Public Health Nutrition: 6(7), 675–681 DOI: 10.1079/PHN2003483

Relationship of tobacco smoking with serum vitamin B_{12}, folic acid and haematological indices in healthy adults

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Submitted 15 July 2002: Accepted 6 May 2003

Abstract

Objectives: To investigate the effects of tobacco smoking on serum vitamin B_{12}, folic acid and haematological parameters in healthy Thai smokers and non-smokers.
Design: Cross-sectional study of smokers and non-smokers in a military unit in Bangkok, Thailand.
Setting: A military unit in Thailand.
Subjects: One hundred and twenty-three male smokers from a military unit in Bangkok, who participated voluntarily in the study, were investigated. Sixty-six male non-smokers from the same unit were selected as controls. Fasting blood samples were collected for investigation of vitamin B_{12}, folic acid and haematological variables.
Results: The serum folic acid concentration of smokers was lower than that of non-smokers, but was not statistically significantly different. Haemoglobin was lower in smokers than in non-smokers; 16.3% of smokers were anaemic compared with only 3.0% of non-smokers. Anaemia was not related to folate deficiency. The white blood cell count was found to be higher in smokers than in non-smokers.
Conclusion: The results of this study suggest that there were low serum folic acid concentrations in smokers compared with non-smokers, which might contribute to the development of vascular and cardiovascular diseases. The higher white blood cell count might be indicative alterations in the immune functions of smokers.

Keywords
Vitamin B_{12}
Folic acid
Haematological parameters
Smokers

The number of smokers in the population of the Third World will increase from 4.5 billion to 7.1 billion by 2025[1,2]. Smokers aged 45–64 years have a three times higher mortality rate compared with non-smokers, and those aged 65–84 years have a doubling of their mortality rate[3]. Smoking is a major health problem and risk factor for chronic disease. Tobacco use is by far the most important risk factor for most respiratory symptoms and chronic bronchitis[4–8]. There are now tens of thousands of studies linking cigarette smoking to increased morbidity and mortality from cardiovascular diseases, various forms of cancer and chronic obstructive pulmonary disease[9].

Abnormal serum folic acid and vitamin B_{12} concentrations might be the cause of homocysteine elevation, which has been recognised as an independent risk factor for vascular disease in smokers[10]. The diseases can be normalised with vitamin supplementation. Polymorphonuclear transit time was found to be delayed in the lungs immediately after smoking[11]. Via chemical inactivation, exposure to cigarette smoke may result in folic acid deficiency that principally affects the bronchial epithelium, rendering it more susceptible to neoplastic transformation by the carcinogenic hydrocarbons of tobacco smoke[12]. Several of the hundreds of chemical components of cigarette smoke, primarily organic nitrites, nitrous oxide, cyanates and isocyanates, have been shown to interact with folic acid and vitamin B_{12} coenzymes, transforming them into biologically inactive compounds[13]. Therefore, the aim of the present study was to determine vitamin B_{12}, folic acid and haematological parameters in healthy Thai smokers compared with non-smokers.

Subjects and methods

Subjects

The subjects used for this study consisted of participants from a military unit in Bangkok, Thailand. The objectives of this study were explained to the volunteers.

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One hundred and twenty-three male smokers, who participated voluntarily in the study, were investigated. Sixty-six male non-smokers from the same unit were selected as controls. The ages of the subjects were in the range of 19–60 years. Information on age, socio-economic status, lifestyle patterns such as consumption of alcohol, smoking and taking of medicines, including past and present illnesses, were obtained by questionnaires. The number of cigarettes smoked per day and the duration of cigarette smoking were multiplied together and expressed as 'cigarette-years'.

The study protocol was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, and informed consent was obtained from each participant.

In the morning, about 10 ml of venous blood was taken from each subject after an overnight fast. Heparinised blood was used to determine haematological variables. Haemoglobin concentration, haematocrit values and mean corpuscular haemoglobin concentration (MCHC) were determined. A serum aliquot was stored frozen at − 20°C for vitamin B_{12} and folic acid determinations.

