## Abstract

Serum total homocysteine levels (tHcy), an emerging risk factor for cardiovascular disease, and its relation to metabolic control in patients with type 2 diabetes mellitus were determined. Sample population was 135 patients with type 2 diabetes without any diabetic complications who attended the diabetic clinic at Pathumthani Hospital and 79 non-diabetics from the population in Pathumthani. Fasting blood samples were obtained in order to determine the level of tHcy, glycated hemoglobin, glucose and lipid profile. Regarding to the degree of glycemic control assessed by hemoglobin A1c (HbA1c), type 2 diabetics were defined to good or fair control (HbA1c ≤ 8.0%, n = 67) and poor control (HbA1c > 8.0%, n=68). The correlations between tHcy levels and glycated hemoglobin and lipid levels were investigated. The results indicated that serum tHcy levels were significantly higher in patients with type 2 diabetes than in the non-diabetes. Eleven percent (15 of 135) of these patients had moderate hyperhomocysteinemia (15-30 μmol/l). There were no significant differences of tHcy levels in the subgroups of type 2 diabetes with regarding to glycemic control. Amongst patients with type 2 diabetes, correlation analysis demonstrated that there was a little significantly correlation between tHcy levels and age ($r = 0.39$: $p < 0.001$) and there was not significantly correlated with both hemoglobin A1c and lipid levels. In conclusion, increased serum tHcy levels were found in type 2 diabetic patients without any diabetic complications as compared with non-diabetic subjects. Improved glycemic control did not reduce tHcy levels and there were no associations between tHcy levels and metabolic control.

**Key words:** Total homocysteine, Glycated hemoglobin, Type 2 diabetes

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# ระดับซีรัมโฮโมซิสทินและการควบคุมเมตาบอลิสม ในผู้ป่วยเบาหวานแบบที่ 2

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## บทคัดย่อ

ตรวจหาระดับซีรัมโฮโมซิสทินซึ่งเป็นปัจจัยเสี่ยงปัญหาใหม่ของโรคหัวใจและหลอดเลือด และศึกษาความสัมพันธ์ระหว่างซีรัมโฮโมซิสทินกับการควบคุมเมตาบอลิสมในผู้ป่วยเบาหวานแบบที่ 2 กลุ่มศึกษาเป็นผู้ป่วยเบาหวานแบบที่ 2 ที่ไม่มีภาวะแทรกซ้อนของโรคเบาหวานซึ่งมารับการตรวจที่คลินิกเบาหวาน โรงพยาบาลปทุมธานี และผู้ที่ไม่เป็นโรคเบาหวานซึ่งอาศัยอยู่ในเขตจังหวัดปทุมธานีจำนวน 135 ราย และ 79 รายตามลำดับ เก็บตัวอย่างเลือดหลังอดอาหารเพื่อตรวจหาระดับซีรัมโฮโมซิสทิน ไกลเคทฮีโมโกลบิน กลูโคส และไขมันชนิดต่าง ๆ จัดแบ่งผู้ป่วยเบาหวานแบบที่ 2 ตามการควบคุมระดับน้ำตาลในเลือดโดยใช้ระดับฮีโมโกลบินเอ วัน ซี เป็นเกณฑ์ตัดสิน ได้เป็น 2 กลุ่ม คือ กลุ่มผู้ป่วยเบาหวานที่ควบคุมระดับน้ำตาลในเลือดได้ดีหรือพอใช้ (ฮีโมโกลบินเอ วัน ซี ≤ 8.0% จำนวน 67 ราย) และกลุ่มผู้ป่วยที่ควบคุมระดับน้ำตาลในเลือดไม่ดี (ฮีโมโกลบินเอ วัน ซี > 8.0% จำนวน 68 ราย) หาความสัมพันธ์ระหว่างระดับซีรัมโฮโมซิสทินกับการควบคุมระดับน้ำตาลในเลือดและระดับไขมันชนิดต่าง ๆ ผลการศึกษาพบว่าระดับซีรัมโฮโมซิสทินในผู้ป่วยเบาหวานสูงกว่าผู้ที่ไม่เป็นโรคเบาหวานอย่างมีนัยสำคัญ โดยร้อยละ 11 (15 ใน 135 ราย) ของผู้ป่วยเบาหวานมีซีรัมโฮโมซิสทินสูงในระดับปานกลาง (15-30 ไมโครโมล/ลิตร) ผู้ป่วยเบาหวานที่มีการควบคุมระดับน้ำตาลได้ดีหรือพอใช้ มีระดับโฮโมซิสทินไม่ต่างกับผู้ป่วยเบาหวานที่มีการควบคุมระดับน้ำตาลได้ไม่ดี ระดับโฮโมซิสทินมีความสัมพันธ์เล็กน้อยกับอายุ ($r = 0.39$, $p < 0.001$) และไม่มีความสัมพันธ์กับระดับฮีโมโกลบินเอ วัน ซี และระดับไขมันชนิดต่าง ๆ การศึกษานี้แสดงว่าผู้ป่วยเบาหวานแบบที่ 2 ที่ไม่มีภาวะแทรกซ้อนของโรคเบาหวานมีระดับซีรัมโฮโมซิสทินสูงกว่าผู้ไม่เป็นโรคเบาหวาน การควบคุมระดับน้ำตาลในเลือดได้ไม่มีผลทำให้ระดับซีรัมโฮโมซิสทินลดลง และระดับซีรัมโฮโมซิสทินในผู้ป่วยเบาหวานแบบที่ 2 ไม่สัมพันธ์กับการควบคุมเมตาบอลิสม

