



UNIVERSITI PUTRA MALAYSIA

**SELECTED B-VITAMINS AND HOMOCYSTEINE LEVELS, AND
METHYLENE TETRAHYDROFOLATE REDUCTASE 677 C>T
POLYMORPHISM IN UNIVERSITI PUTRA MALAYSIA SUBJECTS**

CHEW SIEW CHOO

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CHEW SIEW CHOO

**MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA**

2011

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By

CHEW SIEW CHOO

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfillment of the Requirements for the Degree of Master of Science**

October 2011

This dissertation is dedicated to all my family members. My dear father, mother, brothers and sister. A special dedication also to my beloved partners, Dr Sean Choo and his family, who have been a constant source of encouragement and support to me during my study.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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Chairman: Loh Su Peng, PhD

Faculty: Medicine and Health Sciences

Information on the MTHFR 677 C>T polymorphism and nutritional research involving B vitamins and homocysteine is limited, especially in developing countries such as Malaysia. As a result, this causes paucity in providing an overall picture of folate and homocysteine status by taking into account the genotyping aspect. Thus, this study investigated the nutritional folate intake and its blood status, focusing on the relationship of B-group vitamins (vitamin B₆ and B₁₂), serum homocysteine and the MTHFR 677 C>T polymorphism.

The study subjects were identified by simple random sampling consisting of 54 Chinese and 46 Malays at Universiti Putra Malaysia, Serdang. Dietary folate intake was assessed

with 24-hour dietary recall and a food-frequency questionnaire (FFQ). Biochemical assessment for serum and red blood cell (RBC) folate was carried out using microbiological assay, serum vitamin B₁₂ with electrochemiluminescence immunoassay, and high-performance liquid chromatography for serum vitamin B₆ and homocysteine. Polymerase chain reaction coupled with *HinfI* digestion was used for detection of MTHFR 677 C>T polymorphism.

Based on 24-hour dietary recall, the mean dietary folate intake for men and women were 260.28 and 321.93 µg/day, respectively, which equal to 65% and 81% of Malaysian Recommended Nutrient Intake (RNI). The Chinese subjects had significantly higher folate intake compared to Malay subjects with 325.45 and 261.51 µg/day, respectively, which is equal to 81% and 65% of RNI. Based on FFQ, the main dietary sources of folate were cereals, eggs and dairy products.

Overall, the mean serum folate was 9.42 nmol/L, RBC folate was 209.72 nmol/L, serum vitamin B₁₂ was 460.37 pmol/L, serum vitamin B₆ was 22.14 nmol/L, and serum homocysteine was 14.54 µmol/L. The folate deficiency reported to be 27% for serum and 78% for RBC, while for B₆ and B₁₂, both were 37% and 5%, respectively. As for homocysteine, the percentage elevation was almost half with 54%. Men had significantly lower folate levels than women. Comparing with ethnicity, Chinese showed significantly higher folate and vitamin B₁₂ levels but lower homocysteine and vitamin B₆ levels than the Malay subjects. Serum and RBC folate, serum vitamin B₆, and B₁₂ were significantly inversely related to serum homocysteine for all sexes and ethnicities.

A higher prevalence of homozygous genotype was found among the Chinese subjects. Overall, the distribution of normal, heterozygous and homozygous genotypes was 57, 30, and 13%, respectively. The folate levels differed significantly for heterozygous and homozygous genotypes under different folate intake. Similarly, the effect of homozygous genotype on homocysteine levels was more prominent under low folate intake. Blood folate, vitamin B₆ and B₁₂ were higher in normal genotype for both ethnicities whereas subjects with heterozygous and homozygous genotype showed the highest homocysteine levels.

In conclusion, high dietary folate intake helps to increase serum folate and lower the serum homocysteine levels. Individuals with the homozygous genotype were particularly sensitive to the status of several B vitamins (folate, vitamin B₆, and B₁₂). These results demonstrate an interaction between serum folate and MTHFR genotype in predicting homocysteine levels. This indicates that subjects with homozygous genotype have a higher folate requirement.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

**VITAMIN B TERPILIH DAN KANDUNGAN HOMOSISTEINA, DAN
METHYLTETRAHYDROFOLATE REDUCTASE 677 C>T POLIMORFISME DI
KALANGAN SUBJEK UNIVERSITI PUTRA MALAYSIA**

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Informasi tentang MTHFR 677 C>T polimorfisme dan kajian nutrisi melibatkan vitamin B dan homosisteina adalah terhad, terutamanya di negara membangun seperti Malaysia. Sebagai akibatnya, ini menimbulkan kekurangan dalam memberikan gambaran keseluruhan terhadap status folat dan homosisteina dengan mengambil kira aspek penjenisan gene. Oleh itu, kajian ini mengkaji pengambilan pemakanan folat dan statusnya, memfokuskan kepada hubungkait dengan vitamin B (vitamin B₆ dan B₁₂), homosisteina dan MTHFR 677 C>T polimorfisme.

Kajian ini ditentukan dengan kaedah kajian rentas melibatkan 54 subjek Cina dan 46 subjek Melayu di Universiti Putra Malaysia, Serdang. Pengambilan folat telah dinilai

menggunakan ingatan diet 24 jam dan borang kekerapan pengambilan makanan (FFQ). Penilaian biokimia untuk serum dan sel darah merah folat dianalisa menggunakan kaedah mikrobiologi, serum vitamin B₁₂ dengan *electrochemiluminescence immunoassay*, dan *high-performance liquid chromatography* untuk penentuan serum vitamin B₆ dan homosisteina. *Polymerase chain reaction* dengan pencernaan *HinfI* telah digunakan untuk pengesanan polimorfisme dalam MTHFR 677 C>T.