Laboratory techniques

Haemoglobin concentrations in whole blood were determined by using the modified cyanmethaemoglobin method[14]. The haematocrit values were measured by a micro-method using calibrated heparinised capillary tubes. After filling the capillaries with blood, they were centrifuged for 5 min at 14 000 ***g*** in a micro-haematocrit centrifuge (IEC MB centrifuge model 3412, Massachusetts, USA). Then the haematocrit values were read using a micro-haematocrit reader (Hawksleye Son Ltd, Marlborough, UK). MCHC was also calculated by the formula:

$$\text{MCHC } (\text{g dl}^{-1}) = \frac{\text{haemoglobin}}{\text{haematocrit}} \ (\text{g dl}^{-1}) \times 100.$$

Platelets in peripheral blood smears were counted using the method of Nosanchuk *et al.*[15]. Reticulocytes were counted under an oil-immersion lens[16].

The morphology of both red and white blood cells was determined using the Wedge method, which involved making blood films, staining with Wright's stain and examining under a microscope using an oil-immersion lens (1000×)[16]. A differential leucocyte count was also performed under a microscope using an oil-immersion lens (1000×)[16].

Vitamin B_{12} and folic acid were determined from 200 µl serum samples by radioimmunoassay using commercial kits (Dualcount solid phase no boil assay for vitamin B_{12}/folic acid, Diagnostic Products Corporation, Los Angeles, CA, USA). To further minimise analytical variation, the same technician performed all assays and single lots of reagents were used. The between-run coefficients of variation for each of the parameters were less than 5% ($n = 30$ runs), corresponding to a between-run variance of 0.002. The concentrations of serum folate are reflected in dietary intake, but dietary assessment for folate intake was not recorded in this study and only the exclusion criterion of vitamin tablet intake was examined.

Statistical methods

All continuous data were examined for their distribution, skewness and kurtosis. As these were dispersed from a normal distribution, the results are expressed as median and range and non-parametric statistical analysis was used. For data processing, the Minitab computer program was utilised[7]. The Mann–Whitney *U*-test and the Wilcoxon rank sum *W*-test were used to compare the differences between smokers and non-smokers for continuous variables. The chi-square test was used to compare proportions.

To determine whether smoking was directly related to B_{12} and folic acid levels, a covariance analysis was performed taking folic acid, vitamin B_{12}, alcohol drinking and age as independent variables and smoking as the dependent variable. The statistical software program SPSS 9.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for these computations.

Results

Tables 1 and 2 show the characteristics of the participants (smokers and non-smokers) and the distribution of smokers according to the quantity of cigarettes smoked (units in numbers of cigarette-years). Age and haematological variables of smokers and non-smokers are given in Table 3. Haemoglobin, haematocrit (packed red cell volume) and MCHC in smokers were slightly and significantly lower than in non-smokers. Serum folic acid concentrations of smokers were lower than those of non-smokers, but not statistically significantly different (Table 3). Mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) of smokers were not statistically significantly different from those of non-smokers. Serum vitamin B_{12} levels were statistically significantly higher in the smokers than the non-smokers. The smokers had a significantly higher white blood cell count than the non-smokers, mainly in neutrophil and eosinophil levels (Table 3). Of smokers, 16.3% were anaemic compared with only 3% of non-smokers using a haemoglobin concentration below 13.0 g dl^{-1} as the cut-off point. Of smokers, 17.9% and 4.5% of non-smokers had a haematocrit below the cut-off point of 40%. According to their MCHC, 6.5% of smokers and 16.7% of non-smokers had MCHC below the cut-off point of 33 g dl^{-1} (Table 4). Regarding serum folic acid, 76 out of 122 (61.8%) of the smokers and 30 out of 66 (45.5%) of the non-smokers had serum folic acid levels below the cut-off point of 6.79 nmol l^{-1} (odds ratio = 1.98, $P = 0.026$) (Table 4).

https://doi.org/10.1079/PHN2003483 Published online by Cambridge University Press

