Chapter 1

Introduction

Cerebrovascular accident (CVA) is one of the leading cause of morbidity and mortality in Thailand. One of the most important advances in medicine has been identification of the major risk factors. The major modifiable risk factors include elevated blood pressure, dyslipidemia, smoking and diabetes mellitus. A substantial body of evidence now supports modification these factors to reduce morbidity and mortality.

In recent years, a number of a new candidate risk factors or markers have been proposed as significant predictors of atherosclerosis and its complication. Homocysteine is one of those novel risk factors[1-3].

Homocysteine is a sulfur-containing amino acid in the blood formed during the metabolism of methionine. Homocyst(e)ine is metabolized by one of two pathways: remethylation to methyonine and transsulfuration to cysteine and glutathione. Epidemiological studies have shown that elevated blood levels of homocysteine is associated with increased risk of stroke[4, 5].

Plasma homocysteine levels are strongly influenced by diet, as well as by genetic factors. Homocystinuria is transmitted by a recessive gene. If both parents transmit the gene, the resultant offspring have very high plasma homocysteine levels. People who carry heterozygous gene do not develop the disease but often have a mildly elevated plasma level of homocysteine. About one person in 100 carries one such gene. Abnormal elevation also occurs among people whose diet contains inadequate amounts of folic acid, vitamin B6, or vitamin B12. Such vitamins play role in metabolism of homocysteine in the body. Several studies have found that higher blood levels of B vitamins are related, at least partly, to lower concentrations of homocysteine. Other recent evidence shows that low blood levels of folic acid are linked with a higher risk of fatal coronary heart disease and stroke [6].

Regardless of the cause of the elevation, supplementation with one or more of these vitamins can lower plasma levels of homocysteine [7]. Dietary supplementation with folic acid can reduce elevated homocysteine levels in most patients [6].

Recent findings suggest that laboratory testing for plasma homocysteine levels can improve the assessment of cardiovascular risk. It may be particularly useful in

patients with a personal or family history of cardiovascular disease, but in whom the well-established risk factors (smoking, high blood cholesterol, high blood pressure) do not exist. Blood for measuring serum homocysteine levels is drawn after a 12-hour fast. Levels between 5 and 15 micromoles per liter (umol/L) are considered normal. Abnormal concentrations are classified as moderate (16-30), intermediate (31-100), and severe (greater than 100 umol/L).[8].

Objectives

8.1 To determine total homocysteine level in plasma of ischemic stroke patients, with age range 40-75 years of age

8.2 To compare homocysteine level in 8.1 with those of normal subjects with age and sex match.

Scope of research

In this study, blood samples will be collected from ischemic stroke patients at Srinagarind hospital. Their plasma homocysteine levels will be compared with the age and sex match normal subjects.

Chapter 2

Literature reviews

2.1 Homocysteine structure

Homocysteine is a sulfur-containing amino acid in the blood formed during metabolism of methionine.

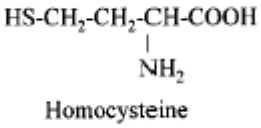

Figure 1 Structure of homocysteine

2.2 Homocysteine metabolism

Homocyst(e)ine is metabolized by one of two pathways: remethylation and transsulfuration (Figure 2).