Berdasarkan ingatan diet 24 jam, purata pengambilan folat untuk lelaki dan wanita adalah 260.28 dan 321.93 µg/hari, masing-masing, bersamaan dengan 65% dan 81% daripada Saranan Pengambilan Nutrien Malaysia (RNI). Subjek Cina menunjukkan signifikansi pengambilan folat yang lebih tinggi berbanding subjek Melayu dengan 325.45 dan 261.51 µg/hari, masing-masing, bersamaan dengan 81% dan 65% daripada RNI. Berdasarkan FFQ, sumber diet utama folat ialah bijirin, telur beserta dengan hasil tenusu.

Secara keseluruhannya, purata serum folat adalah 9.42 nmol/L, sel darah merah folat adalah 209.72 nmol/L, serum vitamin B₁₂ adalah 460.37 pmol/L, serum vitamin B₆ adalah 22.14 nmol/L, dan serum homosisteina adalah 14.54 µmol/L. Kekurangan folat dicatatkan sebanyak 27% untuk serum dan 78% untuk sel darah merah, manakala untuk B₆ dan B₁₂, masing-masing mencatatkan 37% dan 5%. Untuk homosisteina pula, peratus peningkatan adalah lebih kurang separuh iaitu 54%. Subjek lelaki menunjukkan signifikansi serum dan sel darah merah folat yang lebih rendah daripada wanita. Berbanding dengan etnik, kaum Cina menunjukkan signifikansi folat dan vitamin B₁₂ yang lebih tinggi tetapi homosisteina dan vitamin B₆ yang lebih rendah daripada kaum Melayu.

Serum dan sel darah merah folat, serum vitamin B₆ dan B₁₂ adalah berkadar songsang dengan kandungan serum homosisteina untuk kedua-dua jantina dan etnik.

Genotip homozigot adalah lebih tinggi di kalangan subjek Cina. Keseluruhannya, taburan subjek normal, heterozigot dan homozigot ialah 57, 30 dan 13%, masing-masing. Kandungan folat bagi genotip heterozigot dan homozigot berbeza secara signifikan di bawah pemakanan folat berbeza. Demikian juga dengan kandungan homosisteina yang lebih ketara di bawah pengambilan folat yang rendah jika dibandingkan dengan pengambilan folat yang tinggi, dengan kandungan yang tertinggi dicatatkan oleh genotip homozigot. Kandungan folat, vitamin B₆ dan B₁₂ adalah lebih tinggi di kalangan genotip normal untuk kedua-dua etnik manakala subjek genotip heterozigot dan homozigot menunjukkan kandungan homosisteina yang tertinggi.

Kesimpulannya, pengambilan folat yang tinggi membantu untuk meningkatkan serum folat dan menurunkan kandungan homosisteina. Individu dengan genotip homozigot adalah lebih sensitif terhadap status beberapa vitamin B (folat, vitamin B₆, dan B₁₂). Keputusan ini membuktikan hubungan antara serum folat dan genotip MTHFR dalam peramalan kandungan homosisteina. Ini menunjukkan bahawa subjek homozigot memerlukan keperluan folat yang lebih tinggi jika dibandingkan dengan genotip yang lain.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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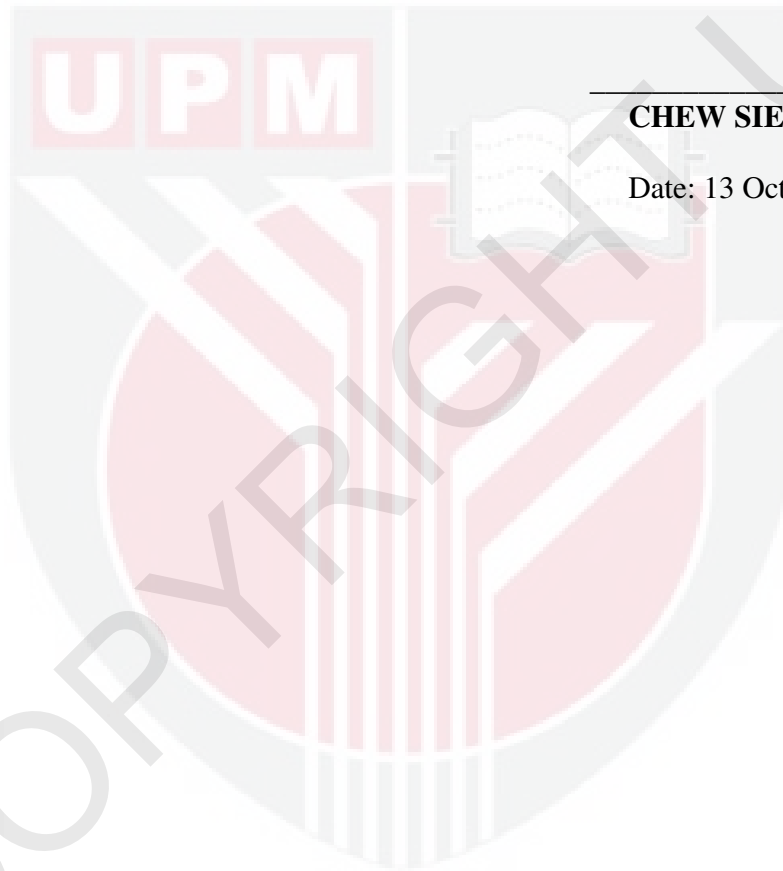
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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



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Date: 13 October 2011

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