The Prevalence of MTHFR 677C→T Missense Mutation, Total Plasma Homocysteine Levels and Associated Risk Factors in Malay Subjects

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Summary

The missense mutation of the methylenetetrahydrofolate reductase (MTHFR) gene 677C→T is associated with modest elevation of homocysteine levels. The bio-ecogenetics factors of total homocysteine levels (tHcy) were investigated in a cross sectional study involving 53 randomly selected healthy Malay subjects. Results indicated that the prevalence of the homozygous 677T/T was 3.8% and heterozygous 677C/T was 17.0%. The levels of tHcy was higher in subjects aged more than 50 years (n=7, 11.53±4.45µmol/l) and in males (10.99±3.77µmol/l) especially smoking males (12.19±3.62µmol/l). THcy levels were low in the 3 pregnant subjects (4.44µmol/l, p=0.036) who were under folate supplementation.

Key Words: Methylenetetrahydrofolate reductase, MTHFR 677C→T, Homocysteine, Bio-ecogenetics

Introduction

Homocysteine is a sulfydryl-containing amino acid, which is formed as an intermediate metabolite in the metabolism of methionine. Methionine is an essential amino acid derived from dietary proteins such as meat, eggs or milk. Excess methionine in the body will be converted into homocysteine. Homocysteine is then further metabolized into beneficial compounds to be utilized or excreted through the normal physiological function of the body [1].

Homocysteine levels in plasma are normally kept low via intracellular enzymatic feedback systems that are dependent on B vitamins [2]. The normal role of homocysteine in the body is to control growth and support bone and tissue formation. Markedly and mildly elevated circulating homocysteine concentrations (hyperhomocysteinemia) are highly associated with the impaired metabolism of the homocysteine. The genetics, biological and environmental factors are cooperatively contributing to the retardation of homocysteine metabolisms. The excessively

retained homocysteine in the cells will be actively excreted into plasma thus causing hyperhomocysteinemia.

Gene defects are important risk factors in contributing to hyperhomocysteinemia. A common missense mutation (C-to-T substitution at nucleotide 677) in the coding region of the gene for methylenetetrahydrofolate reductase (MTHFR) results in conversion of an alanine to valine residue [3], which is associated with a thermolabile MTHFR variant that has nearly 50% of normal enzyme activity [4]. Other genetic defects that are associated with hyperhomocysteinemia are the multiple mutations in cystathionine β synthase (CBS) gene [5] and the methionine synthase (MS) polymorphisms [6].

Folate in its major circulation form, 5-methyltetrahydrofolate (5-MTHF) functions as a methyl donor during the remethylation pathway in the formation of methionine from homocysteine. The MTHFR T/T genotype effect is observed only among subjects with relatively low folate concentration [7] and the association between the thermolabile MTHFR T/T variant and elevated total homocysteine (tHcy) levels is known as a function of folate status [8]. There is a second pathway in which a methyl group is transferred irreversibly from betaine, or trimethylglycine, to homocysteine. This process involves the betaine-homocysteine methyltransferase (BHMT) [9].

Other known risk factors of hyperhomocysteinemia include age [10], male sex [11], smoking[12], physical inactivity[7], poor renal function[13], menopause[14], and circulating cholesterol levels[15]. Besides that, drugs that interfere with homocysteine metabolism such as the nitric oxide, methotrexate, isoniacid, penicillamine, anti-convulsants and various antiepileptic drugs, may give elevated levels of tHcy[16].

Data on the determinants of homocysteine concentrations in Asian populations, particularly the Malay is scarce. The diets and other lifestyle characteristics and the genetic backgrounds of the Malay population are distinct from those of Occidental or other Asian populations. Therefore, the study will describe the distribution of total plasma homocysteine concentrations by age and sex among healthy adult Malay men and women. Furthermore, the study will examine the association of homocysteine concentration with MTHFR677C→T missense mutation, dietary intake high in unsaturated fat, folate, and vitamin B12, lifestyle factors including cigarette smoking and physical activity.

