Effect of Metformin on Plasma Homocysteine, Vitamin B_{12} and Folic Acid: a Cross-Sectional Study in Patients with Type 2 Diabetes Mellitus

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Objective : *To determine the effect of metformin on the levels of plasma homocysteine (Hcy), serum vitamin B_{12} and folic acid in patients with type 2 diabetes mellitus and the relationship between cumulative metformin exposure and levels of plasma homocysteine (Hcy).*

Material and Method : *The cross sectional study was conducted to assess the effect of metformin treatment on plasma homocysteine (Hcy), serum vitamin B_{12} and folic acid in 152 type 2 diabetic out-patients aged between 35-65 years old at the Diabetes Clinic of The Makaruk Hospital, Kanchanaburi, Thailand. Among those, 88 and 64 patients were categorised to the metformin and non-metformin group according to their records of receiving metformin treatment for a period of a minimal 6 consecutive months before the study. Fasting blood was drawn from each patient and analysed for plasma homocysteine using the Fluorescence Polarization Immunoassay (FPIA) method (IMX Analyzer), and serum vitamin B_{12} and folic acid using the radioimmunoassay method.*

Results : *The plasma Hcy levels showed no significant difference ($p = 0.544$) among patients in the metformin group compared with those in the non-metformin group (10.6$\pm$5.8 mol/L vs 10.4 $\pm$ 4.0 mol/L). There was a significant difference ($p = 0.011$) in the levels of serum vitamin B_{12} among patients in the metformin group and among those in the non-metformin group (318.0 $\pm$ 192.2 pg/mL vs 434.3 $\pm$ 300.7 pg/mL). However, there was no significant difference ($p = 0.090$) in serum folic acid levels between patients in the metformin and those in the non-metformin group (8.8 $\pm$ 5.1 ng/mL vs 7.7 $\pm$ 3.8 ng/mL). The plasma Hcy levels showed a significant correlation with the duration of metformin treatment ($p = 0.014$) and the amount of metformin received ($p = 0.015$) with the Spearman correlation coefficient of 0.260 and 0.258 respectively.*

Conclusion : *Even though the direct effect of metformin treatment on the plasma Hcy could not be concluded from the present study, it was found that there was a significant depletion of level of serum vitamin B_{12} among patients who had been on long-term metformin treatment. Therefore, vitamin B_{12} supplement is suggested for diabetic patients who are receiving metformin medication.*

Keywords : *Metformin, Homocysteine, Vitamin B_{12}, Folic Acid, Diabetes Mellitus*

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Metformin is the only one of the biguinide family commercially available both in the United States and in Thailand. Phenformin, another agent in the same family, was withdrawn from the market in the 1970s because of an association with a fatal risk of lactic acidosis[1]. The major mechanisms of metformin are suppressing hepatic glucose production, and to a lesser extent, augmenting insulin-mediated glucose uptake and enhancing insulin responses in peripheral tissues. Furthermore, it was shown that it also reduces

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glucose absorption in the intestine. In addition to lowering blood glucose concentrations, metformin also has a benefit on lowering plasma lipid concentrations and promoting weight loss[2].

In most developed countries, cardiovascular diseases are still the major causes of death in elderly groups regardless of diabetes underlying in their records. However, there was a two to four times greater incidence of myocardial ischaemia and cerebrovascular diseases in the diabetic group than those in non-diabetic[3-5]. Findings in these studies supported a sixteen-year cohort study of Framingham, indicating that there was an increase in both morbidity and mortality from all cardiovascular causes in the diabetic group[6]. There are a large number of risk factors for cardiovascular diseases. Smoking, hypertension and dyslipidemia are the most common major risk factors classified as "classic risk factors". Meanwhile, age and sex are other risk factors and are sometimes called "non-modifiable factors". Neither independent classic nor non-modifiable risk factors could explain a sporadic incidence of developing common complications from diabetes (such as myocardial infarction, cerebrovascular diseases, and other vascular diseases). Therefore, this may imply that there might be more factors involved in the pathogenesis of such complications in diabetes. Among these, an elevation of homocysteine (Hcy) level in plasma or serum has been in focus[7].

