ระดับวิตามินบี 12 โฟเลท และโฮโมซีสเตอีนในกลุ่มผู้สูบบุหรี่ และกลุ่มผู้ไม่สูบบุหรี่แต่ได้รับควันบุหรี่

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3โรงพยาบาลพระมงกุฎเกล้า กรุงเทพมหานคร
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บทคัดย่อ
ความเสี่ยงที่ก่อให้เกิดโรคที่เกิดจากการสูบบุหรี่ไม่ได้จำกัดกับผู้ที่สูบบุหรี่เท่านั้น แต่ยังเกิดกับคนที่ไม่ได้สูบบุหรี่แต่ได้รับควันบุหรี่ด้วย ทั้งการสูบบุหรี่และการได้รับควันบุหรี่มีผลต่อภาวะสุขภาพและเสี่ยงต่อการเกิดโรคหลายชนิด และมีความสัมพันธ์กับการลดลงของปริมาณวิตามินบี 12 ในร่างกาย และโฟเลท นอกจากนี้ยังมีความสัมพันธ์ของการเพิ่มขึ้นของปริมาณโฮโมซีสเตอีนและเพิ่มความเสี่ยงต่อการเกิดโรคหลายชนิดด้วย การวิจัยเพื่อศึกษาผลกระทบของการสูบบุหรี่ต่อปริมาณวิตามินบี 12 ในร่างกาย โฟเลท และโฮโมซีสเตอีนในคนไทยที่สูบบุหรี่ แกนที่ไม่ได้สูบบุหรี่แต่ได้รับควันบุหรี่ และคนที่ไม่สูบบุหรี่ กลุ่มตัวอย่างเป็นเพศชายจำนวน 200 คนซึ่งเป็นอาสาสมัครเข้าร่วมการศึกษาตั้งแต่เดือนเมษายนถึงเดือนกันยายน พ.ศ. 2552 โดยแบ่งเป็นคนที่สูบบุหรี่และคนที่ไม่สูบบุหรี่แต่ได้รับควันบุหรี่กลุ่มละ 50 คนจากโรงพยาบาลพระมงกุฎเกล้า กรุงเทพฯ 50 คนเป็นผู้ที่สูบบุหรี่แบบมวนเอง จังหวัดพิษณุโลก และ 50 คนเป็นผู้ที่ไม่สูบบุหรี่จากพื้นที่เดียวกันที่ผ่านการคัดเลือกเป็นกลุ่มควบคุม โดยเก็บตัวอย่างเลือดตรวจหาปริมาณวิตามินบี 12 โฟเลท และโฮโมซีสเตอีน ผลการคัดเลือกว่าปริมาณของวิตามินบี 12 ในกลุ่มผู้สูบบุหรี่สูงกว่ากลุ่มผู้ที่ไม่สูบบุหรี่อย่างมีนัยสำคัญและ โฟเลทใน คนที่สูบบุหรี่ลดลงอย่างมีนัยสำคัญiolet ปริมาณของวิตามินบี 12 ในกลุ่มผู้สูบบุหรี่สูงกว่ากลุ่มผู้ที่ไม่ได้สูบบุหรี่ กลุ่มวิตามินบี 12 ในกลุ่มผู้สูบบุหรี่สูงกว่ากลุ่มผู้ที่ไม่ได้สูบบุหรี่และกลุ่มผู้ที่ไม่ได้รับควันบุหรี่ ส่วนปริมาณของโฮโมซีสเตอีนในกลุ่มผู้สูบบุหรี่สูงกว่ากลุ่มผู้ที่ไม่ได้สูบบุหรี่อย่างมีนัยสำคัญ เมื่อแยกกลุ่มผู้สูบบุหรี่ประเภทต่างๆ พบว่ากลุ่มผู้สูบบุหรี่จากโรงงานมีปริมาณวิตามินบี 12 ต่ำกว่ากลุ่มคนที่ไม่สูบบุหรี่ แต่กลุ่มคนที่ไม่สูบบุหรี่แต่ได้รับควันบุหรี่และกลุ่มผู้ที่ไม่สูบบุหรี่แบบมานะแต่ได้รับควันบุหรี่มีปริมาณวิตามินบี 12 สูงกว่ากลุ่มคนที่ไม่สูบบุหรี่ ส่วนปริมาณของโฟเลทพบว่า ในกลุ่มผู้สูบบุหรี่มีปริมาณโฟเลทต่ำกว่ากลุ่มคนที่ไม่สูบบุหรี่ ขณะที่ปริมาณของโฮโมซีสเตอีนเพิ่มขึ้นอย่างมีนัยสำคัญในกลุ่มผู้สูบบุหรี่จากโรงงานและกลุ่มคนที่ไม่สูบบุหรี่แต่ได้รับควันบุหรี่ เมื่อปริมาณโฟเลทกับเหตุที่ไม่ได้สูบบุหรี่ และพบว่ากลุ่มคนที่ไม่สูบบุหรี่แบบมานะแต่ได้รับควันบุหรี่
กว่ากลุ่มคนที่ไม่สูบบุหรี่แต่มิได้มีสัญญลักษณะทางสถิติ จากการศึกษาขั้นสรุปได้ว่าวิตามิน B12 และโฟเลทมีปริมาณต่ำในคนที่สูบบุหรี่จากโรงงาน และปริมาณไลโคซิเดอเนียนตุรกีในกลุ่มคนที่สูบบุหรี่จากโรงงานเมื่อเปรียบเทียบกับกลุ่มคนที่ไม่สูบบุหรี่ ซึ่งเป็นปัจจัยสนับสนุนนำไปสู่การเกิดโรคหลอดเลือดและโรคหลอดเลือดหัวใจโดยเฉพาะในกลุ่มผู้สูบบุหรี่จากโรงงาน