With the identification of the polymorphism of MTHFR, dietary and lifestyle factors of the hyperhomocysteinemia subjects, effective intervention programs can be planned and implemented in order to avoid harmful consequences. In addition, this study, which establishes the bio-ecogenetics relationship of hyperhomocysteinemia in the Malay population, will contribute in giving an insight for future studies. It can act as a catalyst for future work on other predisposing factors of hyperhomocysteinemia. It will also help to a certain extent to create awareness that hyperhomocysteinemia is not confined to a certain population but is a global risk factor to the various disease states, as both the mutation and hyperhomocysteinemia are cooperatively or solitarily associated to the risk of neural tubes defects, complicated pregnancy [17], meningomyelocele [18], breast cancer, ovarian cancer, endometrial cancer, leukaemia [19], cardiovascular diseases, cerebrovascular diseases, hyperuricemia [20], acute renal failure, coagulation abnormalities and Alzheimer's disease [21].

Materials and Methods

Study Design

The cross-sectional study was carried out at the Faculty of Medicine and Health Sciences and the Faculty of Veterinary Sciences, Universiti Putra Malaysia. The study involved 53 apparently healthy and randomly selected Malay volunteer

staff from these 2 faculties. The subjects were interviewed and anthropometric data of the subjects providing information on the body mass index (BMI), waist-hip ratio (WHR), and systolic and diastolic blood pressure were also collected after 10 hours overnight fasting. Peripheral fasting blood was taken from the subjects for both the mutation and tHcy analysis. The MTHFR 677C→T missense mutation was identified through polymerase chain reaction (PCR) and restricted fragment length polymorphism (RFLP) analysis.

Sampling of Subjects

The subjects were randomly selected through the lottery method from a list of pre-coded names given by both the faculties. The selected subjects were approached individually with an invitation letter and a booklet that clarified the objectives, methodology and the benefits of the study. Only apparently healthy subjects (with no clinically proven chronic complications such as hypertension, vascular diseases, heart diseases, diabetes, stroke and renal failure) were chosen. The subjects were ethnic Malays aged 20 years old and above. Written consent was obtained from all the subjects.

Instrument of Study

Pre-tested questionnaire in both Malay and English were developed to assess the socio demographic background, medical history, dietary pattern (Food Frequency Questionnaire), physical activities, and smoking practices of the study subjects. The questionnaire consists of five main sections to be filled in by subjects through the semi-assisted method. An additional food frequency questionnaire (FFQ) that consists of a list of 91 food or food ingredients that are common to the Malay society was developed. The frequency of food consumption was classified according to the score method using six-point scale. The modified scoring method by Chee et al., 1996 [22] that calculates the score of each food items was applied in this study. A 7-day physical activity

recall was used to provide information on the physical activity level of the subjects. The physical activity index (PAI) from the physical activity assessment used in the Stanford Five-City project which estimates metabolic equivalent (MET) or ratio of working metabolic rate to resting metabolic rate was adopted and modified [23].

BMI, Blood Pressure and WHR

Height and weight of subjects were obtained by using the TANITA digital weighing scale and the SECA Bodymeter 208 respectively. Body mass index (BMI) of subjects was calculated as weight (kg) / height^2 (m^2). Blood pressure measurements of the subjects were obtained by using an OMRON 330 electronic reader. Blood pressure reading was obtained after the subjects were in resting condition for at least 10 minutes. Waist circumference was measured midway between the lower rib margin and the iliac crest. For hip circumference measurement, the subjects were required to stand erect with arms at the side and feet together. The measurement was taken at the point yielding maximum circumference over the buttocks. All measurements were repeated in order to obtain the average value.