Table 1 Descriptive data for smokers and non-smokers

	Smokers, *n* (%)	Non-smokers, *n* (%)
Age distribution (years)		
18–30	28/123 (22.8)	21/66 (31.8)
31–40	34/123 (27.6)	17/66 (25.8)
41–50	40/123 (32.5)	18/66 (27.3)
51–60	20/123 (16.3)	10/66 (15.2)
> 60	1/123 (0.8)	–
Education		
Primary	33/123 (26.8)	3/66 (5.5)
High	51/123 (41.5)	26/66 (39.4)
Vocational	16/123 (13.0)	14/66 (21.2)
Undergraduate/postgraduate	23/123 (18.7)	23/66 (34.9)
Status		
Low	22/122 (18.0)	13/66 (19.7)
Middle	49/122 (40.2)	20/66 (30.3)
High	51/122 (41.8)	33/66 (50.0)
Marital status		
Single	27/123 (22.0)	23/66 (34.8)
Married	92/123 (74.8)	41/66 (62.1)
Widow	2/123 (1.6)	1/66 (1.5)
Divorced	2/123 (1.6)	1/66 (1.5)
Alcohol drinking		
Not drink	11/123 (8.9)	30/66 (45.5)
Drink	106/123 (86.2)	32/66 (48.5)
Gave up	6/123 (4.9)	4/66 (6.1)
Tonic drink		
Not drink	50/119 (42.0)	49/66 (74.2)
Drink	69/119 (58.0)	17/66 (25.8)

Table 5 shows the correlation coefficients between haematological values, serum vitamin B_{12} and serum folic acid for male smokers. Significant correlations were found between vitamin B_{12}, MCH, MCV and serum folic acid. There were significantly negative correlations between MCV and serum folic acid.

To determine whether smoking is directly related to increased vitamin B_{12} concentration, or whether it is a confounder, a covariance analysis was carried out where folic acid, alcohol consumption and age were taken as independent variables and vitamin B_{12} as the dependent variable (Table 6). Age and folic acid were not found to be significantly related, under these conditions, to vitamin B_{12}. When smoking was added to the model, a significant relationship to serum vitamin B_{12} independent of alcohol consumption was found.

Table 2 Distribution of smokers according to the quantity of cigarettes smoked for the whole period of smoking (units in number of cigarette-years)*

Quantity of cigarettes smoked (cigarette-years)	*n*	%
1–5	36	29.3
6–10	34	27.6
11–15	30	24.4
16–20	18	14.6
>21	5	4.1

*Number of cigarettes per day multiplied by duration of smoking (years).

Discussion

Serum vitamin B_{12} was significantly higher in smokers than in non-smokers. The median serum vitamin B_{12} level was 464.9 pmol l^{-1} in the smokers and 313.7 pmol l^{-1} in the non-smokers (Table 3). It is known that hyperhomocystinaemia is linked to inadequate intake of vitamins, particularly B-group vitamins, and therefore may be amenable to nutritional intervention[18]. The study by Bostom and Lathrop[19] is the only one in which concentrations of all three vitamins known to influence hyperhomocystinaemia were determined. It has been recognised that smoking affects the nutritional status of folic acid, vitamin B_{12} and vitamin B_6[20–23], each of which regulates homocysteine metabolism[24]. Alternatively, it may be because cigarette smokers have poorer diets than non-smokers[25]; smokers are more likely to choose white bread, sugar, meat, butter, whole milk and eggs, and less likely to consume whole-wheat bread, high-fibre breakfast cereals, fruits and vegetables, than non-smokers[26,27]. The usual dietary sources of vitamin B_{12} are meat and meat products (including shellfish, fish, poultry and eggs) and to a lesser extent milk and milk products. The source of vitamin B_{12} in animal products is via the animal's ingestion of micro-organisms containing vitamin B_{12} or the vitamin B_{12}-producing activity of micro-organisms in the animal's alimentary tract being sufficiently high to result in absorption and storage in the animal's tissues[28]. The results obtained in this study confirm that the highest value of serum vitamin B_{12} is found in smokers. However, this result is not in accordance with the results of a study by Pagan *et al.*[29], who found serum vitamin B_{12} concentrations to be significant lower in smokers than in non-smokers. Univariate analysis of local and systemic vitamin B_{12} concentrations showed significantly lower buccal mucosa vitamin B_{12} concentrations in current smokers[22]. The 11.8% prevalence of subnormal cobalamin concentrations (< 140 pmol l^{-1}) confirms that low vitamin B_{12} concentrations are common in the elderly[30], although the prevalence may be even higher in Europe[31], where the population is largely white.