คำรับฟัง: ควันบุหรี่ การได้รับควันบุหรี่ วิตามิน B12 โฟเลท ไลโคซิเดอเนียน
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Vitamin B12, Folate and Homocysteine Levels in Tobacco Smokers and Passive Smokers

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Abstract

Diseases risk due to smoking is not restricted to only active smokers but also passive smokers, those exposed to environmental tobacco smoke. Both types of smoking are associated with health effects and increase the risk of several diseases. They are linked with decreased serum vitamin B12, and folate levels, but are associated with increased homocysteine and also an increased risk of cardiovascular disease. We studied the effects of tobacco smoking on serum vitamin B12, folate and homocysteine parameters in healthy Thai smokers, passive smokers, and non-smokers. Investigations were made on 200 males who participated voluntarily in the study from April to September 2009. These comprised 50 each of male smokers and passive smokers from a military unit of Phramongkutklao Hospital in Bangkok, and 50 male, self local handmade tobacco smokers from a village in Phitsanulok. Additional 50 male non-smokers from the same unit were selected as controls. Fasting blood samples were collected for determination of vitamin B12, folate and homocysteine variables. The results showed that the serum vitamin B12 of smokers were significantly higher than those of non-smokers but folate concentration of smokers were significantly lower than those of non-smokers while the homocysteine levels were significantly higher than those in non-smokers. For more detail of each smoking group the industrial tobacco smokers had serum vitamin B12 lower than those in non-smokers whereas passive smokers and local handmade tobacco smokers had serum vitamin B12 higher than those in non-smokers. For serum folate levels all groups of smokers (industrial tobacco smokers,
passive smokers and local handmade tobacco smokers) had folate levels significantly lower than those in non-smokers. Serum homocysteine levels were significantly higher in the industrial tobacco smokers and passive smokers than in non-smokers but in the local handmade tobacco smokers serum homocysteine levels were non-significantly lower than those in non-smokers. 

In conclusion, the results of this study suggested that there were low serum vitamin B12 and folate concentrations with high levels of homocysteine in the industrial tobacco smokers compared with those in non-smokers, which might contribute to the development of vascular and cardiovascular diseases especially in the industrial tobacco smokers.