Epidemiological studies have shown an association between a moderate elevation of plasma Hcy, which was highly prevalent in the general population, and an increased risk for fatal and nonfatal cardiovascular diseases. This association was usually independent from the most classic cardiovascular risk factors and consistent with significance of biological plausibility[8-10].

According to the metabolic pathway of Hcy, it is clearly demonstrated that folic acid, vitamin B_6 and B_{12} influenced the level of Hcy. Prolonged administration of metformin induced a decreasing of vitamin B12 absorption, indicated by the lowering of serum vitamin B_{12}, found in previous studies[11]. Furthermore, a report from Berchtold *et al*[12] revealed a significant decline in the level of serum folic acid after having metformin for a continuous period of 2 months. Voluntary administration of metformin for a period of 12 to 40 weeks increased the level of Hcy in non-diabetic male patients who were suffering from coronary heart disease[13]. There is one cross-sectional study showed that metfornin have small effect on Hcy in type 2 diabetes mellitus[14]. Furthermore, a study in indigenous Australians, a high risked ethnic population for diabetes, found levels of Hcy increased significantly among the patients who were on metformin[15].

Metformin is one of the most common prescribed oral antihyperglycemic drugs for patients with type 2 diabetes mellitus. The consequence of long-term metformin treatment among the Thai population, primarily affecting the level of vitamin B_{12} and folic acid, which may subsequently influence Hcy levels, has not been studied before. The present study was designed to collect cross-sectional data from patients with type 2 diabetes mellitus who have been on metformin till the time of study and to investigate the effect of metformin on the level of plasma Hcy, folic acid and vitamin B_{12}, and the relationship between the duration and amounts of metformin and the levels of plasma Hcy in patients with type 2 diabetes mellitus.

Material and Method

The study was conducted as a cross sectional study to assess the effect of metformin treatment on plasma Hcy, serum vitamin B_{12} and folic acid levels.

Patients

The patients were selected from clinically proved type 2 diabetic out-patients who attended the Diabetes Clinic of The Makaruk Hospital, Kanchanaburi. All patients were classified into two groups, namely metformin and non-metformin, according to their medical records of metformin treatment on the commencing date of study. The metformin group referred to patients who had a record of receiving metformin treatment for a period of a minimum of 6 consecutive months. Meanwhile, the non-metformin group referred to those who had no records of receiving metformin treatment. Inclusion criteria were the patients with type 2 diabetes mellitus who had received antihyperglycemic agents for at least 6 months and the age was between 35 to 65 years old. The patients, who either were previously diagnosed or had records of the following conditions, were excluded from this study: pregnancy, thyroid diseases, malignancy, gastrectomy, hypertension or taking anti-hypertensive medications, medications for treatment of cerebrovascular and/or coronary heart diseases, receiving folate antagonist medication (*i.e.*

methotrexate), phenytoin and/or carbamazepine medications.

According to the inclusion and exclusion criteria, 232 type 2 diabetic out-patients were preliminary assessed for recruitment but only 152 patients were enrolled in the study. The present study was approved by the Makaruk Hospital Ethics Committee. All patients gave their voluntary consent.

Methods

Patients were asked to fast overnight (*i.e.* about 8-12 hours) prior to their appointment at the Diabetes Clinic. The following morning, 6 mL of blood was drawn from a forearm site of each candidate and placed equally into 2 separate tubes. Blood in the first tube, containing sodium fluoride, was thoroughly mixed and centrifuged. The plasma sample was taken and immediately proceeded for analysis of blood sugar. Meanwhile, 2 ml of the plasma sample was kept separately in a -20 C freezer for further analysis of plasma Hcy. In the second tube, serum was prepared and immediately proceeded for analysis of creatinine, cholesterol and triglyceride. Similar to the plasma sample, another 2 mL of serum was saved in a -20 C freezer for further analysis of vitamin B_{12} and folic acid. All frozen plasma and serum samples were stored for a period of 2-5.5 months before performing a laboratory analysis. Blood pressure and weight were measured on the day that blood was drawn. The demographic data (*i.e.* history of medi-cations, sex, age, smoking habit, menopause) was collected from combined sources of medical records, computer database for medication, and individual interview with patients.

