Prevalence of Hyperhomocysteinemia in Normal Healthy Thai Subjects

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Abstract

The concentration of circulating total homocysteine is a sensitive marker of inadequate folate and vitamin B12 status. The elevations of plasma homocysteine concentration are associated with an increased risk of vascular disease. The primary goals of this study were to identify plasma homocysteine concentrations in Thai residents and to test for differences in homocysteine levels among sex and age categories. The authors measured plasma total homocysteine concentrations in 3,345 Shinawatra employees (1,133 males, 2,212 females aged between 20-65 years) by using fluorescence polarization immunoassay (FPIA) method. The mean plasma homocysteine concentrations of males and females were 11.495 and 8.547 μmol/L respectively. Plasma homocysteine concentrations were significantly lower in females than in males ($p < 0.0001$). The age-specific plasma homocysteine levels were lower in females than in males for each group, but the levels of each group was not significantly different both in males and females. When more than 12 μmol/L was used as the cut-off value, it was found that 33.6 per cent of males and 6.69 per cent of females were classified as hyperhomocysteinemia subjects. The authors concluded that the prevalence of hyperhomocysteinemia in Thai males is more common than in females. Further investigation should be done to clarify the association between serum folate, vitamin B12, vitamin B6 concentrations and plasma homocysteine concentration.

Key word : Homocysteine, Folate, Vitamin B12, Prevalence, Hyperhomocysteinemia

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Epidemiological studies have identified moderately raised concentrations of homocysteine as a potentially modifiable risk factor for coronary artery disease[1-5]. Hyperhomocysteinemia is a recently recognized risk factor that is independent of major risk factors such as diabetes, hypertension, hypercholesterolemia, and smoking. The prevalence estimates of hyperhomocysteinemia vary between 2-17 per cent in the general population and 9-38 per cent in coronary artery disease patients[6-11]. Although the mechanisms by which homocysteine promotes atherothrombosis are unknown, the epidemiological evidence for the association of hyperhomocysteinemia with atherothombotic disease is strong[12]. Evidence to support a direct role for homocysteine in the pathogenesis of vascular disease has emerged from studies showing a dynamic and inverse relationship between plasma homocysteine and vascular endothelial function. Acute hyperhomocysteinemia is associated with rapid-onset vascular endothelial dysfunction, an early manifestation of atherosclerosis[13,14]. These observations are consistent with reports of dose and time dependent effects of homocysteine on endothelial cellular function *in vitro*[15,16]. These findings suggest that even diet-related increments in plasma homocysteine contribute to the development and progression of atherosclerosis.

The aim of the present study was to establish the prevalence of hyperhomocysteinemia in healthy Thai subjects.

MATERIAL AND METHOD

Subjects

A cluster sampling survey was performed on 3,345 highly educated Shinawatra employees (1,133 males and 2,221 females) who had a high socioeconomic status. Informed consent was obtained from all respondents. This project was approved by the ethic committee, Faculty of Medicine Siriraj Hospital, Mahidol University.

Determination of plasma homocysteine concentration

Blood was drawn from fasted subjects and transferred into EDTA tubes. Plasma was immediately obtained by centrifugation at room temperature for 15 minutes at 3,000 g. Aliquots of plasma were then transferred to a -80°C freezer within 1 hour of sampling and stored for batch analysis. Plasma total homocysteine, which included the sum of protein-bound and free homocysteine, was measured by fluorescence polarization immunoassay (FPIA) method (Imx, Abbott Thailand). The coefficients of variation within and between days for the assays was ≤ 5 per cent.

Statistical analysis

All results are expressed as the mean and standard derivation (SD). Unpaired *t*-tests were carried out to compare parameters between different groups. StatView (Abacus, USA.) statistical packages were used for statistical analysis. The significant p-valve was < 0.05.

RESULTS

Plasma total homocysteine concentrations were measured for 3,345 individuals aged ≥ 20 years old. The distribution of participants by age, sex, mean plasma total homocysteine concentrations and standard deviations are presented in Table 1. The age-specific total homocysteine concentrations were lower in females than in males for each age group, but the concentrations of each group were not significantly different between males and females. The authors did not find a pattern of increased concentrations of total homocysteine with age from 20-60 years old. The percentile values of plasma total homocysteine concentrations of males and females are shown in Table 2. The 95th percentiles of the male and female group were 18.750 and 12.500 µmol/L respectively. The percentile-specific total homocysteine concentrations were lower in females than in males for each percentile level. Histograms of plasma total homocysteine concentrations of males and females are displayed in Fig. 1. Box plot of plasma total homocysteine levels of males and females are shown in Fig. 2. The mean of total homocysteine values of males and females were 11.495 and 8.547 µmol/L respectively. Plasma homocysteine concentrations were significantly lower in females than in males ($p<0.0001$). If 12 µmol/L of plasma homocysteine concentration was used as the cut-off value, 33.36 per cent and 6.69 per cent of males and females were classified as hyperhomocysteinemia (Fig. 3).