**Key words:** Tobacco smoke, Passive smoking, Vitamin B12, Folate, Homocysteine

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Introduction

Tobacco consumption is one of the world’s leading causes of preventable death in the United States. In addition to economic consequences, more than 5 million deaths per year are attributed to tobacco smoking according to the World Health Organization (WHO).\(^{(1)}\) It accounts for more than 440,000 of the more than 2.4 million annual deaths. More data are currently available on the use of cigarettes and other smoked products than on smokeless tobacco. Worldwide, approximately 1.3 billion people currently smoke cigarettes or other products (almost 1 billion men, 250 million women). The number of smokers in the population of the Third World will increase from 4.5 billion to 7.1 billion by 2025.\(^{(2-4)}\) Both active and passive smoking have been related to the development of cancer and cardiovascular diseases with tobacco’s plethora of carcinogenic and volatile chemical responsible for its negative effect on human health. In 1992, the American Health Association concluded that the risk of death due to heart disease is increased by about 30% in those exposed to environmental tobacco smoke at home, and that this risk could be much higher for people exposed at the workplace, where higher levels of environmental tobacco smoke may be present.\(^{(5)}\) In 2004, the International Agency for Research on Cancer (IARC) of the World Health Organization reviewed all significant published evidence related to tobacco smoking and cancer. These meta-analyses showed that there is a statistically significant and consistent association between lung cancer risk in spouses of smokers and exposure to second-hand tobacco smoke from the spouse who smokes. The excess risk is of the order of 20% for women and 30% for men and remains after controlling for some potential sources of bias and confounding.\(^{(6)}\) The US Surgeon General, in his 2006 report, estimated that living or working in a place where smoking is permitted increases the non-smokers’ risk of developing heart disease by 25-30% and lung cancer by 20-30%.\(^{(6)}\) Cigarette smoking is associated with increased plasma levels of homocysteine\(^{(9-11)}\) and both are associated with an increased risk of cardiovascular disease. Smokers also tend to have lower levels of folate, vitamin B6, and vitamin B12,\(^{(10,12)}\) which are cofactors (vitamins B6 and B12) or cosubstrates (folate) for enzymes that control homocysteine metabolism.\(^{(13-14)}\) Folate is critical for DNA synthesis, methylation, and repair in all cells. It plays an important role in the activation of the enzyme methylenetetrahydrofolate reductase (MTHFR) and is also associated with the homocysteineremethylation pathway. Folic acid, as known of vitamin B9, is the most oxidized and stable form of folate, the water-soluble form of folic acid. Therefore, the aim of the present study was to examine vitamin B12, folate and homocysteine
parameters in healthy Thai smokers and passive smokers compared with non-smokers.

Materials and methods

The subjects in this study comprised 100 male smokers (50 smokers and 50 passive smokers) from a military unit of Phramongkutklao Hospital and College of Medicine in Bangkok, and 50 male self local handmade tobacco smokers from a village in Phitsanulok. Another 50 males who self reported as never-smokers from the same unit were selected as controls. The volunteers with diabetes mellitus, liver, lung, kidney, hypertension and cardiovascular diseases were excluded from further analysis as they were found to have abnormal biochemical parameters. None of the subjects had any major complaints of ill health and judging from their appearance and general check up they seemed to be healthy. The subjects participated in the study between April to September 2009. This study protocol was approved by the Ethics Committee of Phramongkutklao Hospital and College of Medicine for Health Statistics and informed consent was obtained before participation.

The age, marital status, socio-economic status, drinking, smoking and medicines, including past and present illnesses, were assessed through standardized questionnaires.

About 20 mL of venous blood was drawn from the study subjects in the morning, after their overnight fast. Heparinised blood was used to analyze haematological variables. A serum aliquot was stored frozen at -80 °C then analyzed for vitamin B12, folate and homocysteine within 1 month of collection to ensure the stability of the compounds.

Laboratory techniques

Haemoglobin (Hb) concentrations in whole blood were determined by using the modified cyanmethaemoglobin method. The haematocrit (Hct) values were analyzed by a micro-method using calibrated heparinised capillary tubes. The capillary with blood were centrifuged at 14,000g for 5 min then the Hct values were read using a micro-haematocrit reader (Hawksleye Son Ltd, Marlborough, UK). Platelets in peripheral blood smears were counted using the method of Nosanchuk et al. Reticulocytes were counted under an oil-immersion lens. The morphology of both red and white blood cells were determined using the Wedge method. Serum vitamin B12 and folate were measured in 200 μL samples by using radioimmunoassay commercial kits (Dualcount solid phase no boil assay for vitamin B12/folate, Diagnostic Products Corporation, Los Angeles, CA, USA). The reduced levels of vitamin B12 and folate were defined as a concentration <200 pmolL⁻¹ and 6.79 nmolL⁻¹, respectively. Homocysteine levels were measured using automated fluorescence polarization immunoassay. An elevated homocysteine level was defined as a concentration >15 μmolL⁻¹.
Statistical analysis

All data were checked for the distribution. There were abnormal distribution, the results were expressed as median, range and 95% confidence interval (CI) and non-parametric statistical analysis were calculated. The Mann Whitney U-Wilcoxon Rank Sum W-test (two tailed) was used to compare the statistical differences between groups for continuous variables. The statistical analyses were performed by using software program SPSS 12.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

The distribution of smokers according to the quantity of cigarettes smoked was shown in Fig. 1 and the characteristics of the participants both smokers and non-smokers were shown in Table 1.