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## Introduction

Diabetes mellitus (DM) is an important health problem worldwide. It is projected that the number of individuals with DM will rise to 300 million in 2025. The greatest increase is projected for economically developing countries, such as Thailand, that already account for approximately two-thirds of all individuals with DM[1]. The estimated national prevalence of DM in Thai adults has been reported to be 9.6 % (2.4 million people)[2]. Most of the diabetics (> 95 %) are type 2[3]. Cardiovascular diseases (CVD), the most common and clinical important complications, are a major cause of morbidity and mortality in type 2 DM[4]. The mechanisms by which DM increase vascular risks observed in type 2 are still not completely understood. Elevated levels of total and LDL cholesterol, triglyceride, lipoprotein (a) and decreased levels of HDL cholesterol are well-known CVD risk factors[5]. Recently, homocysteine which is a thiol containing amino acid produced by the intracellular demethylation of methionine has been emerged as a novel independent risk factor for the development of CVD[6]. The association of hyperhomocysteinemia with an increased risk for the premature development of atherosclerotic CVD in general population has been identified by several studies[7-8]. Amongst type 2 DM, plasma total homocysteine (tHcy) levels in patients with macrovascular complications have been reported to be higher than in those without and in healthy non-diabetic controls[9]. Each 5 μmol/l increment of tHcy after adjustment for the others known CVD risk factors has found to increase the risk of coronary events by 28 % with the hazard ratio of 1.28[10]. For microvascular complications, the relation between an increased serum tHcy levels with the severity of retinopathy, nephropathy and peripheral arterial disease in type 2 DM has also been reported[11-13]. However, control of hyperglycemia in type 2 DM is mandatory for the prevention of development of both macro and micro-vascular complications[14]. Current recommendation for glycemic control includes reducing fasting glucose to near normal with a hemoglobin A1c of less than 7%[15]. In addition, a 3-year follow-up study in type 2 DM patients has also shown that plasma tHcy levels decrease even with modest improvement of glycemic control[16]. Therefore, the aims of this study are to determine serum tHcy levels and evaluate its relation to metabolic control in patients with type 2 diabetes without any vascular complications.

## Materials and methods

### Subjects

Patients were comprised of 135 type 2 diabetes without any diabetic complications, who attended the diabetic clinic at Pathumthani Hospital during June to August 2004. Type 2 diabetes was diagnosed according to the World Health Organization definition: those having fasting plasma glucose > 7.0 mmol/l (126 mg/dl) or taking antidiabetic medications[17]. Non-diabetics consisted of 79 healthy individuals of whom were from the general population in Pathumthani. They had normal fasting plasma glucose (< 7.0 mmol/l) and hemoglobin A1c (4.3 to 5.9 %), normal hematological and liver/renal function tests and no history of diabetes mellitus and coronary artery disease. Exclusion criteria for both groups were individuals with pregnancy or having liver/renal pathology or cancer or thyroid disease or taking lipid-lowering drugs (fibric acid derivative and gemfibrosil) or group B vitamins supplement. Informed consents were obtained from all participants. Measurements of body weight, height and blood pressure were taken. Body mass index was calculated from the weight in kilograms divided by the height in metres squared. Hypertension was defined as a blood pressure greater than 140/90 mmHg or a history of taking antihypertensive medications[18]. All patients were asked to complete the questionnaires including information on smoking habit, medications used and duration of diabetes.