Laboratory analysis

The plasma Hcy was measured using the Fluorescence Polarization Immunoassay (FPIA) method (Abbott IMX assay) at the Faculty of Pharmacy, Silpakorn University. The serum vitamin B_{12} and folic acid were respectively measured by ^{57}Co and ^{125}I radioimmunoassay method (Dual count, Solid Phase, No Boil Assay Kit, DPC, USA), at the Department of Tropical Nutrition and Food Science, Faculty of Tropical Medicine, Mahidol University.

Meanwhile, the plasma glucose, serum creatinine, cholesterol and triglyceride were analyzed using the spectrophotometry method (Hitachi 902 Automatic Analyzer, Boehringer Manheim, Germany) at the Makaruk Hospital, Kanchanaburi.

Statistical Analysis

Results are expressed as mean $\pm$ SD otherwise stated. A non-parametric method, Mann-Whitney U test, was used to compare non-normally distributed variables between the two groups, while a non-parametric method Chi-square test was used to compare the categorical variable between the two groups. Since the Hcy levels were not normally distributed, Spearman correlation was used to determine the relationship between Hcy levels and some variables. The level of significant of all tests was 0.05 (two-sided). All statistical tests were performed using SPSS software.

Results

After the preliminary assessment for anticipated patients, the initial 232 patients with type 2 diabetes mellitus were included as previously described. However, before commencing of the study, 11 patients refused participation. In addition, for the whole period of this study, 44 patients were absent from their appointments and another 25 patients were excluded from the list. Among those excluded were 9 patients for having recently been on metformin less than a period of 6 months, 4 for having anti-hypertensive medications, 3 for having thyroid disorder medications, 5 for having ischaemic heart disease medications and 1 each for having congestive heart failure, psychotic disorder, cerebrovascular attack and transient ischaemic attack, respectively. The final total number was 152 patients throughout the study.

Among those 152 patients, 88 (17 male and 71 female) and 64 (20 male and 44 female) were, respectively, assigned to metformin and non-metformin groups according to their recent record of metformin therapy. There were 51 and 34 menopause, and 8 and 5 current smokers, among those patients in the metformin and non-metformin groups, respectively (Table 1).

Demographic and clinical characteristic data between metformin and non-metformin group revealed no significant differences in sex, percentages of menopause, age, current smoking habit, weight, systolic blood pressure (SBP) and diastolic blood pressure (DBP). The duration of diabetes, which describes the period since being diagnosed until the time of study, was significantly different between the metformin group and the non-metformin group ($p <$ 0.001). The daily metformin doses ranged between 500 mg to 3000 mg (data not shown).The amount of metformin was calculated from a multiplication of

Table 1. Summary of demographic and clinical characteristic data of the patients

	Metformin group	Non Metformin group	p value
Total number of patients	88	64	
Percentage of female	80.7	68.7	0.091
Percentages of menopause	71.8	77.3	0.518
Years of age ($\bar{x} \pm$ SD)	52.5 ± 7.8 (36.0-65.0) #	54.3 ± 7.6 (39.0-65.0)	0.145
Percentages of current smoker (%)	9.1	7.8	0.781
Body weight (kg)	61.7 ± 10.0 (40.0-100.0)#	62.3 ± 9.2 (42.0-82.0)	0.539
SBP (mmHg)	126 ± 18 (90-170)	126 ± 17 (100-170)	0.908
DBP (mmHg)	77 ± 10 (60-100)	77 ± 11 (60-100)	0.968
Duration of DM (month)	58.2 ± 37.0 (8.0-129.0)	26.4 ± 22.7 (6.0-113.0)	< 0.001
Duration of metformin therapy (month)	32* (6.00-127.00)	-	-
Amount of metformin (gm)	1376.25* (126.00-8792.50)	-	-

* presented as median, # denotes the range (paretheses)

total daily doses (grams) with the number of days for the whole period of metformin administration at the time of blood sampling.

