Short Communication
Homocysteine, Folic Acid and Vitamin C in Young Malaysian Smokers and Non Smokers: a Preliminary Report

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Abstract
Homocysteine, folic acid and vitamin C have been shown to play a role in cardiovascular diseases. As smoking is on the rise in Malaysia and the smokers are getting younger it was decided to determine the levels of homocysteine, folic acid and vitamin C in some young Malaysian smokers. Sera from 105 subjects (65 smokers, 40 non-smokers, ages 20-30 years) were analyzed for homocysteine, folic acid and vitamin C. In non smokers the mean serum folate concentration was 11.9 ± 3.1 ng/ml while the mean serum homocysteine concentration was 9.2 ± 3.1 µmol/L. In smokers the mean serum folate concentration was 7.6 ± 3.7 ng/ml while the homocysteine concentration was 10.7 ± 2.8 µmol/L. Statistical analysis revealed that the smokers had significantly lower folate concentration and higher homocysteine concentration compared to non-smokers (p ≤ 0.05). Homocysteine and vitamin C concentrations in erythrocyte were also determined. The mean erythrocyte homocysteine concentration in non-smokers was 0.29 ± 0.2 µmol/L and in smokers it was 0.24 ± 0.1 µmol/L. However no significant difference was observed between the smokers and non-smokers. Vitamin C analysis showed that there was no difference in its concentration between sera (2.51 ± 2.7 vs 1.02 ± 0.8 ng/ml) and erythrocytes (6.76 ± 2.7 vs 7.17 ± 1.2 ng/ml) of smokers and non-smokers.

Keywords: homocysteine, folic acid, vitamin C, smokers

Introduction
Homocysteine, folate and vitamin C have been shown to play a role in cardiovascular diseases. Homocysteine itself is an independent risk factor for atherosclerosis and other cardiovascular diseases. Smoking, a major public health concern, has been shown to be one of the risk factors too. In addition, researchers revealed that smoking caused the depletion or reduction of some of the important constituents in the human body, eg. folate and vitamin C were identified as two vitamins affected by smoking [1].

Folate is the generic term used to describe all compounds, found naturally in food and produced during metabolism, that display the activity of folic acid [2]. Folate is crucial in the synthesis and repair of DNA [3]. The deficiency of folate in a human body can lead to anaemia related diseases. Folate is also known to be associated with neural tube defect (NTD) in newborns, as research has shown that NTD could be reduced by the periconceptional ingestion of elevated amount of folic acid [2]. Folate deficiency also contributes to increased cardiovascular risk associated with hyperhomocysteinemia, which can eventually lead to atherosclerosis [4].

Vitamin C is crucial for its role as an antioxidant in the body to guard against harmful oxidants; such as those present in cigarette smoke [5]. Smokers are under increased oxidative stress from the toxins in cigarette smoke [6]. Antioxidant depletion has been shown to increase individual vulnerability to free radicals and other oxidant species produced by cigarette smoking and therefore lead to elevated morbidity, aging and death [6].

Apart from the decrease in some nutrient levels, smoking has also been associated with the elevation of homocysteine. Oxidation of homocysteine can generate free radicals such as superoxide and copper-catalysed hydrogen peroxide that can damage arterial endothelium [7]. Homocysteine also promotes the oxidation of low density lipoprotein (LDL) cholesterol, which can lead to heart disease [8]. It has been found that folate has an inverse relationship with homocysteine [4].

In view of the growing population of young smokers in Malaysia, it was decided to carry out a preliminary study on the concentrations of folate, vitamin C and homocysteine in a group of young smoking Malaysians. The concentration of homocysteine and vitamin C were also determined in erythrocytes as no work has been done on them.

Materials and Methods
Blood from 105 individuals (20-30 years, smokers: 65, non-smokers: 40) were collected into heparinized
Homocysteine, FA, vit c in young smokers

Informed oral consent was given by the blood donors. The collected blood was then immediately spun at 3500 rpm for 15 minutes to separate the plasma from the red blood cells. The plasma was kept separately from the red blood cells in different tubes at -20ºC until required for analysis.