### Blood collection and biochemical determinations

Blood samples were drawn into EDTA, NaF and no anticoagulant tubes from the antecubital vein in the morning after an overnight fast. Plasma and serum were separated by centrifugation at 3000 r.p.m. for 10 minutes. Glucose, hemoglobin A1c, lipids levels were determined immediately. The sera for total homocysteine determination were stored at -20°C for 2 weeks. Serum total homocysteine levels were determined by a fluorescence polarization immunoassay technique using a commercial reagent on an automated IMx analyzer (Abbott Laboratories, Illinois, USA)[19]. Internal control samples at three concentrations of tHcy levels (7.0, 12.5 and 25 μmol/l) were used for an individual run. The inter-assay coefficients of variation [CVs] were 4.6%, 3.3% and 4.5 % at 7, 12.5 and 25 μmol/l of tHcy, respectively. External quality assessment samples were purchased from the College of American Pathologists (CAP) surveys and anatomic pathology education programs. The evaluated results were within the acceptable limits at all tHcy levels. Hemoglobin A1c in EDTA whole blood was measured by the high performance liquid chromatography method on

the Hb C 723 G 7 (Tosoh corporation, Chuo-ku, Tokyo) using the calibrator certified by the National Glycohemoglobin Standardization Program (NGSP) and anchored to the DCCT reference method[20]. The inter-assay CVs were 0.85 % and 0.92 % at 5.6 % and 10.1 % of HbA1c levels, respectively. Plasma glucose, serum total and HDL cholesterol and triglyceride concentrations were analyzed by enzymatic methods using commercial reagents (Ecoline, Merck, Germany). All of these tests were performed on an automated chemistry analyzer (Merck, Mega, Germany). LDL cholesterol levels were calculated from the Freidewald's equation[21]. The inter-assay CVs of these tests were less than 5%. The external quality control samples for these tests were also purchased from the CAP proficiency testing programs and the evaluated results were within the acceptable limits.

**Statistical analyses**

Data were expressed as mean ± SE for continuous variables with normal distribution or median and interquartile range for variables with skewed distribution or geometric mean for variables with markedly skewed distribution after ln-transformed or percentage. Comparisons between groups were performed with Student's unpaired-*t*-test or Mann-Whitney U test or $\chi^2$- test for categorical variables. The associations of serum tHcy levels with glycemic control index and lipid levels were assessed by Pearson's and Spearman's correlations. Two tailed p values less than 0.05 were considered significant. All analyses were performed using MINITAB statistical program for Windows (release 13; Minitab Inc, Philadelphia).

Serum tHcy level of 16 μmol/l corresponded to the 95$^{th}$ percentile of the distribution in non-diabetic group was the cut-off point for hyperhomocysteinemia.

## Results

The characteristics and biochemical data of patients and control subjects were presented in Table 1. There were significant differences of age, percentage of current cigarette smokers, FPG, HbA1c, TG and HDL-C levels but no significant differences of gender ratio, BMI, systolic blood pressure, diastolic blood pressure, percentage of hypertension, TC and LDL-C levels between the two groups. Of all type 2 DM, 10 (7.4 %) were newly diagnosed and 125 (92.6 %) were known to have diabetes and 96 % were treated with oral hypoglycemic agent (metformin), 2 % with insulin and 2 % with both oral hypoglycemic agent and insulin. The median known duration of diabetes of subjects in

whom type 2 DM had previously been diagnosed was 7 years with an interquartile range of 3 to 10 years.