Among the metformin group, 85.2% of the patients had a high level of blood glucose (*i.e.* 140 mg/dL) compared with 60.9 % in the non-metformin group. The serum creatinine levels of 78 patients in the metformin group were within the normal range while 8 patients in this group were shown to have levels between 1.21-1.5 mg/dL and 2 patients had greater levels than 1.5 mg/dL. Of the 64 patients in the non-metformin group, only 2 patients were shown to have serum creatinine levels greater than 1.2 but not 1.5 mg/dL.

Comparison of plasma Hcy and laboratory findings between two groups

There was no significant difference (p = 0.544) in plasma Hcy levels between the patients in the metformin group (10.6 ± 5.8 mol/L) and those in the non-metformin group (10.4 ± 4.0 mol/L).

The high levels of plasma Hcy (more than 15 mol/L) were found in 13 and 8 patients (14.8 and 12.5 %) in the metformin and non-metformin group respectively. The average Hcy levels in this subgroup were 18.11 mol/L in the metformin group and 18.09 mol/L in the non-metformin group and this difference was not significant (p = 0.885). Furthermore, the remaining patients whose average Hcy levels were less than 15 mol/L (8.42 mol/L and 9.23 mol/L in the metformin and non-metformin groups, respectively) showed no significant difference (p = 0.288).

For laboratory findings, fasting blood sugar (FBS) and serum vitamin B12 levels were significantly different between the patients in the metformin group and those in the non-metformin group. When 56 and 32 patients in the metformin group were further categorised into the metformin therapy continuously over and under 4 years according to the time that can induce vitamin B_{12} deficiency was induced. It was found that the mean serum vitamin B_{12} levels were 235.0 and 300.0 pg/mL in over and under 4 years of metformin therapy respectively. Even though there was no significant difference between these two subgroups (p = 0.111), the mean serum vitamin B_{12} level among patients having metformin therapy over 4 years were significantly decreased (p = 0.002) compared with those in the non-metformin group (434 pg/mL) (Table 2). Neither serum creatinine, cholesterol,

Table 2. Summary $\bar{x} \pm$ SD of laboratory findings

Laboratory value (normal range)	Metformin group N = 88	Non Metformin group N = 64	p value
Plasma homocysteine (5 -15 μmol/ L)	10.6 ± 5.8 (4.1-42.0) #	10.4 ± 4.0 (4.1-24.0)	0.544
Serum creatinine (0.5-1.2 mg/dl)	0.9 ± 0.2 (0.5-1.8)	0.9 ± 0.2 (0.4-1.4)	0.460
Fasting blood sugar (70-110 mg/dl)	192 ± 59 (109-387)	165 ± 52 (108-402)	0.001
Serum cholesterol (130-200 mg/dl)	238 ± 51 (129-391)	244 ± 48 (82-389)	0.239
Serum triglyceride (50-155 mg/dl)	238 ± 181 (54-1298)	183 ± 93 (52-469)	0.052
Serum folic acid (2-14 ng/ml)	8.8 ± 5.1 (1.9-40.0)	7.7 ± 3.8 (2.520.0)	0.090
Serum vitamin B_{12} (200-1100 pg/ml)	319.0 ± 192.2 (50.0-1000.0)	434.3 ± 300.8 (50.0-1900.0)	0.011

# denotes the range (paretheses)

triglyceride nor folic acid levels was found to be significantly different between two groups (Table 2).

Pooled data from both groups were re-analysed to determine the correlation between the level of serum vitamin B_{12} and plasma Hcy. All participating patients were categorised into two groups according to the level of their serum vitamin B_{12} that may affect the Hcy levels (cutoff point 300 pg/mL). The results from the present study were consistent with those previously reported. The significant difference (p = 0.009) of mean plasma Hcy levels of 9.9 and 8.9 mol/L were, respectively, found in 78 (vitamin B_{12} < 300 pg/mL) and 74 (vitamin B_{12} 300 pg/mL) patients. Furthermore, it was found that 8 (out of 13) and 5 (out of 8) patients in the metformin and non-metformin groups, respectively, whose plasma Hcy levels greater than 15.0 mol/L, presented a serum vitamin B_{12} of less than 300 pg/mL.