Total Homocysteine levels (tHcy) and MTHFR Polymorphisms

Subjects were required to fast (overnight) for at least 10 hours before blood sampling was carried out. Four millilitres (4 ml) of fasting peripheral venous blood was collected for tHcy enzyme immunoassay and MTHFR variants genotyping. The collected blood was kept at 4°C and was centrifuged into plasma and whole blood within an hour. Buffy coat was separated and was kept at -20°C until further genotyping analysis. Plasma was stored at -80°C until further analysis of total homocysteine levels.

Genomic DNA extraction was carried out using the Perfect gDNA Blood Mini kit by Eppendorf®. The MTHFR 677C→T missense mutation site was

amplified using a set of primers previously described by Frosst et al., 1995 [4]; the forward primer (5'-TGA AGG AGA AGG TGT CTG CGG GA-3') corresponds to the sequence 653-676 of MTHFR exonic region and the reverse primer (5'-AGG ACG GTG CGG TGA GAG TG-3') corresponds to the sequence 832-851 of MTHFR intronic region. 'Hot-start' PCR was performed in a Mastercycler Gradient Thermalcycler (Eppendorf®) for 35 cycles. The temperature for the initial denaturation of DNA was 95°C for 30 s, annealing at 53°C for 90 s, and extension at 72°C for 90 s and a final extension at 72°C for 7 minutes following the last cycle. The PCR products were purified by using PURE-GENE PCR Purification Kit (BST Techlab). Upon purification, the samples were subjected to HinfI digestion and electrophoresed using 5.0% agarose gel (Figure 1). Positive samples were sent for automated sequencing (ABI PRISM Model 377) at the Institute of Bioscience, Universiti Putra Malaysia.

Total homocysteine levels were determined using the Axis® Homocysteine EIA (IBL Hamburg). The entire enzyme immunoassay was controlled with low, medium and high tHcy controls provided in the Axis® Homocysteine Control Kit (IBL-Hamburg). Results were then interpreted incorporating all other test results and clinical status of the subjects. The logarithmic curve fit was used for preparing the calibration curve and calculation of unknown samples.

Analysis of Results

The Statistical Package for Social Sciences (SPSS) for Windows® version 10.0.1 was used to analyse the data. Descriptive statistics were used to analyse all the studied variables such as socio-economic characteristics, dietary pattern, physical activities and smoking practices, anthropometric measurements, biological parameters and the MTHFR genotypes of subjects. Pearson's Correlation was used to test the association between two normally distributed continuous variables (plasma tHcy, BMI, WHR, diastolic and systolic blood pressures, and physical activity index). The independent t-test was carried out to compare means between categorical independent variables with continuous dependent variables (BMI, WHR, MTHFR variants, folate supplemented and non-supplemented female, smoking practices with tHcy). One-way ANOVA was applied for assessing the differences between various age groups with tHcy levels. A statistical probability level of $p < 0.05$ was considered as significant.

Results

Of the 53 subjects in this study, 49% of them were males and 51% were females. The age of the subjects ranged from 21 to 60 years old with a mean age of 37.58±9.72 years. All subjects (n=53) were included in the prevalence study regardless of their age, health status and medication background.

The prevalence of MTHFR 677C→T missense mutation in the Malay subjects was 3.8% (2 females) for homozygous mutation (T/T), 17.0% (5 females and 3 males) for heterozygous mutation (T/C) and 79.2% (17 females and 23 males) for normal wild type (C/C). The frequency of MTHFR 677T allele was low compared to MTHFR 677C allele with 0.13 and 0.87 in males and 0.06 and 0.94 in females respectively. There were no significant differences in MTHFR 677T between both sexes (Table I).

As shown in Table II, none of the measured MTHFR 677 alleles contributed significantly to tHcy levels. However, there was an observation in female subjects that homozygotes with thermolabile MTHFR 677C→T mutation had higher tHcy levels. The presence of MTHFR 677T allele in the heterozygous males was also associated with tHcy levels. However, this comparison did not take into account age, sex and smoking factors due to insufficient number of subjects. These factors cannot be excluded.