Folic acid concentration was determined by using the AxSYM Folate kit (Abbott Laboratories, Illinois, USA) which was based on ion capture technology. Homocysteine level was determined by the AxSYM homocysteine kit (Abbott Laboratory, Illinois, USA) based on fluorescence Polarization Immunoassay (FPIA) while vitamin C was determined by spectrophotometric detection of the complex 2,4-dinitrophenylosazone [9].

The results were analyzed using Microsoft Excel 2000, with an added on software known as PHStat and \( p \leq 0.05 \) was considered significant.

Table 1: Mean plasma homocysteine, folate and vitamin C levels in smokers and non smokers

<table>
<thead>
<tr>
<th></th>
<th>Folate (ng/mL)</th>
<th>Vitamin C (ng/mL)</th>
<th>Homocysteine (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers</td>
<td>7.6 ± 3.7</td>
<td>1.02 ± 0.8</td>
<td>10.7 ± 2.8</td>
</tr>
<tr>
<td>Non smokers</td>
<td>11.9 ± 3.1</td>
<td>2.51 ± 2.7</td>
<td>9.20 ± 3.1</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Mean erythrocyte homocysteine and vitamin C levels in smokers and non-smokers

<table>
<thead>
<tr>
<th></th>
<th>Vitamin C (ng/mL)</th>
<th>Homocysteine (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers</td>
<td>0.68 ± 0.3</td>
<td>0.24 ± 0.1</td>
</tr>
<tr>
<td>Non smokers</td>
<td>0.72 ± 0.2</td>
<td>0.29 ± 0.2</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results and Discussion

As seen in Table 1, smokers had significantly lower folate concentration and higher homocysteine concentration in plasma compared to non smokers \( (p \leq 0.05) \). These results were parallel to what was found by Cafolla et. al. [4]. This essentially showed that smoking depleted the folate concentration but elevated the homocysteine levels in a smoker. Even young smokers were susceptible to folate depletion and sustained increase in homocysteine levels and hence maybe at risk to cardiovascular diseases. Therefore, smokers are encouraged to take supplements containing folate.

The vitamin C levels in plasma of smokers was also lower than those of non-smokers (Table 1). This was expected as tobacco in cigarettes contains the metal cadmium which, when combined with ascorbate (vitamin C), becomes more susceptible to attack by oxygen [10].

Table 2: Mean erythrocyte homocysteine and vitamin C levels in smokers and non-smokers

This will lead to the production of free radicals which can cause oxidative stress. Vitamin C is an anti-oxidant that protects the body from oxidative stress. In smokers, larger amounts of vitamin C are needed to protect the human body from the damaging effects of smoking and therefore smokers tend to have lower vitamin C levels as more vitamin C is used up by the body.

The vitamin C and homocysteine levels in erythrocytes of smokers and non-smokers showed no significant differences (Table 2). This is one of the first trials ever carried out on measurement of homocysteine and vitamin C in erythrocytes. From previous studies carried out on homocysteine and vitamin C levels in plasma, it was assumed that the same results would be expected in erythrocytes where the levels of vitamin C in smokers is lower while the homocysteine levels in smokers is higher [4]. However, contrary to what was expected, the results showed no significant differences in vitamin C and homocysteine levels in erythrocytes. The most likely explanation to this could be due to the amount of vitamin C and homocysteine present in erythrocytes being too low to be a good indicator of the actual amount of vitamin C and homocysteine present in the blood.

In conclusion smokers had significantly lower plasma folate concentration and higher homocysteine concentration compared to non smokers. The vitamin C levels in plasma of smokers was also lower than non-smokers. In erythrocytes however, there was no significant differences in the vitamin C levels and homocysteine levels between smokers and non-smokers.
References

1. Alberg A. The influence of cigarette smoking on circulating concentrations of antioxidant micronutrients, Toxicology, 2002; 180 (2): 121-137.