Table 1. Means and standard deviation of plasma total homocysteine concentrations by age group and sex.

	Age	Mean	Standard deviation
		μmol/L	
Males	20-25 (n=218)	11.899	5.113
	26-30 (n=384)	11.618	4.373
	31-35 (n=269)	11.377	3.962
	36-40 (n=150)	11.065	3.789
	41-45 (n=63)	10.961	2.642
	46-50 (n=32)	11.321	3.558
	51-55 (n=9)	11.008	1.537
	56-60 (n=8)	12.123	2.819
Females	20-25 (n=493)	8.719	2.453
	26-30 (n=969)	8.544	2.877
	31-35 (n=538)	8.404	2.722
	36-40 (n=140)	8.553	2.574
	41-45 (n=51)	8.419	1.793
	46-50 (n=16)	8.534	2.322
	51-55 (n=2)	8.955	0.841
	56-60 (n=3)	8.737	1.156

Table 2. Percentile values used to define categories of plasma total homocysteine by sex.

	Percentile					
	10th	25th	50th	75th	90th	95th
Males	7.892	9.168	10.700	12.803	15.124	18.750
Females	6.017	6.940	8.140	9.600	11.290	12.500

DISCUSSION

This study presents the first reference information on plasma total homocysteine concentrations in a representative sample of the Thai population. The results established that total homocysteine concentrations were not different across age groups from 20 to 60 years old, and were significantly higher in males than in females. The sex differences are consistent with observations from other studies[17-23]. The Hordaland Homocysteine Study examined 7,591 males and 8,585 females aged 40-67 years with no history of vascular disease, diabetes, or hypertension[2]. In the youngest participants (40-42 years), total plasma homocysteine concentrations in males (10.8 μmol/L) were 19 per cent higher than in females (9.1 μmol/L). Of 1,160 members of the original Framingham Heart Study cohort aged between 67-96 years, total plasma homocysteine concentrations were 10 per cent higher in males (11.8 μmol/L) than in females (10.7 μmol/L) for those aged less than 75 years[24]. The present study also demonstrated higher plasma total homocysteine concentrations in males (11.495 μmol/L) than in females (8.547 μmol/L), the difference was 34.5 per cent. The reason for the higher homocysteine concentrations in males may be explained by differences in body size, estrogen status and vitamin status. Moreover, Brattstrom L et al, established the correlation between plasma homocysteine concentrations and serum creatinine[25]. This association may indicate increased homocysteine production as a consequence of methyl group transfer during creatinine metabolism.

The homocysteine data from the present study present a unique opportunity to establish population reference ranges in a nationally representative sample of Thai subjects. In general, investigators