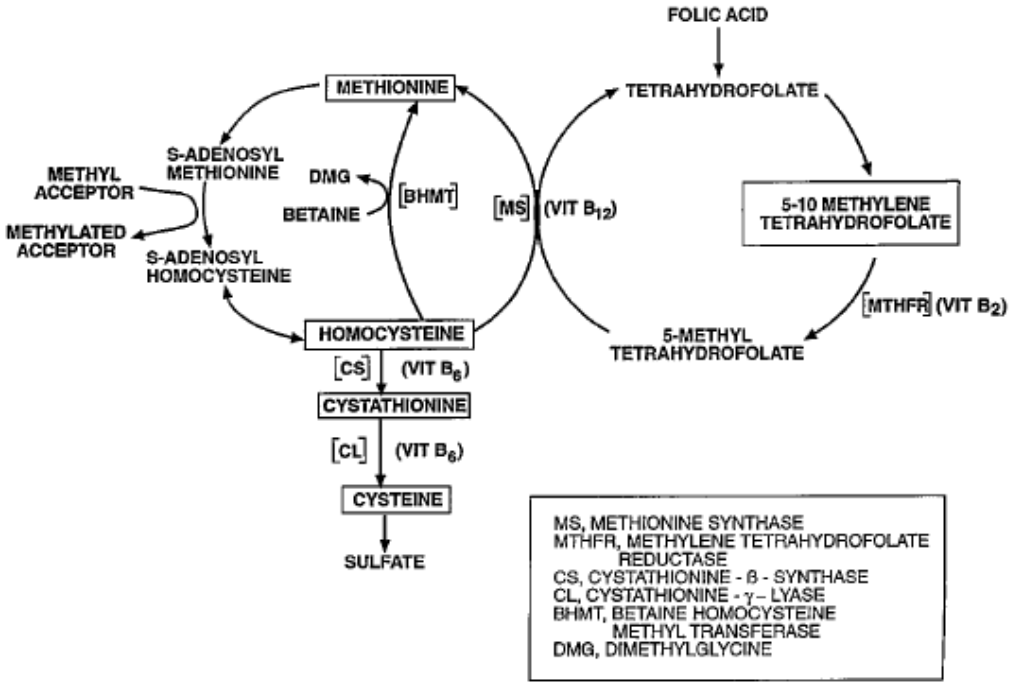

Figure 2 metabolism of homocysteine

In the remethylation cycle, homocyst(e)ine is salvaged by the acquisition of a methyl group in a reaction catalyzed by methionine synthase. Vitamin B12 (cobalamin) is an essential cofactor for methionine synthase, N_5-methyl-tetrahydrofolate is the methyl donor in this reaction, and N5,N10-methylenetetrahydrofolate reductase functions as a catalyst in the remethylation process. Under conditions in which an excess of methionine is present or cysteine synthesis is required, homocyst(e)ine enters the transsulfuration pathway. In this pathway, homocyst(e)ine condenses with serine to form cystathionine in a reaction catalyzed by the vitamin B 6–dependent enzyme cystathionine β-synthase. Cystathionine is subsequently hydrolyzed to form cysteine, which may in turn be incorporated into glutathione or further metabolized to sulfate and excreted in the urine [9].

2.3 Role of genetic factor on homocysteine level

Elevations in plasma homocyst(e)ine are typically caused either by genetic defects in the enzymes involved in homocysteine metabolism or by nutritional deficiencies in vitamin cofactors. Homocystinuria and severe hyperhomocyst(e)inemia are caused by rare inborn errors of metabolism resulting in marked elevations of plasma and urine homocyst(e)ine concentrations. Cystathionine *b*-synthase deficiency is the most common genetic cause of severe hyperhomocyst(e)inemia. The homozygous form of this disease, congenital homocystinuria, can be associated with plasma homocyst(e)ine concentrations of up to 400 μ mol per liter during fasting. The homozygous trait is rare (occurring in 1 in 200,000 births), and clinical manifestations include ectopiaentis, skeletal deformities, mental retardation, thromboembolism, and severe, premature atherosclerosis. Mudd and colleagues have estimated that approximately 50 percent of untreated patients with homocystinuria will have a thromboembolic event before the age of 30 and that overall, the disease-related mortality is approximately 20 percent. Heterozygotes typically have much less marked hyperhomocyst(e)inemia, with plasma homocyst(e)ine concentrations in the range of 20 to 40 *m* mol per liter, approximately two to four times greater than the normal concentration of homocyst(e)ine in plasma [5, 10].