![Figure 1](image.png)

**Fig. 1** Distribution of smokers according to the quantity of cigarettes smoked for the whole period of smoking (units in number of cigarette per day multiplied by duration of smoking years)
Haemoglobin and haematocrit in smokers were significantly lower than in non-smokers. Mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) of smokers were not statistically significantly different from those of non-smokers. Serum folate concentrations of all groups of smokers were statistically significantly lower than those of non-smokers, but not statistically significantly different from passive smokers. Serum vitamin B12 and homocysteine levels were statistically significantly higher in the total smokers than in the non-smokers. The highest serum vitamin B12 levels were found in local handmade tobacco smokers. In addition the smokers had a significantly higher white blood cell count than the non-smokers whereas serum folate levels were statistically significantly lower in the total smokers than in the non-smokers (Table 2).
Table 2  Median, range and 95% confidence interval (CI) of age, hematological measurements, vitamin B12, folate and homocysteine in smokers, passive smokers and non-smokers

<table>
<thead>
<tr>
<th>Variable</th>
<th>Smokers</th>
<th>Non-smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Industrial Tobacco Smokers (n=50)</td>
<td>Passive Smokers (n=50)</td>
</tr>
<tr>
<td></td>
<td>Median (95% CI)</td>
<td>Median (95% CI)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>21.0d (18.0-24.5)</td>
<td>21.0ce (19.0-24.5)</td>
</tr>
<tr>
<td>Hb (gdL⁻¹)</td>
<td>13.2abd (12.5-13.9)</td>
<td>14.3 (13.5-15.9)</td>
</tr>
<tr>
<td>Hct</td>
<td>0.421abd (0.400-0.442)</td>
<td>0.435e (0.413-0.456)</td>
</tr>
<tr>
<td>MCHC (gdL⁻¹)</td>
<td>31.6abd (30.0-33.2)</td>
<td>32.6 (30.9-34.2)</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>27.9 (27.5-28.8)</td>
<td>27.6 (26.7-29.0)</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>88.4ad (84.0-92.8)</td>
<td>84.2 (81.6-87.4)</td>
</tr>
<tr>
<td>WBC count (cellµL⁻¹)</td>
<td>7000 (6500-7385)</td>
<td>6700 (6365-7035)</td>
</tr>
<tr>
<td>Platelet count (cellµL⁻¹)</td>
<td>277300 (263400-291200)</td>
<td>254600 (241800-268000)</td>
</tr>
<tr>
<td>Vitamin B12 (pmolL⁻¹)</td>
<td>385.8b (366.51-405.09)</td>
<td>434.00c (412.30-455.70)</td>
</tr>
<tr>
<td>Serum folate (nmolL⁻¹)</td>
<td>5.28abdf (5.02-5.54)</td>
<td>6.39 (6.07-6.71)</td>
</tr>
<tr>
<td>Homocysteine (µmolL⁻¹)</td>
<td>15.33ad (14.56-16.10)</td>
<td>17.71ce (16.82-18.6)</td>
</tr>
</tbody>
</table>