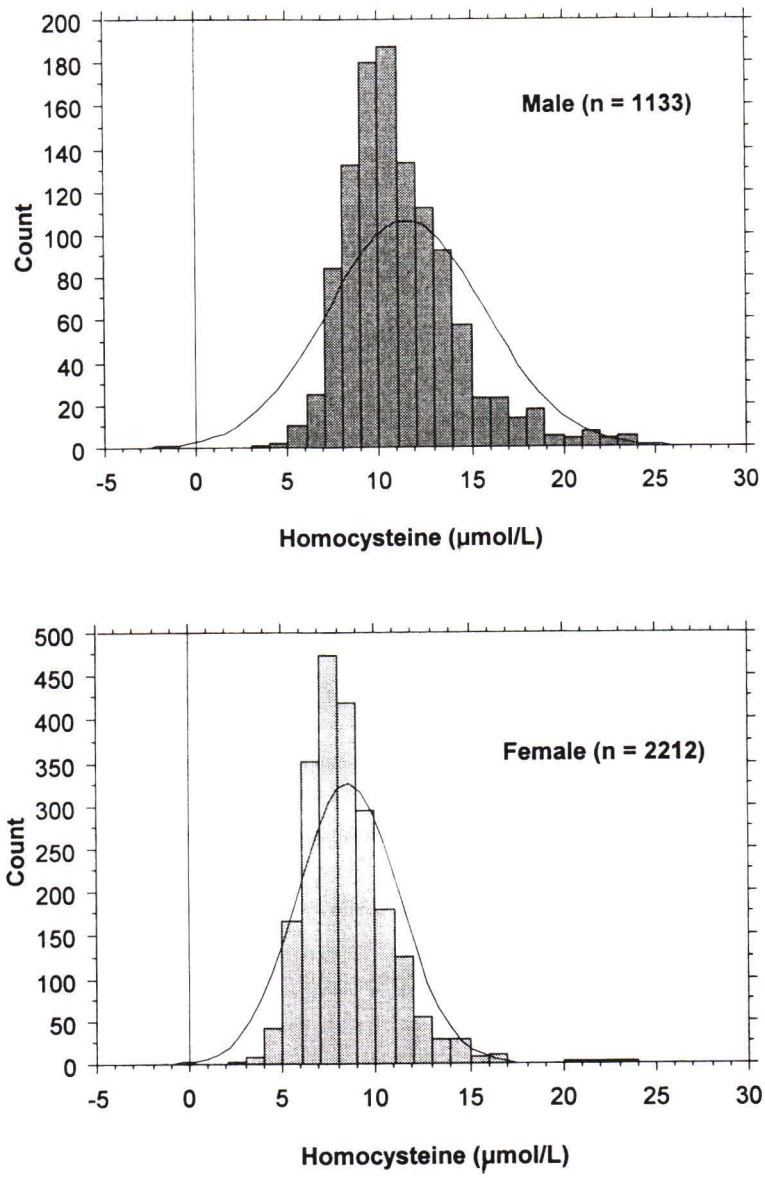

Fig. 1. Histograms of plasma total homocysteine concentrations by sex.

have recommended that reference ranges for total homocysteine concentrations should be established in populations with apparently adequate vitamin status. However, previously published references were based on various samples, for example the 97.5 percentile from a population-based sample of Norwegians was 12.6 μmol/L in females and 14.5 μmol/L in males aged between 40-42 years, and 14.5 μmol/L in females and 17.8 μmol/L in males aged between 65-67 years[26]. Other investigators calculated 95 per cent reference intervals as the mean total homocysteine + 2 standard deviations from smaller convenience samples[27,28]. Ubbink et al suggested a reference range of 4.9-11.7 μmol/L, which was calculated from the expected effects of vitamin supplementation in South African males aged

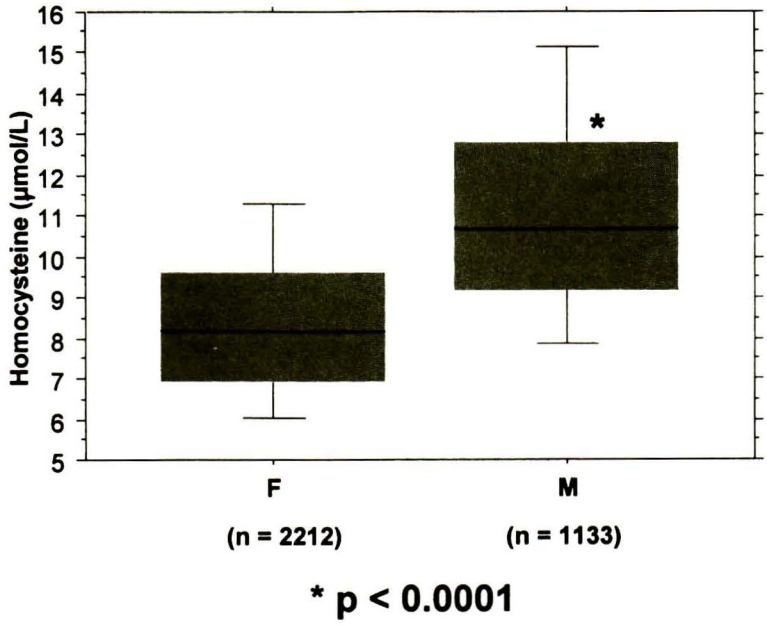

Fig. 2. Box plot of plasma total homocysteine concentrations compared between males and females.

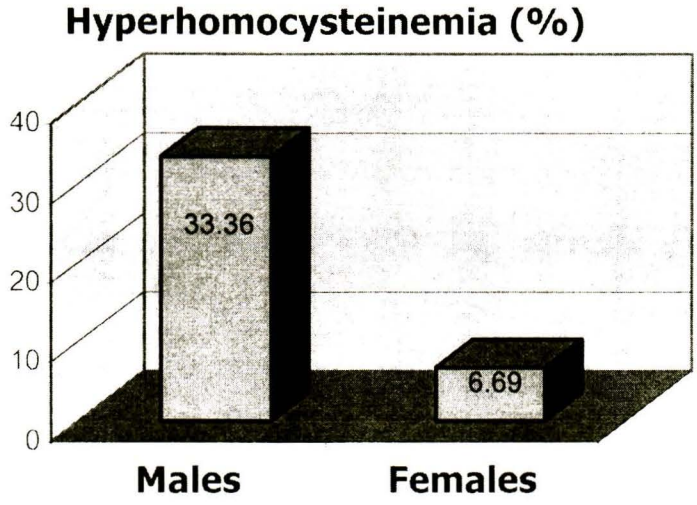

Fig. 3. Bar chart of prevalence of hyperhomocysteinemia in males and females.