Table III shows, for each age group, male subjects were found to have higher levels of tHcy compared to females but these differences were not statistically significant. Comparison between both the sexes shows significantly higher tHcy in male after adjusting for the genotypes (Table II). From Table III, after adjusting for smoking practise, non-smoking males have significantly higher tHcy levels compared to non-smoking females. As shown in Table III, tHcy levels were found higher in male compared to female subjects in all the age groups but these differences were not statistically significant.

On the other hand, there were no significant differences in tHcy levels among all the age groups after controlling for the gender. However, tHcy levels were higher in both males and females aged more than 50 years (n=4, 12.75±5.70µmol/l and n=3, 9.92±1.95µmol/l respectively) compared to the other age groups but these differences were not statistically significant. The mean tHcy level for those aged more than 50 years (n=7) was 11.53±4.45µmol/l compared to those subjects below 50 years (n=43, 9.20±3.39µmol/l) disregarded the genotypes and smoking factors.

According to Table IV, the majority of the subjects have never smoked (86%) while 16% of them are smokers. All of the smokers are male. The mean years of smoking were 17.38±11.92 and the number of cigarettes smoked per day was 11.13±5.44. Smokers show higher tHcy levels (n=8, 12.19±3.62µmol/l) compared to non-smoking males (n=18, 10.45±3.81µmol/l) and females

(n=24, 7.95±2.67µmol/l) but these differences were not statistically significant.

The mean WHR for the subjects was 0.835 and the Pearson's Correlation test (Table V) shows significant but weak linear correlation between WHR, systolic and diastolic blood pressure with tHcy levels but no linear association with BMI and PAI. The insignificant results probably are due to the low number of subjects. Among the subjects, 3 were pregnant and taking folate supplements. They have significantly (p=0.036) lowered tHcy levels (4.44±1.30µmol/l) compared to 24 non-pregnant females (7.95±2.67µmol/l). However, the differences might be due to unequal number of subjects in the groups.

Based on the frequency of food intake obtained from the questionnaire, the twenty most frequently consumed food are fresh non-processed fish, hen eggs, cucumber, cabbage or cauliflower, orange, mustard, string bean, lady's finger, tomato, carrot, sprout, swamp cabbage, red or green apple, fried rice, *nasi lemak*, guava, papaya, banana, watermelon and spinach. The frequently consumed food contains high levels of folic acid and sufficient amount of vitamins B_{12} and B_6. The least consumed foods are chicken, daun *ubi kembili*, internal organs of mutton, canned mutton curry with potatoes, betel leaf, eel, *kundur*, mutton, fermented shrimp, asparagus, canned beef curry with potatoes, *mengkudu*, fish roe, clam, kiwi fruit, bambooshoots, quail eggs and duck eggs.

Table I : Frequency of allele 677T and 677C in male and female

Gender	MTHFR 677 allele		$^a\chi^2$
	T (%)	C (%)	
Male	3 (25)	35 (44)	1.015
Female	9 (75)	45 (56)	

$^a\chi^2$ values were obtained by Pearson Chi-Square (df = 1; p = 0.314).

Table II : Mean total homocysteine levels in male and female with MTHFR genotypes

Gender	MTHFR variants (µmol/l)			p
	Homozygote mutation (T/T)	Heterozygote (C/T)	Wild type (C/C)	
[a]Male	–	14.7±6.30	10.5±3.23	0.068
n (%)	0 (0)	3 (12)	23 (88)	
[b]Female	9.39±2.33	7.74±2.46	7.84±2.85	0.743
n (%)	2 (8)	5 (21)	17 (71)	
[a]p	–	0.034*	0.010*	

*. Means difference is significant at the 0.05 level (2-tailed).
[a]. p (2-tailed) values were obtained by independent T-test.
[b]. p (2-tailed) values were obtained by one-way ANOVA.