A homozygous deficiency of N5,N10-methylenetetrahydrofolatereductase, the enzymeinvolved in the vitamin B 12–dependent remethylation of homocysteine to methionine, may also lead to severe hyperhomocyst(e)inemia [11]. Patients with this type of deficiency tend to have a worse prognosis than those with cystathionine*b*-synthase deficiency,in part because of the complete lack of effective therapy [12]. In addition, Kang and colleagues [13] have reported a thermolabile variant of N5,N10 –methylenetetrahydrofolate reductase that is caused by a point mutation (C677T) the coding region for the *N*5,*N*10methylenetetrahydrofolate binding site, leading to the substitution valine for alanine [14]. This mutation was found in 38 percent of French Canadians and 5 to 15 percent of the general population in Canada and correlated with elevated plasma homocyst(e)ine concentrations [14, 15]. Although this variant of the *N* 5,*N*10-methylenetetrahydrofolate reductase gene is quite common, it does not appear to be a significant, independent risk factor for atherothrombotic vascular disease [16, 17]. Persons who are homozygous for this mutation appear to have an exaggerated hyperhomocyst(e)inemic response to the depletion of folic acid and with folic acid depletion may be at increased risk for vascular disease. Other abnormalities of the remethylation cycle that are associated with hyperhomocyst(e)inemia include methionine synthase deficiency and disorders of vitamin B 12 metabolism that impair methionine synthase activity.

2.4 Role of homocysteine in vascular diseases

The relationship between homocysteine and vascular disease was suspected almost 25 years ago by McCully [18] when it was observed that people with a rare condition called homocystinuria developed severe cardiovascular and thromboembolic diseases in their teens and twenties [19-21]. In this condition, an enzyme deficiency [20] results in severe hypoerhomocysteinemia and be excreted in urine (homocysteinurea). Recent studies suggest that mild to moderate hyperhomocysteinemia is as important as high blood cholesterol levels and can operate independently [22-25]. Some 10% to 20% of cases of coronary heart disease have been linked to elevated homocysteine levels [23]. Both hereditary and dietary factors may be involved.

Other evidence suggests that homocysteine may have an effect on atherosclerosis by damaging the inner lining of arteries and promoting blood clots.

However, a direct causal link hasn't been established. Abnormal homocysteine levels appear to contribute to atherosclerosis in at least three ways [26-28]: (1) a direct toxic effect that damages the vascular endothelium, (2) interference with clotting mechanism, and (3) oxidation of low-density lipoproteins (LDL).

Selhub and colleagues[29] have recently demonstrated that the prevalence of carotid-artery stenosis increases with increasing plasma concentrations of homocyst(e)ine. In a cross-sectional study of 1041 elderly subjects in the Framingham Heart Study, they found a strong association between elevated homocyst(e)ine concentrations and occlusive vascular disease that remained even after adjustment for other conventional coronary risk factors. There was a graded, rather than a threshold, relation between plasma homocyst(e)ine and the risk of carotid stenosis. The risk of carotid stenosis was increased even at lower plasma concentrations of homocyst(e)ine (between 11.4 and 14.3 *m*mol per liter) that had previously been considered to be normal. Malinow and colleagues[30] reported similar results in an earlier study. A graded response has also been demonstrated between homocyst(e)ine concentrations and the risk of coronary artery disease or cerebrovascular accident[31, 32].

Case-control studies have shown higher levels of total homocysteine in patients with premature peripheral and cerebrovascular disease and atherosclerosis[25, 33]. Most but not all studies have demonstrated an association between elevated levels of total homocysteine and stroke [34, 35]. Bots ML and colleques [36] reported that the risk of stroke and myocardial infarction increased directly with total homocysteine. The linear coefficient suggested that a risk increase by 6 to 7 % for every 1 micromole/litre increase in total homocysteine. The risk by quintiles of total homocysteine level was significantly increase only in the group with levels above 18.6 micromole/litre. Odd ratios were 2.42 (95% confidence interval, 1.11 -5.35) for myocardial infarction and 2.53 (95% confidence interval, 1.19-5.35) for stroke. In a meta-analysis, odd ratios for stroke was 2.5 in 9 studies (95% confidence interval, 2.0 – 3.0) [37]. Another study found that elevated homocysteine levels may be associated with an increased risk of stroke in people who already have coronary heart disease [38].

Several clinical trials are underway to test whether lowering homocysteine will reduce CHD risk. Recent data show that the institution of folate fortification of foods has reduced the average level of homocysteine in the U.S. population [6].