**Table 1** Characteristics and biochemical data of study subjects

| Characteristics | Type 2 DM | Non-diabetics | p-value | 95 % CI |
|---|---|---|---|---|
| Number of subjects | 135 | 79 | | |
| Gender, female/male | 104/31 | 64/15 | 0.494 | |
| Age[a], years | 58.72 ± 0.91 | 54.14 ±1.37 | 0.004 | 1.45 to 7.70 |
| (min. - max.) | (27 - 81) | (28 - 82) | | |
| BMI[a], kg/m$^2$ | 26.32 ± 0.38 | 25.29 ± 0.44 | 0.096 | -0.18 to 2.26 |
| Blood pressure[b], mmHg | | | | |
| Systolic | 130 (120-140) | 130 (120-140) | 0.333 | |
| Diastolic | 80 (75-80) | 80 (70-80) | 0.381 | |
| Hypertension, % | 11.9 | 13.9 | 0.660 | |
| Cigarette smoker, % | 20.7 | 7.6 | 0.011 | |
| Biochemical data | | | | |
| FPG[c], mmol/l | 8.61 | 5.05 | < 0.001 | |
| HbA1c[c], % | 7.99 | 5.30 | < 0.001 | |
| Lipid levels, mmol/l | | | | |
| TC[a] | 5.78 ± 0.10 | 5.96 ± 0.13 | 0.262 | -0.50 to 0.14 |
| TG[c] | 1.86 | 1.35 | < 0.001 | |
| LDL-C[a] | 3.52 ± 0.08 | 3.75 ± 0.11 | 0.077 | -0.52 to 0.03 |
| HDL-C[c] | 1.32 | 1.50 | < 0.001 | |

*[a]Mean ± SE, [b]Median (Interquatile range), [c]Geometric mean*

*BMI: Body mass index, FPG: Fasting plasma glucose, HbA1c: hemoglobin A1c, TC: Total cholesterol, TG: Triglyceride. LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol*

The serum tHcy level in type 2 DM was significantly higher than those in the control subjects (mean ± SE; 12.01 ± 0.30 *vs* 10.07 ± 0.33 μmol/l; p = 0.046 and 95 % CI; 0.019 to 1.857) as shown in Table 2. Hyperhomocysteinemia (tHcy > 16 μmol/l) was found in 15 of 135 diabetics. Good and fair degree of glycemic control (HbA1c < 8 %) and poor glycemic control (HbA1c > 8 %) were no significant differences of serum tHcy levels (12.23 ± 0.42 *vs* 11.78 ± 0.43 μmol/l, p = 0.463) (Table 2).

Pearson's correlation coefficients between serum tHcy levels and index of glycemic control and other CVD risk factors in type 2 DM patients were shown in Table 3. Serum tHcy levels were significantly correlated positively with age (r = 0.39, p < 0.001) and

negatively with BMI (r = -0.25, p = 0.003) but were not significantly correlated with systolic and diastolic blood pressure, diabetes duration, hemoglobin A1c, serum total and LDL cholesterol, triglyceride and HDL cholesterol levels (r = 0.09, -0.05, 0.12, -0.07, 0.14, 0.16, 0.11 and 0.11, respectively; p > 0.05).

**Table 2** Serum total homocysteine levels of the study group

| Study group | Homocysteine levels[a] (μmol/l) | p-value | 95 % CI |
|---|---|---|---|
| Non-diabetics (n = 79) | 10.07 ± 0.33 | | |
| Type 2 DM (n = 135) | 12.01 ± 0.30* | 0.046 | 0.019 to 1.857 |
| Glycemic control | | | |
| Good and fair (HbA1c < 8 %) (n = 67) | 12.23 ± 0.42 | | |
| Poor (HbA1c > 8 %) (n = 68) | 11.78 ± 0.43** | 0.463 | -0.17 to -0.06 |

[a] *Mean ± SE*

**Unpaired-t-test when compared to controls (non-diabetics)*

***compared to type 2 DM with good and fair glycemic control*

**Table 3** Correlation between serum total homocysteine levels and index of glycemic control and other cardiovascular risk factors in type 2 diabetes

| Variables | Homocysteine | |
|---|---|---|
| | Correlation coefficients (r) | p-value |
| Age | 0.39 | < 0.001 |
| Body mass index | -0.25 | 0.003 |
| Systolic blood pressure | 0.09 | 0.282 |
| Diastolic blood pressure | -0.05 | 0.594 |
| Duration of diabetes | 0.12 | 0.157 |
| ln HbA1c | -0.07 | 0.335 |
| Total cholesterol | 0.14 | 0.114 |
| LDL cholesterol | 0.16 | 0.065 |
| ln Triglyceride | 0.11 | 0.217 |
| ln HDL cholesterol | -0.11 | 0.225 |