Table III : Mean total homocysteine levels in male and female according to age groups

Age groups	Gender (µmol/l)		[a]p
	Male	Female	
20 – 29	11.57±0.96	8.16±3.03	0.101
n (%)	3 (30)	7 (70)	
30 – 39	10.66±4.74	7.29±3.04	0.086
n (%)	10 (53)	9 (47)	
40 – 49	10.37±2.12	7.65±1.66	0.030*
n (%)	9 (64)	5 (36)	
> 50	12.75±5.70	9.92±1.95	0.456
n (%)	4 (57)	3 (43)	
[b]p	0.761	0.545	

*. Means difference is significant at the 0.05 level (2-tailed).
[a]. p (2-tailed) values were obtained by independent T-test.
[b]. p (2-tailed) values were obtained by one-way ANOVA.

Table IV : Mean total homocysteine levels in male and female smokers

Gender	Smoking status (µmol/l)		[a]p
	Smoker	Non-smoker	
Male	12.19±3.62	10.45±3.81	0.285
n (%)	8 (31)	18 (69)	
Female	–	7.95±2.67	–
n (%)	0 (0)	24 (100)	
[a]p	–	0.017*	

*. Means difference is significant at the 0.05 level (2-tailed).
[a]. p (2-tailed) values were obtained by independent T-test.

Table V : Correlation between total homocysteine levels and selected variables

Variables	n	$^a r$	$^b R^2$	$^c p$
Waist Hp Ratio (WHR)	50	0.428	0.183	0.002*
Body Mass Index (BMI)	50	0.089	0.008	0.540
Systolic blood pressure	50	0.332	0.110	0.018*
Diastolic blood pressure	50	0.303	0.092	0.032*
Physical Activity Index (PAI)	50	0.141	0.020	0.328
Age	50	0.145	0.021	0.317

*. Correlation is significant at the 0.05 level (2-tailed).

^{a}. r (Pearson's correlation coefficient) values were obtained by Pearson Correlation test.

^{b}. R^2 (Coefficient of determination) values were obtained by squaring the r.

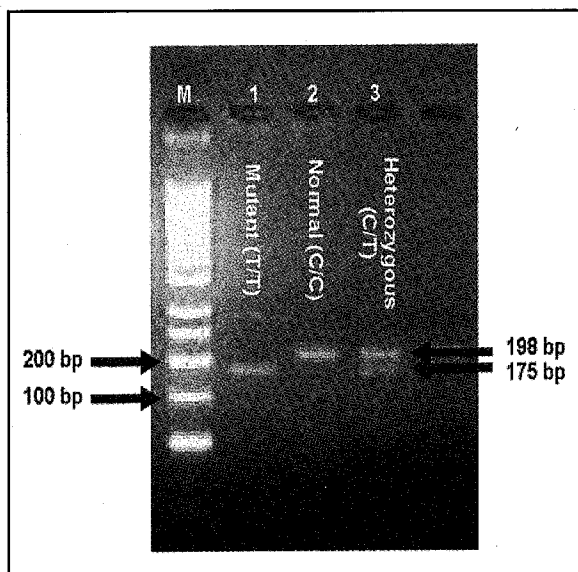^{c}. p (2-tailed) values were obtained by Pearson Correlation test.

Fig. 1 : 5.0% agarose gel electrophoresis to determine the restricted PCR products for RFLP analysis. MTHFR 677C→T missense mutation created a new restriction site GANTC for HinfI. HinfI digested the fragment into 2 parts, the shorter fragment, 23bp, and the longer fragment, 175bp. However, the 5.0% agarose gel was unable to retain the shorter fragment and it was suspected to have migrated out of the gel. Therefore, one band at 175bp (lane 1) indicates homozygous mutation (T/T), one band at 198bp (lane 2) indicates homozygous wild type (C/C) and two bands with 198bp and 175bp (lane 3) indicate the heterozygous mutation.