18-65 years[29]. Rasmussen et al provided age-specific reference ranges based on vitamin-replete Danish males and females aged between 20-29 years to be 4.6-8.1 µmol/L, 4.5-7.9 µmol/L for females and 6.3-11.2 µmol/L for males aged between 30-59 years, and 5.8-11.9 µmol/L for males and females aged 60 years or older[30]. Joosten et al reported a reference range of 5.3-12.7 µmol/L for vitamin-

supplemented Belgian, German and Dutch participants aged between 65-95 years[28].

These data show that the prevalence of hyperhomocysteinemia in Thai males is common. However, a threshold of total homocysteine concentrations associated with an increased risk for cardiovascular disease has not been clearly established. Most studies suggested that homocysteine concentrations of 10-12.5 μmol/L or higher are associated with a significantly increased risk of vascular disease [3,31,32]. Moreover, Malinow et al reported an elevated risk of thickening of the carotid artery intimal media starting at total homocysteine concentrations as low as 8.3 μmol/L[33]. The limitation of the present study was, measured serum folate and vitamin B12 concentrations were not.

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อุบัติการณ์ของภาวะโฮโมซีสทีนในเลือดสูงในประชากรไทยสุขภาพดี

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พบว่าระดับความเข้มข้นของโฮโมซีสทีนในเลือดเป็นตัวบ่งชี้ที่ดีในการบอกถึงภาวะขาดโฟเลทและวิตามิน บี 12 นอกเหนือจากนั้นยังพบว่าการเพิ่มสูงขึ้นของระดับความเข้มข้นของโฮโมซีสทีนในเลือดยังมีความสัมพันธ์กับการเพิ่มความเสี่ยงแห่งการเกิดโรคหลอดเลือดต่าง ๆ โดยเฉพาะอย่างยิ่งโรคหลอดเลือดหัวใจ จุดประสงค์ในการศึกษาวิจัยในครั้งนี้เพื่อให้ทราบค่าความเข้มข้นของโฮโมซีสทีนในเลือดของประชากรไทยสุขภาพดีโดยเปรียบเทียบค่าดังกล่าวในช่วงอายุต่าง ๆ ตลอดจนเปรียบเทียบระหว่างเพศหญิงและเพศชาย คณะผู้วิจัยได้ทำการวัดระดับความเข้มข้นของโฮโมซีสทีนในเลือดของพนักงานบริษัทชินวัตร ที่มีสุขภาพดีจำนวน 3,345 คน ประกอบด้วยผู้ชาย 1,133 คน ผู้หญิง 2,212 คน ช่วงอายุระหว่าง 20–65 ปี โดยใช้วิธีฟลูออเรสเซ็นท์โพลาไรเซชั่นอิมมูโนแอสเส (เอฟพีไอเอ) พบว่าค่าเฉลี่ยความเข้มข้นของโฮโมซีสทีนในพลาสม่าของเพศชายและเพศหญิงมีค่าเท่ากับ 11.495 และ 8.547 ไมโครโมล/ลิตร ตามลำดับ โดยพบว่าค่าดังกล่าวในเพศชายสูงกว่าในเพศหญิงอย่างมีนัยสำคัญทางสถิติ (พี < 0.0001) ถ้าเปรียบเทียบในแต่ละกลุ่มอายุก็พบว่าในทุกกลุ่มอายุค่าความเข้มข้นของโฮโมซีสทีนในเลือดเพศชายจะสูงกว่าในเพศหญิงอย่างชัดเจนแต่ถ้าเปรียบเทียบในเพศเดียวกันพบว่าไม่พบความแตกต่างระหว่างกลุ่มอายุต่าง ๆ เมื่อใช้ค่าสูงสุดที่ระดับ 12 ไมโครโมล/ลิตร เพื่อแยกภาวะโฮโมซีสทีนในเลือดสูงออกจากภาวะปกติ พบว่าเพศชายมีผู้ที่มีระดับโฮโมซีสทีนในเลือดสูงกว่า 12 ไมโครโมล/ลิตร ถึง 33.6 เปอร์เซ็นต์ ในขณะที่เพศหญิงพบเพียง 6.69 เปอร์เซ็นต์ คณะผู้วิจัยสรุปว่า อุบัติการณ์ของการเกิดภาวะโฮโมซีสทีนในเลือดสูงพบได้บ่อยมากในประชากรเพศชาย และควรมีการศึกษาเพิ่มเติมเพื่อแสดงให้ทราบว่าการเพิ่มสูงขึ้นของระดับโฮโมซีสทีนในเลือดดังกล่าวมีความสัมพันธ์อย่างไรกับระดับของโฟเลท วิตามิน บี 12 และ วิตามิน บี 6 ในซีรั่ม

คำสำคัญ : โฮโมซีสทีน, โฟเลท, วิตามิน บี 12, อุบัติการณ์, ภาวะโฮโมซีสทีนในเลือดสูง

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