## Discussion

This study was conducted to determine serum tHcy levels in type 2 DM Thai patients without any diabetic complications and assess its relation to metabolic control and other known CVD risk factors. The higher ratio of females than males among cases was studied

because of higher prevalence of type 2 DM found in women[3]. The diabetics were rather older than non-diabetics. A significantly increased mean serum tHcy level in type 2 DM compared to those in non-diabetics was observed. Our results are in line with those of the other studies[13,22], whereas no significant difference of plasma tHcy levels between smaller sample size of patients with type 2 diabetes without vascular complications and healthy subjects had been reported[23,24]. Hyperhomocysteinemia was seen in only 11 % of the patients. The lower prevalence of hyperhomocysteinemia in our patients had been found when compared to the other studies using the same or different-tHcy cut-off points[23,25]. Additionally, a graded CHD mortality risk associated with and an elevated plasma tHcy levels was classified by the American Heart Assocation's Statement[26]. Both of them had moderate hyperhomocysteinemia, none had intermediate or severe hyperhomocysteinemia. Furthermore, Goldstein and colleagues have reported higher prevalence of hyperhomocysteinemia (> 15 μmol/l) found in type 2 DM patient with retinopathy than those without retinopathy[23]. Moreover, many prospective studies dealing with a possible relation between hyperhomocysteinemia and vascular complications in type 2 diabetic patients have appeared over the past few years. These results have indicated that moderately elevated plasma tHcy levels are independently related to an increased incidence of fatal and nonfatal CHD events[11,27]. Hyperhomocysteinemia has also been reported to be a risk factor for microvascular complications in type 2 diabetes.[9,12]

The mechanism by which homocysteine promotes atherothrombosis in type 2 DM are proposed by several studies[28,29]. Hyperhomocysteinemia causes endothelial dysfunction by increasing oxidative stress and decreasing the release of nitric oxide resulting in an impaired vasodilatation[30]. An excess of homocysteine stimulates smooth muscle cell proliferation and collagen synthesis promoting intima-media thickening[31]. Hyperhomocysteinemia also causes abnormalities in the coagulation system by increasing platelet aggregation[32].

Regarding to the degree of glycemic control, our findings showed the similar serum tHcy levels in a subgroup of type 2 DM patients with poor control and those with good and fair control. The results have indicated no effect of degree of glycemic control on serum tHcy levels. However, a conflicting result has shown higher serum tHcy level in 26 poorly-controlled type 2 DM patients with no symptom of any cardiovascular diseases than those in 18 well-controlled patients[33].

In relation to metabolic control, serum tHcy levels positively correlated with age and negatively correlate with body mass index but did not correlate with blood pressure, diabetes duration, glycated hemoglobin and lipid levels. These results have indicated that serum tHcy

level increases with ages, decreases when BMI increases and is an independent of other major CVD risk factors in type 2 DM. Results from previous studies have been conflicting, ranging from no association or weak association to strong association between serum tHcy levels and glycemic control and other major CVD risk factors[22,34,35]. These discrepancies may be related to the differences in the heterogeneity of patients' characteristics and the methodologies used.

In addition, there are other factors affecting homocysteine metabolism. A mutation of C677T gene appears to have an impaired activity of 5, 10 methylenetetra-hydrofolate reductase which is a key enzyme in methionine-homocysteine metabolism leading to an exaggerated hyperhomocysteinemia[36]. Certain medications such as lipid- lowering drugs (fibric acid derivative and gemfibrosil) increase serum tHcy levels[37,38]. In our study, most patients have taken metformins and statins. None has taken fibrate and gemfibrosil because of no availability for use. Both drugs have no effects on homocysteine metabolism[37-39]. Therefore bias from drug treatment has not occurred.

Strengths of our study compared with prior studies include (1) an adequate sample size for each study group (2) fasting specimens used (3) an accurate and precise method for tHcy, HbA1c determination used (4) standard protocol for diagnosis of DM and classification of glycemic control used. However, the limitations are (1) not age and sex matched for non-diabetic group, (2) no assessment for folate, B6 and B12 vitamin status, given that effective metabolism of homocysteine requires an adequate supply of these vitamins. However, our study suggests that hyperhomocysteinemia in patients with type 2 diabetes may be an important modifiable CVD risk factor; consequently it is presumed that studies on tHcy in patients with vascular complications must be confirmed and the tHcy reduction for preventing the development of atherosclerosis in type 2 DM patients need further investigation.

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