Discussion

The prevalence of the T allele in the Malay subjects were low compared to most of the other studies on Asian population. The frequency of the MTHFR 677C→T allele shows geographic and ethnic or racial variations. The majority of studies of Asians were done on Japanese people [24]. The prevalence of MTHFR 677 T/T mutation in Japanese (pooled) was 10% [25-31]. Recently, studies have been carried out on Chinese in Singapore (8%) [7] and Taiwan (15%) [12].

In occidental countries, the frequency appears to be high in white Hispanics from California (21%) [32], Italy (pooled estimate, 19%), Britain (pooled estimate, 13%) [24], France (10%) [33], Sweden (10%) [34], USA (pooled estimate, 9%) [24], Germany (7%) [35] and in Norway (6%) [36]. Since most of the studies were based on either convenient or relatively undefined or undocumented sampling, the applied simple random sampling of 53 subjects in this study was a comparable one.

Results in this study found that genotype frequency is not significantly different in males and females of the Malay subjects. Most published studies either do not specify the gender composition of the samples or do not comment on differences of genotype frequencies by sex. Besides that, there is also no correlation between the 677C→T allele and age. A study from Japan reported a lower C677T allele frequency in older people than in younger people and both in males than in females [37]. To some extent, Laurence et al., 1997 [38] tried to establish the relationship between the prevalence of MTHFR thermolabile variant and human longevity and he found that the frequency of MTHFR 677C→T mutation was low in centenarians and nonagenarians compared to adults (<70 years).

Thermolabile MTHFR is characterized by increased sensitivity of the MTHFR protein to increased temperature. Results show no significant differences between tHcy levels among MTHFR variants but the heterozygosity of the MTHFR

677C→T missense mutation in the study appears to have significantly (p<0.05) higher tHcy levels in non-smoking heterozygous males. Astrid and associates [39] found that the thermolabile form of MTHFR was observed in 11 of 39 premature-vascular-disease patients with mild hyperhomocysteinemia after methionine loading. Nine of those 11 patients were hyperhomocysteinemic in the fasting state. No thermolabile MTHFR was observed among 29 normohomocysteinemic patients with premature-vascular disease, and of 23 healthy subjects 1 case had thermolabile MTHFR. These findings indicate that thermolabile MTHFR is one of the causes leading to mild hyperhomocysteinemia, established by methionine loading but subjected to patients with vascular disease only.

Our results show higher tHcy level for subjects aged more than 50 years compared to other age groups but these differences were not significant. As demonstrated by Herrmann et al., 1999 [10] tHcy levels increase with age. This is associated with the age-related change in gastric and renal functions. Vitamin B_{12} deficiency could be attributed to a severe atrophic gastritis or low intake of the vitamin. The absorption of vitamin B_{12} is also reduced as a consequence of hypo- and achlorhydria. Besides that, energy requirements decrease with age and as a consequence food intake also decreases by approximately 30% by the age of 80 [40]. This in turn, lowers the intake of vitamins especially folate, B_{12} and B_6 which are essential for keeping tHcy within normal range.

Fukagawa and associates, 2000 [11] showed that females have higher remethylation rate compared to males. However, the study failed to show significant sex-related differences in fasting tHcy concentrations, responses to the oral methionine load, or rates of methionine flux based on carboxyl or methyl labels. As proposed by them, the remethylation rate might be due to sex-related differences in the methionine synthase (5-methyltetrahydrofolate-homocysteine S-methyltransferase) or the betaine-homocysteine S-methyltransferase pathway, or both. However, the

influence of sex on these pathways has not been investigated nor have sex-related differences in the activity of the enzymes involved been implicated. Besides that, lower tHcy in females may be due to higher methionine transamination and homocysteine-lowering effect of estrogen. According to Hak and associates, homocysteine was observed to be lower during the high hormonal (estrogen) phase than the low hormonal phase in women using oral contraceptives [14].