2.5 Relationship between nutrients and homocysteine level

There are 3 majors vitamins involve in homocysteine metabolism. They are folic acid, vitamin B12 and vitamin B6 which are co-factors for homocysteine metabolism as shown in Figure 2. Nutritional deficiencies in the vitamin cofactors (folate, vitamin B12, and vitamin B6) required for homocysteine metabolism may promote hyperhomocyst(e)inemia. Markedly elevated homocyst(e)ine concentrations have been observed in patients with nutritional deficiencies of the essential cofactor vitamin B 12 [39] and the cosubstrate folate[40, 41]. Negative correlations between serum vitamin B12, folate, and vitamin B6 concentrations and plasma homocyst(e)ine concentrations have been observed in normal subjects[42]. Selhub and colleagues[42] have suggested that inadequate plasma concentrations of one more B vitamins are contributing factors in approximately two thirds of all cases of hyperhomocyst(e)inemia. Vitamin supplementation can normalize high homocyst(e)ine concentrations (see below); however, it remains to be determined whether normalizing homocyst(e)ine concentrations will improve cardiovascular morbidity and mortality. Other evidence shows that low blood levels of folic acid are linked with a higher risk of fatal coronary heart disease and stroke.

Several studies found that higher blood levels of B vitamins are related, at least in part, to lower concentrations of homocysteine[7, 43].

So far, no controlled treatment study has shown that folic acid supplements reduce the risk of atherosclerosis or that taking these vitamins affects the development or recurrence of cardiovascular disease[44]. Researchers are trying to find out how much folic acid, B-6 and/or B-12 are needed to lower homocysteine levels. Screening for homocysteine levels in the blood may be useful in patients with a personal or family history of cardiovascular disease but who don't have the well-established risk factors (smoking, high blood cholesterol, high blood pressure, physical inactivity, obesity and diabetes).

2.6 Homocysteine lowering and vascular outcome

Homocysteine lowering in patients with homocystinuria has been proven to reduce the risk of adverse cardiovascular events. A recent study that followed 80,000 women for 14 years found that the incidence of heart attacks was lowest among those using multivitamins or had the highest intake of folic acid and B6 from diet [45]. This data parallels the finding that elevated homocysteine levels are associated with a higher incidence of heart disease. However, the researchers measured folic acid blood levels but did not measure B12 or homocysteine levels.

Homocysteine screening is advisable for individuals who manifest coronary artery disease that is out of proportion to their traditional risk factors or for people who have a family history of premature atherosclerotic disease. Levels above 9 or 10 μmol/l warrant treatment. The effect of supplementation is usually apparent within a month. A recent study of the effect on homocysteine of either folic acid or B12 alone found that the body adjusts its reliance on one or the other and that supplementing with both provides a more certain way to improve homocysteine levels [46].

Large-scale studies of more than 60,000 people are underway in the United States, Canada, and Europe to examine the effects of lowering blood homocysteine levels on the incidence of heart attacks and/or strokes [47, 48]. The longest one so far involved 553 patients who had had successful angioplasty has found that lowering homocysteine levels significantly decreased the incidence of major cardiac events after angioplasty. The participants were randomly assigned to receive a combination of folic acid, vitamin B12, and vitamin B6 or a placebo for 6 months and were followed for about six more months. The study found that the incidence of heart attacks, death and need for repeat revascularization were about one third less in the vitamin group than in the control group[49].

Studies have not yet determined whether lowering homocysteine levels reduces the incidence of heart attacks or strokes among people with mildly elevated homocysteine levels [50, 51] but many experts believe that scientific studies will prove that it does. This belief has been strongly supported by a four-year study in which 101 men with vascular disease were given supplementary doses of folic acid, B6 , and B12. Ultrasound examinations of their carotid arteries found a decrease in the amount of

carortid plaque in their arteries, with the greatest effect in those whose homocyteine levels had been highest before the treatment began [48].

Although evidence for the benefit of lowering homocysteine levels is lacking, patients at high risk should be strongly advised to be sure to get enough folic acid and vitamins B6 and B12 in their diet. Foods high in folic acid include green, leafy vegetables and grain products fortified with folic acid. But this is just one risk factor. A physician taking any type of nutritional approach to reducing risk should consider a person's overall risk factor profile and total diet.

2.7 Plasma homocysteine

The majority homocysteine in plasma conjugated to protein through disulphide bonding (more than 80%); as the symmetrical disulphide homocysteine; as the mixed disulphide homocysteine-cysteine; and, as the free thiol (less than 2%). The free thiol can undergo a reversible conversion to homocysteine thiolactone but it is present in very minor amounts in plasma, probably at nanomolar levels due to non-specific enzymatic hydrolysis [52].