Although there is wide documentation of the adverse effects of cigarette smoking on a variety of diseases and disturbances, the direct effects of smoking on nutrient concentrations are less well studied[32]. Detrimental effects of cigarette smoke on systemic concentrations of folic acid and vitamin B_{12} have been known for decades. Many of these published studies, however, have not considered other factors, including dietary intake, which might explain the differences in folic acid or vitamin B_{12} status among smokers and non-smokers[20,33–36]. In this study, higher serum B_{12} levels in smokers could not explain the relationships between the increased levels of white blood cells found in smokers. However, Bunting *et al.*[37] reported that high apoptotic leucocyte levels were found in subjects who had low serum cobalamin. Therefore, a high level of

https://doi.org/10.1079/PHN2003483 Published online by Cambridge University Press

Table 3 Median, range and 95% confidence interval (CI) of age, anthropometric variables, haematological measurements, vitamin B_{12} and folic acid in smokers and non-smokers

	Smokers ($n = 123$)		Non-smokers ($n = 66$)		
Variable	Median (range)	95% CI	Median (range)	95% CI	*P*-value
Age (years)	40.0 (19.0–68.0)	38.0–42.0	37.0 (19–59)	32.0–42.0	0.326
Haemoglobin ($g\,dl^{-1}$)	13.9 (11.4–16.6)	13.6–14.3	14.6 (12.3–15.8)	14.2–14.8	0.009*
Haematocrit	0.430 (0.350–0.525)	0.423–0.438	0.445 (0.380–0.484)	0.432–0.450	0.027*
MCHC ($g\,dl^{-1}$)	32.6 (29.5–37.2)	32.2–32.8	33.0 (30–35.4)	32.8–32.2	0.046
MCH (pg)	28.4 (19.3–37)	27.5–28.8	28.3 (3.9–34.4)	27.6	0.855
MCV (fl)	86.3 (62.5–100.9)	81.6–87.4	85.5 (66.7–97.3)	84.0	0.828
Basophils (cells/100 WBC)	0 (0–12)	0–0	0 (0–1)	0–0	0.087
Eosinophils (cells/100 WBC)	3.0 (0–30)	2.0–3.0	2.0 (0–14)	1.0–3.0	0.070
Lymphocytes (cells/100 WBC)	33.0 (10–55)	30.00–35.00	35.50 (21–50)	32.60–38.0	0.215
Monocytes (cells/100 WBC)	5.0 (1–10)	4.0–5.0	4.0 (1–18)	4.0–5.0	0.455
Neutrophils (cells/100 WBC)	58.0 (23–85)	56.15–60.0	56.5 (38–74)	54.0–60.0	0.933
WBC count ($\times 10^9\,l^{-1}$)	7000 (3600–13 500)	6500–7385	6300 (3800–10 100)	5800–6840	0.013*
Platelet count ($\times 10^9\,l^{-1}$)	255 000 (63 000–585 000)	243 000–268 392	250 500 (138 000–365 000)	238 601–264 789	0.525
Serum B_{12} ($pmol\,l^{-1}$)	464.9 (36.9–2435.4)	373.1–546.6	313.7 (31.0–1439.1)	279.2–333.4	0.001*
Serum folic acid ($nmol\,l^{-1}$)	8.84 (0.99–32.86)	7.48–10.06	9.74 (0.48–23.79)	8.12–11.35	0.457

MCHC – mean corpuscular haemoglobin concentration; MCH – mean corpuscular haemoglobin; MCV – mean corpuscular volume; WBC – white blood cell.
*Significant difference using the Mann–Whitney *U*-test.