Correlation between plasma Hcy and duration and amount of metformin

Among 88 patients in the metformin group, plasma Hcy had a significant positive correlation with the duration of metformin therapy (Spearman correlation coefficient = 0.260, p = 0.014) and amount of metformin (Spearman correlation coefficient = 0.258, p = 0.015) There was a trend of higher plasma Hcy with longer duration and higher amount of metformin received (data not shown).

Discussion

Plasma Hcy and related factors (serum vitamin B_{12} and folic acid) between the two groups

It was revealed that the plasma Hcy levels found among patients in the metformin group were not significantly different (p = 0.544) from those in the non-metformin group. The present finding was in agreement with the results from a cross-sectional study of Hoogeveen et al[14] which studied 111 type 2 diabetic patients aged between 40-75 years old, despit the fact that the longest duration of metformin therapy in the present study was 10 years and 7 months, compared with the maximal period of 2 years in Hoogeven's study. Moreover, in the present study there were 13 and 8 patients (14.8 and 12.5 %) in the metformin and non-metformin groups, respectively, who presented with plasma Hcy over the normal range of 15 mol/L.

There was no significant change in serum folic acid levels between patients in the metformin and non-metformin groups from the present study. Compared with vitamin B_{12}, there were a few, controversial reports about the effect of long-term metformin therapy on the level of serum folic acid. The serum folic acid level was decreased among patients taking long-term metformin therapy[12] whilst it was also found to be increased in the low serum vitamin B_{12} patients[11]. There are a few reports to support the effect of metformin therapy on the level of serum folic acid. In a study of Aarsand and Carlsen[16], 28 type 2 diabetic patients who received metformin for more than a year (mean of 4.1 years) had normal ranges of serum folic acid levels (*i.e.* between 1.8-8.2 ng/mL). There have been only a few reports on metformin induced megaloblastic anemia[17] which agrees with the results from the present study that the folate deficiency is a rare complication of long term metformin therapy.

In contrast, the levels of serum vitamin B_{12} were significantly different between the two groups (p = 0.011). Interestingly, it was lower in patients taking metformin. This result could also support the previous findings indicating metformin treatment can decrease absorption of vitamin B_{12}[11, 12]. There are two different mechanisms responsible for the event in these patients. Firstly, there is a transient effect, possibly mediated by changes in intestinal bacteria that end up with an increase of microbacterial intrinsic factor utilization. Secondly, there is a depression of intrinsic factor secretion that can have a long-lasting effect, even permanent, in some patients[12, 18].

There is an abundance of the total body vitamin B_{12} pool (*i.e.* about 2-3 mg). Daily losses of vitamin B_{12}, is approximately 0.1% of the body pool which results in a long half-life of vitamin B_{12} of 480-1360 days. In addition, vitamin B_{12} pool is partly supplied by food and partly by reabsorption of vitamin B_{12} in the biliary system. Therefore, it takes several years (4 -7 yrs) to induce vitamin B_{12} deficiency even with a complete block in the vitamin B_{12} absorption[19]. In the present study, the depletion of vitamin B_{12} in these patients may be due to their long-term administration of metformin.

According to the metabolic pathway of Hcy, a depletion of serum vitamin B_{12} would elevate Hcy levels as described by Carlsen *et al*[13]. They demonstrated a significant decrease in serum vitamin B_{12}, and an increase in Hcy levels, among those patients in the metformin group compared with those in the non-metformin group. In general, to observe an apparent increased level of plasma Hcy, the level of serum vitamin B_{12} must be lower than 339 pg/ml

(equivalent to 250 pmol/L)[20]. The figure of serum vitamin B_{12} was even lower in other studies by Pancharuniti *et al*[21] (*i.e.* 305 pg/mL) and Ueland et al[22] (*i.e.* 176 pg/mL). As previously mentioned, the main source of vitamin B_{12} comes from food and it would be a few years before the effect of serum vitamin B_{12} depletion on the elevation of plasma Hcy to be recognised. Furthermore, most of the participating patients were still continuing on their normal daily intake of food before and during the study. All of that may contribute only a slight increase in plasma Hcy in those patients.

Therefore, the fact that there was no significant difference of plasma Hcy between the metformin and non-metformin groups in the present study may explain these two reasons: the effect of period of time to induce vitamin B_{12} deficiency and the level of serum vitamin B_{12} to increase plasma Hcy.