The term, total homocysteine in plasma refers to the sum of the concentrations of the free and oxidised forms, measured after reduction of the disulphide bond to liberate the free form. The main source of homocysteine in plasma is probably the liver and proliferating cells. Only a very minor fraction of the homocysteine produced by the cells is excreted in the urine, degradation in the renal tissue after tubular reabsorption seems to account for the major part of the homocysteine clearance [52].

Elevated concentration of plasma homocysteine has been a focus of research interest since it was established that it plays a major role in the cause and effect chain linking lifestyle, nutrition and cardiovascular disease. During the seven years following the publication of the first meta-analysis of the relationship between plasma total homocysteine and cardiovascular disease [53], the study has been cited 1255 times, reflecting the enormous research activity involved in the further clarification of the many questions that remain unanswered. While we wait for the results of the crucial intervention studies on the effect of vitamin supplementation in the prevention of cardiovascular disease [54], measurement of total homocysteine in plasma has found its way into risk assessment of individuals in many clinical settings.

Intake of vitamins B6, B12 and folate in the diet and the amount of methionine in the dietary proteins will influence the total plasma homocysteine as well as the age and gender. Factors determined by lifestyle do play a role too: Coffee consumption, cigarette smoking and lack of exercise is associated to an increase in total plasma homocysteine [55].

Several polymorphisms and mutations in the metabolism of homocysteine have been described and the total plasma homocysteine is influenced by genetic factors. Since there are race differences in the prevalence of these polymorphisms and mutations, there are also ethnic differences in the total plasma homocysteine. The common C677T polymorphism in the gene for methylenetetrahydrofolate reductase (MTHFR) is present in approximately 10% of white Caucasians and nearly absent in Africans-Americans [55].

Several drugs may interfere with homocysteine metabolism and folate antagonists, such as methotrexate, induce hyperhomocysteinaemia.

Renal function has a strong influence on total plasma homocysteine and hyperhomocysteinaemia is seen in patients with hypothyroidism. Other diseases with impaired folate status such as hepatic diseases, some cancers, and psoriasis are associated to increased total plasma homocysteine [52].

Because the essential amino acid methionine is the only source of homocysteine, food intake would be expected to influence total plasma homocysteine. Homocysteine is slowly eliminated from plasma with a half-life of 3-4 hours; hence an elevated total plasma homocysteine would be expected 12-20 hours after a protein-rich meal. Samples for the measurement of total plasma homocysteine should be taken in the morning, after a light breakfast and following a light meal the evening before [52, 56, 57].

As most homocysteine in plasma is protein-bound, posture and stasis during sampling will influence total plasma homocysteine. Total plasma homocysteine declined by 6.3% on average in 24 healthy subjects after 30 minutes in supine position and a moderate stasis of 3 minutes duration increased total plasma homocysteine 2.8% on average. Since most sampling is done in ambulatory subjects or walking in-patients, blood collection should be standardised to patients sitting down and avoided in supine

subjects if possible. A short, moderate stasis will not add an appreciable variation to the measurement of homocysteine [58].

2.8 Determination of total plasma homocysteine

Stability of plasma homocysteine was reported. The release is time and temperature dependent and amounts to about 10% at room temperature. The absolute increase is, to some degree, independent of total plasma homocysteine, so non-optimal sample handling tends to reduce the difference between high and low total plasma homocysteine. Refrigerating EDTA or heparin anti-coagulated samples on ice delays the release for up to an hour. To stabilise samples at room temperature, various additives have been used: acidic citrate, sodium fluoride, and 3-deazaadenosine. All of these have their different drawbacks: acidic citrate results in a systematic decrease in concentration of about 1 μmol/L, part of the apparent effect of sodium fluoride is due to the osmotic effects of the high concentrations of sodium diluting the sample, and 3-deazaadenosine interferes with the enzymatic conversion in the immunoassays (see below). Currently, the best option seems to be to anticoagulate the samples with EDTA or Heparin, refrigerate and centrifuge as soon as possible [59-66].