Table 4 Haematological parameters indicating anaemia* in smokers and non-smokers

	Smokers		Non-smokers			
Variable	*n*	%	*n*	%	Odds ratio	*P*-value
Haemoglobin	20/122	16.3	2/66	3.0	6.27	0.006
Haematocrit	22/122	17.9	3/66	4.5	4.53	0.010
MCHC	8/122	6.5	11/66	16.7	0.35	0.028
Serum B_{12}	35/122	28.5	18/66	27.3	1.07	0.837
Serum folic acid	76/122	61.8	30/66	45.5	1.98	0.026

MCHC – mean corpuscular haemoglobin concentration.
*Values indicating haematological deficiencies are, for males: haemoglobin $<13\,g\,dl^{-1}$; haematocrit <0.400; MCHC $<33\,g\,dl^{-1}$; serum $B_{12} < 147.6\,pmol\,l^{-1}$; serum folic acid $<6.79\,nmol\,l^{-1}$.

serum cobalamin might be a factor in extending the lifetime of leucocytes and related to higher numbers of white blood cells. When computing covariance, where folic acid, alcohol consumption and age were taken as independent variables and vitamin B_{12} as a dependent variable, the model with the two independent variables did not significantly determine the variation of the dependent variable, vitamin B_{12}. However, when smoking was added to the model, this variable significantly determined the variation in serum vitamin B_{12} (Table 6). Therefore, an increase in vitamin B_{12} can be assumed to be a useful protective reaction for smokers. Another reason for the increased leucocytes found in smokers may be the accumulation of polymorphonuclear white blood cells in upper and lower respiratory tract infections, which most frequently occurs in smokers. This hypothesis may require further study.

Serum folic acid concentrations of the smokers were lower than those of the non-smokers, but not significantly different. The median of serum folic acid levels in smokers was $8.84\,nmol\,l^{-1}$, and $9.74\,nmol\,l^{-1}$ in non-smokers (Table 3). It is important to mention that plasma folic

Table 5 Correlation coefficients of haematological parameters, anthropometric parameters, serum B_{12} and serum folic acid in smokers

	B_{12}	Folic acid	Haemoglobin	Haematocrit	MCH	MCHC	Platelet	MCV
B_{12}	1.000	0.274**	−0.096	−0.061	−0.045	−0.079	−0.051	−0.071
Folic acid	0.274**	1.000	0.010	0.034	−0.160*	−0.113	0.051	−0.174**
Haemoglobin	−0.096	0.010	1.000	0.794**	0.192**	0.160*	−0.058	0.217**
Haematocrit	−0.061	0.034	0.794**	1.000	0.027	−0.045	−0.036	0.090
MCH	−0.045	−0.160*	0.192**	0.027	1.000	0.598**	−0.157*	0.797**
MCHC	−0.079	−0.113	0.160*	−0.045	0.598**	1.000	−0.133*	0.435**
Platelet	−0.051	0.051	−0.058	−0.036	−0.157*	−0.133*	1.000	−0.119
MCV	−0.071	−0.174**	0.217**	0.090	0.797**	0.435**	−0.119	1.000

MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular haemoglobin concentration; MCV – mean corpuscular volume.
Significance level: *, $P < 0.05$; **, $P < 0.01$.

https://doi.org/10.1079/PHN2003483 Published online by Cambridge University Press

Table 6 Covariance analysis of folic acid, alcohol drinking and age as independent variables, and vitamin B_{12} as dependent variable, and smoking as an additional independent variable to the model