To date, there are no comprehensive studies regarding the underlying mechanisms of smoking in elevating tHcy levels. However, smoking has always been associated with coffee or alcohol consumption and sedentary lifestyles [41]. Notably, caffeine from coffee is a methyl xanthine which is known to act as a vitamin B_6 antagonist [17]. This will cause impaired transsulfuration pathway of homocysteine metabolism thus resulting in increased tHcy levels.

The weak positive correlation between WHR and tHcy levels might reflect that the tHcy levels were associated with truncal obesity instead of BMI as shown by Lone et al., 1999 [42]. According to Lone and associates, the BMI was positively associated with log tHcy in a study involving 290 young women and 288 older women. The cause and effect relationship can be hardly established between blood pressure and tHcy levels. As reported by Haynes, 2000 [43], homocysteine causes artherothrombotic events by increasing systemic blood pressure. In minipigs, experimental hyperhomocysteinemia due to chronic methionine administration increases systolic and diastolic arterial pressure. This is accompanied by vascular wall thickening, disruption of the elastic component of the vessel wall and endothelial cell hypertrophy and disruption.

The lowered tHcy levels showed by the pregnant women with folate supplementation agree with many studies that demonstrated lowered tHcy levels in subjects given folate supplementation. Lone et al., 2000 [42] found that the total folate intake from supplements and total folate intake were

inversely associated with log tHcy. Among the vitamins studied during the Homocysteine Lowering Trialists' Collaboration [44], folic acid had the dominant blood homocysteine lowering effect, and this effect was greater among subjects with higher blood homocysteine concentration or lower blood folate concentrations before treatment.

The twenty most frequently consumed foods contain necessary vitamins especially the vitamins B_{12}, B_6 and folate for maintaining the tHcy levels within the normal range. Most of the least consumed foods are main sources of protein and more specifically, methionine. Low intake of methionine based foods coupled with frequently consumed green leafy vegetables and fruits can enhance the nutritional therapeutics of hyperhomocysteinemia.

The dietary pattern of the Malay subjects could be the marked determinant that explains the normohomocyteinemia condition in most of the subjects. Since the food frequency questionnaire is a fully qualitative measurement, further association can be hardly established. A Semi-quantitative food questionnaire may be a better measurement in revealing the association between vitamin B_{12}, B_6, folate and other nutrients with tHcy levels.

Conclusion

The prevalence of MTHFR 677C→T missense mutation was 3.8% for homozygous mutation, 17.0% for heterozygous and 79.2% remains normal. Total homocysteine levels increased in subjects aged more than 50 years (12.75±5.70μmol/l for male and 9.92±1.95μmol/l for female). THcy was found to be significantly higher in male (10.45±3.81μmol/l) compared to female (7.95±2.67μmol/l) after adjusting for smoking practices. Male smokers have higher tHcy levels (12.19±3.62μmol/l) compared to non-smoking males (10.45±3.81μmol/l) but it was not statistically significant. There were only weak linear correlations between WHR, systolic and

diastolic blood pressure with tHcy. The 3 pregnant subjects under folate supplementation have lower tHcy levels (4.44±1.30µmol/l) compared to non-pregnant females without folate supplementation (7.95±2.67µmol/l).

The study could not establish association between MTHFR 677C→T variants and tHcy levels. The dietary pattern of the Malay subjects reflects a healthy diet with high preference on green leafy vegetables and fruits. The dietary pattern could be the stronger determinant of tHcy compared to genetic factors. However, tHcy levels seem to be weakly correlated with smoking, WHR, BMI, systolic and diastolic blood pressure. The finding of lowered tHcy in the 3 pregnant subjects with folate supplementation is a cost effective treatment for preventing hyperhomocysteinemia in pregnancy. Therefore, MTHFR 677C→T missense mutation in the Malay subjects did not affect the tHcy levels most probably due to the more prominent biological and environmental factors. Thus, by adjusting the lifestyles of the subjects even with the presence of predisposing genetic and irreversible biological factors, the occurrence of the adverse effects of hyperhomocysteinemia can be prevented.

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