For biological vatiation, Intra-individual variations have been found to be from 7.0 to 9.4% and inter-individual variations from 24 to 34% [67-70].

All methods for the determination of total plasma homocysteine include a reduction step before separation and detection. The use of sulphydryl reagents such as dithiothreitol, dithioerythritol, mercaptoethanol with sodium or potassium borohydride or tri-n-butylphosphine (TBP) is being used in many methods and has been reviewed by Ueland et al [71].

The common methods for determination of total homocysteine include Capillary Gas Chromatography – Mass Spectrometry with Selected Ion Monitoring according to the method of Stabbler et al [72], Liquid chromatography electrospray tandem mass spectrometry by Magera et al [73] [74]. Capillary Electrophoresis [87, 88] , Immunoassays [90], ELISA and Automated methods [94-97] A version for the Abbott AxSym platform has recently been introduced [98]. An immunoluminescence version using S-adenosylhomocysteine conjugated with alkaline phosphatse has been available

since 2001 for the DPC IMMULITE systems [99] and a method for the Advia Centaur is currently under evaluation [100].

High pressure liquid chromatography (HPLC) using amino acid analysers and photometric detection [75], electrochemical detection [10] and fluorometric detection [76] . Several modifications have been published [77, 78],

A fully automated method was described by Fiskerstrand et al using reduction with dithioerythritol and sodiumborohydride and derivatisation with monobromobimane. In the version later described by Jacobsen et al reduction with sodiumborohydride was done simultaneously with derivatisation with monobromobimane [79, 80].

Reagents and method descriptions for HPLC equipment are readily available from several manufacturers and kits and HPLC systems designed for the analysis of P-tHcy has been evaluated, i.e. from BioRad [81-85] and Drew Scientific [86].

Effort is made for the development of analytical methods that can be adapted to mainframe automated clinical chemistry analysers such as direct enzymatic methods with photometric detection or immuno-turbidimetric methods. In the future the new applications will be seen in making homocysteine methods available to a large number of non-specialised routine clinical chemistry laboratories.

Chapter 3

Materials and Methods

Subjects

The study was carried out on a sample group of ischemic stroke patients diagnosed by clinical and brain CT scan or brain MRI in Srinagarind hospital. Their total plasma homocysteine was determined before vitamin supplementation compared with control group with the match age and sex. Risk factor of atherosclerotic disease, diabetes, hypertension, previous vascular disease and atrial fibrillation were evaluated. Fasting plasma glucose, fasting lipid profile(total cholesterol, HDL, LDL, triglycerides), renal function (blood urea nitrogen, creatinine) were tested. The exclusion criteria were brain tumor, chronic kidney disease, intracerebral hemorrhage, and encephalitis

Ethics

The Ethics Committee, Faculty of Medicine, Khon Kaen University, approved the study protocols.

Collection of plasma samples

Five millilitres of blood samples were collected by venipuncture in the morning from fasting subjects. Plasma samples were obtained by addition of ethylenediamine tetraacetic acid (EDTA) in the blood. The blood was then centrifuged at 2,500 rpm at 4^{o}C for 10 min to obtain serum. Hemolyzed samples were excluded. The serum was stored at –20^{o}C until analysis.

Determination of plasma homocysteine

Total plasma homocysteine will be determined by HPLC according to the method of Ubbink [101].

Statistical analysis

Statistical analysis will be conducted using the Prism, SPSS and Sigma stat. Differences among each group were determined using Turkey's pairwise comparisons of the analysis of variance (ANOVA), t-test, a and Pearsons depend on variables. Means were considered significantly different if *p*-values were less than or equal to 0.05.