Hb= Hemoglobin   Hct=Heamatocrit  (if use unit volume %, multiply the result with 100)
MCHC=Mean corpuscular haemoglobin concentration
MCH=Mean corpuscular haemoglobin   MCV=Mean corpuscular volume   WBC=White blood cell
a= significant between Industrial Tobacco Smokers and Passive Smokers
b= significant between Industrial Tobacco Smokers and local handmade tobacco smokers
c= significant between Passive Smokers and local handmade tobacco smokers
d= significant between Industrial Tobacco Smokers and non-smokers
e= significant between Passive Smokers and non-smokers
f= significant between local handmade tobacco smokers and non-smokers
*= significant between total smokers and non-smokers at p-value <0.05
**= significant between total smokers and non-smokers at p-value <0.01
Table 3 shows the proportion of smokers and non-smokers who had the abnormal haematological parameters, vitamin B12 and folate deficiency, and hyperhomocysteinemia. Of the total participants 24.2% of smokers were anaemic compared with only 4% of non-smokers using a haemoglobin concentration below 13.0 g dL\(^{-1}\) as the cut-off point. For haemoglobin the odds ratio was 6.00 which means that the smokers had 6 times significantly higher risk for anaemia than non-smokers \((p=0.006)\). For haematocrit, 19.3% of smokers and 4% of non-smokers had a haematocrit below the cut-off point of 40%. According to their MCHC, 6.7% of smokers and 8.0% of non-smokers had MCHC below the cut-off point of 33 g dL\(^{-1}\). With the cut-off points for serum vitamin B12 deficiency which levels below 150 pmolL\(^{-1}\)(203 pgmL\(^{-1}\), conversion factor 0.737)\(^{(16)}\) 28.7% of the smokers and 8.0% of the non-smokers had serum vitamin B12 deficiency. When using folate concentration below 10 nmolL\(^{-1}\)(4 ng mL\(^{-1}\), conversion factor 2.265)\(^{(16)}\) \((p= 0.026)\) as the cut-off points for serum folate deficiency 31.3% of smokers had serum folate deficiency whereas no serum folate deficiency was found in non-smokers. For serum folate the odds ratio was 0 which means that there was a large difference significantly at \(p\)-value < 0.05 between smokers and non-smokers for folate deficiency. For homocysteine 37.6% of smokers and 20% of non-smokers had hyperhomocysteinemia (homocysteine concentration >15µmolL\(^{-1}\))\(^{(17)}\).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Smokers</th>
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<th>Non-smokers</th>
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<th>Odds ratio</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heamoglobin</td>
<td>36/150</td>
<td>24.2</td>
<td>2/50</td>
<td>4.0</td>
<td>6.00</td>
<td>0.006</td>
</tr>
<tr>
<td>Heamatocrit</td>
<td>29/150</td>
<td>19.3</td>
<td>2/50</td>
<td>4.0</td>
<td>4.83</td>
<td>0.010</td>
</tr>
<tr>
<td>MCHC</td>
<td>10/150</td>
<td>6.7</td>
<td>4/50</td>
<td>8.0</td>
<td>0.83</td>
<td>0.028</td>
</tr>
<tr>
<td>Serum B12</td>
<td>43/150</td>
<td>28.7</td>
<td>4/50</td>
<td>8.0</td>
<td>3.58</td>
<td>0.008</td>
</tr>
<tr>
<td>Serum folate</td>
<td>47/150</td>
<td>31.3</td>
<td>0/50</td>
<td>0.0</td>
<td>0.00</td>
<td>0.026</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>56/150</td>
<td>37.6</td>
<td>10/50</td>
<td>20.0</td>
<td>1.87</td>
<td>0.017</td>
</tr>
</tbody>
</table>
Discussion