Relationship between plasma Hcy and duration and amount of metformin therapy

Data from the present study could not demonstrate a significant difference of plasma Hcy levels between patients in the metformin and those in the non-metformin groups. They also presented a low correlation, albeit significant, between plasma Hcy levels and duration of metformin treatment and amounts of metformin received (the rho value of 0.258 and 0.260, respectively). Plasma Hcy appeared to increase with longer duration and a higher amount of metformin therapy. A follow up study in patients with type 2 diabetes mellitus, who have been taking metformin for an average of 4.1 years revealed no significant correlation between plasma Hcy and the current dose of metformin treatment[16]. In the present study, the authors focused on only the amount of metformin, not the current dose as it could be adjusted by physicians at anytime during the study depending on the clinical condition of the patients. In addition, Hoogeven *et al*[14] could not demonstrate the dose-response relationship between cumulative exposure to metformin (dose multiplied by duration of treatment) and Hcy levels for the range of 2 years of metformin therapy.

Fasting blood sugar was found to have no correlation with plasma Hcy in this study. However, the actual mechanism behind these relations are still not known[23].

There have been several reports that support the present findings of the negative correlation between the plasma Hcy and serum folic acid and vitamin B_{12}, which confirms the well-established role of folic acid and vitamin B_{12} in the Hcy metabolism[24-26]. Such reports are The National Diet and Nutrition Survey (performed in 972 British patients aged over 65)[26], Hordaland County in Norway (performed in 11,941 subjects aged between 40-67)[24] and Framingham Heart Study in Framingham, Massachusetts, USA (performed in 1,160 local residents aged between 67-97)[25].

The duration of diabetes was found to be significantly different among patients in the metformin and those in the non-metformin groups. However, there was no significant correlation between the duration of diabetes and the plasma Hcy in the pooled data as also shown in a study[27]. This implies that the duration of diabetes in either group may not have a direct effect on the plasma Hcy.

Limitation of the study

The results of present study showing no significant difference in plasma Hcy but a significant correlation between duration of metformin treatment and amount of metformin received and plasma Hcy may be due to a few constraints of this present study design. Since this study was constructed as a cross-sectional study, there were no data for a baseline comparison and adjustment. The patients might have uncontrollable supplement of vitamins or trace elements from food or any daily intake, which may alter the level of serum folic acid and/ or vitamin B12. Furthermore, there was a relatively low number of the sample size which could not be categorised into subgroups, such as different period of metformin treatment, different levels of serum folic acid and vitamin B_{12}, and so on. This may be confounded in an interpretation of the present finding of plasma Hcy in these patients. Further studies (*i.e.* a prospective cohort/ clinical studies), by monitoring all defined factors since the beginning and at a persistent period of follow up, will lead to more understanding than the present study.

Conclusion

Although the present study could not conclude any significant difference in the plasma Hcy levels between patients in the metformin and non-metformin groups, it showed a significant difference in serum vitamin B_{12} levels between the two groups, particularly patients in the metformin group who were apparently shown to have lower levels of the vitamin. Therefore, giving vitamin therapy, particularly vitamin

B_{12}, which is inexpensive and has a very low risk of toxicity or any complications, would provide better health benefits to these patients, as has been proved to reduce Hcy levels in many studies[28].

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ผลของยาเม็ทฟอร์มิน ต่อระดับ พลาสมาโฮโมซิสเทอีน วิตามินบี12 และกรดโฟลิค การศึกษาภาคตัดขวางในผู้ป่วยเบาหวานชนิดที่ 2

มนัส พงศ์ชัยเดชา, วินิดา ศรีกุศลานุกูล, อภิสิทธิ์ ฉัตรทนานนท์, สมพงษ์ ตันจริยพร

วัตถุประสงค์ *: เพื่อศึกษาผลของยาเม็ทฟอร์มินต่อระดับโฮโมซิสเทอีนในน้ำเลือด วิตามินบี 12 และกรดโฟลิค ในซีรั่มในผู้ป่วยเบาหวานชนิดที่ 2 และความสัมพันธ์ระหว่างปริมาณสะสมของยาเม็ทฟอร์มินกับระดับโฮโมซิสเทอีน*