Chapter 4

Results and discussion

From June 2006- May 2007, a total 49 patients (34 male and 15 female) and age between 31-86 years, were included in this study. The mean hospital length of stay of these patients were 8.1 days. According to TOAST criteria, incidence of large vessel disease, small vessel disease and cardioembolic stroke was 28.57, 67.3 and 4.1%, respectively

Most of these ischemic stroke subjects, 86%, have traditional risk factors of stroke. The most common risk factor found in this study was dyslipidemia, 73.5%. In the present study, the incidence of hypertension, smoking, diabetes, previous stroke, atrial fibrillation and coronary artery disease was 49, 40.8, 30, 16.7, and 4.1%, respectively **(Table 1.)**

Table 1. Traditional risk factors of ischemic stroke patients

Risk factor	No. (%)
Dyslipidemia	36(73.5)
Hypertension	24(49.0)
Smoking	20(40.8)
Diabetes	15(30.6)
Previous TIA or stroke	8(16.7)
Atrial fibrillation	2(4.1)
CAD of CHF	2(4.1)

- CAD; coronary artery disease
- CHF; congestive heart failure

When classify the level of plasma homocystein level to under 5, 5-15, 15-25, 25-50, and above 50 µmol/L as low, normal , normal, moderately high and very high homocysteine level, most of patients (79.6%) had homocystein level under 15 µmol/L.**(Table 2.)**

Table 2. Patients' homocysteine level according to homocysteine range

Homocysteine level range (μmol/L)	No of patients (%)
Under 5	1 (2)
5-15	39 (79.6)
15-25	8 (16.3)
25-50	1 (2)
More than 50	0

The risk factors and total homocysteine level were described in **Table 2.**

Table 3. Total homocysteine level (μmol/L) according to risk factors.

Risk factors	Risk factor (No.)	Percent of patients with risk factor	Mean plasma homocysteine (μmol/L)	P value*
Dyslipidemia	Yes 36	73.5	11.58	0.489
	No 13		12.94	
Hypertension	Yes 24	49	11.7	0.220
	No 25		13.51	
Smoking	Yes 20	40.8	12.61	0.986
	No 29		12.56	
Diabetes	Yes 30	30	12.94	0.478
	No 34		11.80	
Previous TIA or stroke	Yes 8	16.7	12.72	0.762
	No 41		12.11	
Atrial fibrillation	Yes 2	4.1	12.69	0.490
	No 47		10.12	
CAD or CHF	Yes 2	4.1	12.61	0.885
	No 47		12.00	

- compare between subject who had and had not risk factors

The mean plasma homocystein level of the patients was 12.59 ± 5.11 μmol/L.The highest level of homocysteine level in the present study was 30.44 μmol/L while the lowest level was 4.97 μmol/L. The standard level of plasma homocysteine level in the present study was 123.5 μmol/L. In the present study, 36.7 % of these ischemic stroke patients had hyperhomocysteinemia (plasma homocysteine level above 12.5 μmol/L). However, the age, sex and hostpital stay of patients who had hyperhomocysteinemia were not different from the patients who had normal homocysteine level.

Consideration of type of ischemic stroke according to TOAST criteria, the mean plasma homocystein level of patients with large vessel disease, small vessel disease and cardioembolic stroke patient homocysteine level, the homocysteine levels were not significant different from the group.

This was the first study of homocysteine level in stroke patients in the north-east of Thailand and was the third report of homocysteine level in Thai stroke patients. The mean homocysteine level of ischemic stroke patients in this study was quite lower than the previous study of Thai people[106]. Most of ischemic stroke patients in this study did not have hyperhomocysteinemia (plasma homocysteine level above 12.5 μmol/L). The incidence of hyperhomocysteine level in Northeast Thai patients was lower than in the previous report [106] from central part of Thailand. This could explain that Northeast people had lower prevalence of ischemic stroke than those from other part of Thailand[107]. The lower homocystein level of the patients in this study may be explained by the high vegetable consumption of Northeast people which contain high folate content. The study of folate rich food consumption and such as vegetables and homocysteine level of northeast patients may be warranted to explain the lower prevalence of hyperhomocysteinemia

Moreover, hyperhomocysteine patients in the present study were not associated with other traditional risk factor. The other studies demonstrated that hyperhomocysteinemia was an independent risk factor of ischemic stroke and homocysteine level [5,32,34]. The traditional risk factors of stroke are still stronger and more important than the weaker hyperhomocysteine level.

Chapter 5

Conclusion

The data of homocysteine level in this study is may give an advantage as the reference value for Northeast patients of Thailand which have lower prevalence of hyperhomocysteinemia. Homocysteine lowering treatment should not routinely given in ischemic stroke but may be warrantted in stroke patients who have hyperhomocysteine level to prevent the recurrent of stroke.

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