	Coefficient			
Model	β	Standard error	*T*	Significance level
(Constant)	1.162	0.150	7.755	0.000
Folic acid	-1.214×10^{-2}	0.014	−0.862	0.390
Alcohol drinking	2.590×10^{-4}	0.039	3.255	0.001
Age	4.912×10^{-3}	0.003	1.523	0.130
(Constant)	1.284	0.093	13.766	0.000
Alcohol drinking	2.476×10^{-4}	0.000	3.525	0.001
Smoking	23.007	6.546	3.515	0.001

acid is related to recent consumption, while red blood cell folic acid is an indicator of folic acid stores[38]. People who smoke cigarettes are known to differ from persons who have never smoked with respect to several lifestyle behaviours, including eating less healthful diets and drinking more alcohol[39–43]. This study found that 86.2% of the smokers drank alcohol compared with 48.5% of the non-smokers. Alcohol is a known antagonist of folic acid metabolism[44] and an interaction between alcohol and folic acid intake was reported in prospective studies of coronary heart disease, colon cancer and breast cancer. For each of these diseases, the benefit of folic acid intake in decreasing risk is stronger in alcohol users than in non-users[45–48]. With respect to folic acid metabolism, Sullivan and Herbert[49] were among the first investigators to recognise that ethanol has an effect on folic acid status that cannot be explained simply by a diminished intake of folic acid. The causes for this deficiency are presently unclear although a number of mechanisms have been proposed, including diminished dietary intake, poor absorption of polyglutamyl folic acids, decreased hepatic uptake and retention, increased urinary excretion of folic acid, impaired formation or hydrolysis of polyglutamates, and increased folic acid catabolism[50–56]. Several of the hundreds of chemical components of tobacco smoke have been shown to interact with folic acid coenzymes, transforming them into biologically inactive compounds[13]. These chemical interactions may have physiological significance, which is supported by reports of lowering circulating folic acid levels in smokers[34]. Reactive oxygen species (ROS) can be produced by cigarette smoke-induced phagocytic cells and cause oxidative damage to DNA, proteins and lipids, which may be closely related to cancer, ageing and cardiovascular disease. ROS may act as an initiator or promoter in multi-stage chemical carcinogenesis[57]. Our study also reported results in accordance with other studies in which white blood cell count in smokers was significantly higher than in non-smokers (30.0% higher leucocyte count). The higher white blood cell count might also cause the alteration of immune function.

In this study, 16.3% of smokers were anaemic compared with only 3% of non-smokers. Anaemia was not related to folic acid deficiency because the MCV of the smokers was not statistically different from that of the non-smokers (Table 3). Folic acid malabsorption also causes folic acid deficiency, although anaemia and macrocytosis are not seen consistently. This malabsorption is caused by diseases affecting the jejunum, such as celiac diseases, tropical sprue, jejunal resection or infiltrative disease such as lymphoma of the upper intestine[58]. Folic acid deficiency can also be due to an increased demand of any kind. The demand may be physiological such as haemolytic anaemia, malignant disease, inflammatory disease or psoriasis. Liver disease and alcoholism are usually associated with folic acid deficiency but the mechanisms are often multi-factorial. In addition to dietary inadequacy, varying degrees of intestinal malabsorption are induced by excessive alcohol consumption. Altered hepatic function is also a potential cause of folic acid deficiency because the liver is the major site of folic acid storage and metabolism. A recent report documented the elevation of plasma homocysteine in alcoholism[59].

This result implies that nutritional intervention plans that are specifically designed for the enhancement of folic acid status might be necessary for cigarette smokers in public health programmes. In the future, such programmes should face the challenge of providing appropriate care to maintain a high quality of life for this population group.

Acknowledgements

The authors wish to express their sincere thanks to Mr Paul Adams, for valuable suggestions to the manuscript; to volunteers from Military Unit 13, Samsen Road, Bangkok, who participated in this project; and to all staff of the Department of Tropical Nutrition and Food Science, Faculty of Tropical Medicine, Mahidol University for their co-operation in this research. This work was partly supported by funds from Mahidol University and the Free University, Berlin, Germany.

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https://doi.org/10.1079/PHN2003483 Published online by Cambridge University Press

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