This is the study to identify a positive independent relationship between tobacco smoke, passive smoking and serum vitamin B12, folate and plasma homocysteine. Cigarette smoking is established as a major risk factor for coronary heart disease. The effect is caused by various factors such as sympathetic activation and inflammation.\textsuperscript{(18-19)} It has already been shown that passive smoking was linked to cardiovascular events, and in that study being a passive smoker was determined by interview alone.\textsuperscript{(20)} Folate, vitamin B6 and vitamin B12 are involved in the regulation of homocysteine, and elevation of homocysteine has been shown to be an independent risk factor for coronary heart disease.\textsuperscript{(21)} Homocysteine lies at the branch point of methionine metabolism, between the remethylation and trans-sulfuration pathway, and forms methionine and cystathionine, respectively. Hyperhomocysteinemia could be associated with the process of atherosclerosis via modification of low-density lipoprotein (LDL), a decrease in antioxidative protection, lipid peroxidation, and thrombosis. Chemical compounds found in tobacco smoke interact with the above and transform them into inactive compounds reducing their active concentration in biological fluids and possibly alter the ability of the cell to store and metabolize folate.\textsuperscript{(22)} The significantly lower serum folate levels in all groups of smokers (5.28-6.39 nmolL\textsuperscript{-1}) compared with 7.84 nmolL\textsuperscript{-1} of non-smokers found in our study most likely follow the mentioned mechanism, and other studies have confirmed the finding.\textsuperscript{(22-24)} Regarding B12 levels, we found that only the group of handmade tobacco smokers had significantly higher vitamin B12 (754.9 pmolL\textsuperscript{-1}) than non-smokers (409.22 pmolL\textsuperscript{-1}). No significant differences were found among the other groups of smokers and non-smokers except for the group of industrial tobacco smokers that had serum vitamin B12 lower than in non-smokers but not significantly. The explanation of this phenomenon is based on the studies on differences in lifestyle and poor dietary habits between smokers and non-smokers.\textsuperscript{(25)} Industrial tobacco smokers have a lesser tendency to consume whole-wheat bread, high-fiber breakfast cereals, fruits and vegetables than other groups and non-smokers. The usual dietary sources of vitamin B12 are meat and meat products, seafood, shellfish, fish, poultry and eggs. On the other hand, passive smokers and local handmade tobacco smokers (farmers and gardeners) are more likely to consume a high dietary intake. However, the method for a quantitative dietary assessment used in this investigation did not allow the measurement of folate and vitamin B12. Correspondingly were the number of cigarettes smoked per day, the industrial tobacco smokers, the time of exposure to environmental tobacco smoke, the passive smokers, and the composition of rolling paper used as a base material for local handmade
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Further study is needed in order to investigate these multifactors such as lifestyle, nutritional status especially in fruits and vegetables, exposure time to environmental tobacco smoke and composition of rolling paper that defines the roles of vitamin B12, folate and homocysteine in different smokers. The existing literature is vague regarding the relationship between smoking and serum cobalamin levels (vitamin B12). Two studies investigated into smoking during pregnancy and vitamin status suggested that there may be a dose related relationship between smoking and the metabolism of vitamin B6 and B12 while a third study (among men only) found significantly higher B12 levels among smokers (465 pmol L\(^{-1}\) vs 314 pmolL\(^{-1}\) in non-smokers) although it has been stated, that elevated B12 levels among smokers might be attributed to higher meat consumption, (since smokers are more likely to choose meat instead of fruits and vegetables). A lack of dietary folic acid leads to many health problems. Folate deficiency affects the haematopoietic system, resulting in megaloblastic anemia, which is also induced by a vitamin B12 deficiency. In addition, folate deficiency has been implicated in the carcinogenesis of several tumor types, including acute lymphoblastic leukemia, breast cancer, and gastric cancer. It is known that hyperhomocysteinemia is linked to inadequate intake of vitamins, particularly B-group vitamins, and therefore may be amenable to nutritional intervention.

Alternatively, it may be because cigarette smokers have poorer diets than non-smokers. According to several studies, the differences in lifestyle dietary habits between smokers and non-smokers could potentially account for these disparities. Smokers tend to consume fewer fruits and vegetables as a source of these vitamins. In general, smokers are known to consume fewer food items rich in antioxidants, and tend to prefer a meat and alcohol dietary pattern in comparison to non-smokers.

A relationship between blood folate and homocysteine concentration has been reported in terms of second hand exposure to cigarette smoke, i.e., passive smoking. In our study, blood folate concentration was decreased in men exposed to tobacco smoke (6.39 ± 0.32 vs 7.84 ± 1.26 nmolL\(^{-1}\) in non-smokers). In addition to Ortega et al. study, blood folate concentration was decreased in young women exposed to tobacco smoke (16.7 ± 6.5 vs 18.4 ± 6.7 nmolL\(^{-1}\) in non-smokers).

Similarly, second hand workplace exposure also increased homocysteine concentration.

We obtained the same result in our study, homocysteine concentration was increased in passive smokers (17.71 ± 0.84 vs 14.48 ± 0.72 µmolL\(^{-1}\) in non-smokers). Folate and hyperhomocysteinemia are typically found in smokers, but their role in this pathophysiological process remains controversial. Future work is clearly needed in order to comprehensively investigate and define the roles of folate and homocysteine in smokers.
Conclusion

Blood folate levels are decreased by tobacco smoking and reasonably by second-hand smoke. Low folate concentration is an independent risk factor for cardiovascular disease. The mechanism of low folate depends on the lifestyle and dietary habits, of which smokers may be consuming low folate-rich fruits and vegetables. Although hyperhomocysteinemia usually comes with low folate, the significance of hyperhomocysteinemia still remains to be illustrated in smokers. Low folate and hyperhomocysteinemia are typically found in smokers and passive smokers, but their role in the pathophysiological process is still controversial. These findings indicate that passive smoke exposure in never-smokers is associated with plasma homocysteine levels in a positive manner and may support why passive smoking is a risk factor for cardiovascular events. Further study is needed in order to investigate lifestyle, nutritional status especially in fruits and vegetables and define the roles of folate and homocysteine in smokers.

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