วิธีการ *: การศึกษาแบบตัดขวางนี้มีวัตถุประสงค์เพื่อศึกษาถึงผลของยาเม็ทฟอร์มินต่อระดับโฮโมซิสเทอีนในผู้ป่วยเบาหวานชนิดที่ 2 จำนวน 152 รายที่มีอายุระหว่าง 35 ถึง 65 ปี ณ แผนกผู้ป่วยนอก โรงพยาบาลมะการักษ์ จังหวัดกาญจนบุรี ผู้ป่วยจะถูกแบ่งเป็น 2 กลุ่ม คือกลุ่มที่ได้รับยาเม็ทฟอร์มิน อย่างน้อย 6 เดือน จำนวน 88 ราย และกลุ่มที่ไม่เคยได้รับการรักษาด้วยยาเม็ทฟอร์มิน จำนวน 64 ราย หลังจากผู้ป่วยอดอาหาร10-12ชั่วโมงแล้วจะได้รับการเจาะเลือดเพื่อนำพลาสมาไปวิเคราะห์ระดับ โฮโมซิสเทอีน ด้วยวิธี Fluorescence Polarization Immunoassay (FPIA) ด้วยเครื่อง IMX Analyzer และซีรั่มจะถูกนำไปวิเคราะห์หาระดับวิตามินบี 12 และ กรดโฟลิคด้วยวิธี Radioimmunoassay*

ผลการศึกษา *: ผลจากการตรวจระดับโฮโมซิสเทอีนในน้ำเลือด พบว่าในกลุ่มผู้ใช้ยา เม็ทฟอร์มิน มีระดับ โฮโมซิสเทอีน 10.6 $\pm$ 5.8 ไมโครโมล/ ลิตร ในขณะที่กลุ่มผู้ที่ไม่เคยรับยาเม็ทฟอร์มินนี้มีค่าระดับโฮโมซิสเทอีน $\bar{x}$ $\pm$ SD 10.4 $\pm$ 4.0 ไมโครโมล/ลิตร โดยที่ความแตกต่างนี้ไม่มีนัยสำคัญทางสถิติ (p = 0.544) และพบว่าระดับวิตามินบี12 ในซีรั่มของกลุ่มผู้ใช้ยา และไม่ใช้ยาเม็ทฟอร์มิน เป็น 318.0 $\pm$ 192.2 พิโครกรัม / มิลลิลิตร และ 434.3 $\pm$ 300.7 พิโครกรัม/มิลลิลิตร ตามลำดับ ซึ่งเป็นความแตกต่างที่มีนัยสำคัญทางสถิติ (p = 0.011) ในขณะที่ระดับกรดโฟลิคในซีรั่มของ 2 กลุ่มเป็น 8.8 $\pm$ 5.1 นาโนกรัม/มิลลิลิตรและ 7.7 $\pm$ 3.8 นาโนกรัม/มิลลิลิตร ในกลุ่มของผู้ใช้ยาและไม่ใช้ยาเม็ทฟอร์มินตามลำดับ และเป็นความแตกต่างที่ไม่มีนัยสำคัญทางสถิติ (p = 0.090) ในการศึกษานี้ยังพบว่าระดับ โฮโมซิสเทอีนมีความสัมพันธ์กับระยะเวลาและปริมาณการได้รับยา เม็ทฟอร์มินอย่างมีนัยสำคัญ โดยมีค่าสัมประสิทธิ์สหสัมพันธ์สเปียร์แมนเท่ากับ 0.260 (p = 0.014) และ 0.258 (p = 0.015) ตามลำดับ*

สรุป *: ถึงแม้ว่าการศึกษานี้ไม่สามารถแสดงถึงผลของการใช้ยา เม็ทฟอร์มินต่อระดับโฮโมซิสเทอีนในน้ำเลือด แต่พบว่าการใช้ยาในระยะเวลายาวนานทำให้ระดับวิตามินบี 12 ในซีรั่มลดลง จึงเสนอแนะให้มีการเสริมวิตามินบี 12 ในผู้ป่วยเบาหวานที่ได้รับยาเม็ทฟอร์มิน*