

# Effect of Cigarette Smoking on Serum Homocysteine and Vitamin B<sub>12</sub> Levels in Apparently Healthy Male Smokers

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### ABSTRACT

A cross-sectional, analytical study was carried out, between July 2019 and June 2020, to evaluate the effect of cigarette smoking on serum homocysteine and vitamin B<sub>12</sub> levels in apparently healthy male smokers. A total of 90 male subjects were included in this study. Among them, 60 apparently healthy male smoker subjects aged between 20 and 60 years were considered as study group (group A); smokers were subdivided into two groups according to the pack-years of smoking history: group A<sub>1</sub> (smokers with 5-10 pack-years of smoking history) and group A<sub>2</sub> (smokers with >10 pack-years of smoking history). Another 30 apparently healthy non-smoker male subjects (age and BMI matched) were considered as comparison group (group B). All the subjects were selected from the hospital staff members of Mitford hospital and Bangabandhu Sheikh Mujib Medical University (BSMMU) hospital, Dhaka, Bangladesh, and also some from personal contact. All biochemical tests were performed in the Department of Biochemistry and Molecular Biology of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. The study parameters were serum homocysteine, and serum vitamin B<sub>12</sub>. Serum homocysteine level was found significantly higher among smokers compared to non-smokers ( $p < 0.001$ ), while vitamin B<sub>12</sub> level was found significantly lower among smokers ( $p < 0.001$ ). Moreover, these changes were more profound in the smokers with >10 pack-year of smoking history. Beside this, serum homocysteine level was positively correlated ( $r = +0.948$ ) with pack-year of smoking history ( $p < 0.001$ ), while vitamin B<sub>12</sub> level was negatively correlated ( $r = -0.844$ ) with pack-year of smoking history ( $p < 0.001$ ). Our study revealed that long term smoking has significant effects on serum homocysteine and vitamin B<sub>12</sub> levels.

**Keywords:** Cigarette smoking, duration of smoking, homocysteine, vitamin B<sub>12</sub>, smoker and non-smoker

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### INTRODUCTION

Smoking defined as a chemical toxicosis, which is able to cause detrimental effects either of acute or chronic type on different structures of the body being some of these like cardiovascular system, respiratory system and epithelial glands target organs. Smoking also causes physical addiction, primarily due to nicotine, that adversely influences smoking cessation.<sup>1</sup> A smoker is a person who smokes any tobacco product either daily or occasionally. Non smoker is a person who does not smoke at all.<sup>2</sup>

Cigarette smoking is one of the major leading causes of death throughout the world. It is responsible for a

majority of cases of chronic obstructive pulmonary disease and lung cancer and most smokers die either from these or from ischaemic heart disease. Smoking also causes cancers of the upper respiratory and gastrointestinal tracts, pancreas, bladder and kidney and increase risk of peripheral vascular disease, stroke and peptic ulceration.<sup>3</sup>

Tobacco products contain 50 established carcinogens which increase the risk of cancer. About 20% of all cancers are caused by smoking.<sup>4</sup> The chief pharmacologically active ingredients of cigarette smoke are nicotine, responsible for acute effects (1-2 mg per cigarette) and tars responsible for chronic effects (10-15 mg per cigarette). Tobacco smoke also contains carbon monoxide (1-5%) and carcinogenic substances (polycyclic hydrocarbons and nicotine derived N-nitrosamines).<sup>5</sup> Cigarettes are the most common form of tobacco consumption. Other smoking implements include pipes, cigars, biris and hookahs.<sup>6</sup> The prevalence rate of current cigarette smoking in our country is 14 %. Current cigarette smoking has been reported to be very high among males as compared to females.<sup>7</sup> One pack-year means 20 cigarettes per day for one year.

Homocysteine is a nonessential sulfur-containing amino acid derived from essential amino acid methionine.<sup>8</sup> Smokers have significantly increase plasma homocysteine level in comparison to those of non-smokers.<sup>9</sup> Cigarette smokers with high plasma homocysteine are at greatly increased risk of cardiovascular disease.<sup>10</sup> Elevated plasma homocysteine levels promote oxidative damage, inflammation, and endothelial dysfunction and are an independent risk factor for occlusive vascular disease such as cardiovascular disease (CVD) and stroke.<sup>11</sup>

Smokers have lower serum levels of vitamin B<sub>12</sub> in comparison to those of non-smokers.<sup>12</sup> Vitamin B<sub>12</sub> is essential for remethylation pathway of homocysteine which converts homocysteine into methionine.<sup>11</sup> Vitamin B<sub>12</sub> is an essential coenzyme for two enzymes involved in one-carbon metabolism: methylmalonyl-CoA-mutase and methionine synthetase.<sup>13</sup> The studies have shown that supplement of vitamin B<sub>12</sub> in patients with hyperhomocysteinaemia could reduce homocysteine levels.<sup>14</sup> Serum vitamin B<sub>12</sub> deficiency and hyperhomocysteinemia are related with cardiovascular risk factors in patients with coronary diseases.<sup>15</sup> Therefore, the present study aimed to evaluate the effect of cigarette smoking on serum homocysteine and vitamin B<sub>12</sub> levels in apparently healthy male smokers.

## METHODS

This cross-sectional, analytical study was carried out between July 2019 and June 2020. A total number of 90 male subjects (aged between 20 and 60 years) were selected purposively. Inclusion criteria for the study group (smokers) was apparently healthy male regular cigarette smokers taking at least 10 sticks per day for 10 years or e"5 pack-years of smoking history. For comparison group (non-smokers), we selected apparently healthy non-smoker male subjects. Exclusion criteria for both groups were: i) BMI e"30 kg/m<sup>2</sup>; ii) history of preexisting hypertension, diabetes mellitus, heart disease, chronic hepatic dysfunction, renal disease, nutritional derangements, malignancy, acute infection, pernicious anaemia, inflammatory bowel disease, celiac disease, malabsorption, biliary or pancreatic disease; iii) history of alcoholism or drug addiction or ex-smoker; iv) history of taking multivitamins, metformin and other drugs affecting homocysteine and vitamin B<sub>12</sub> levels.

A total of 90 male subjects were divided into 2 groups; group A (study group, smokers) consisted of 60 apparently healthy male cigarette smokers, and group B (comparison group, non-smokers) consisted of 30 apparently healthy non-smoker male subjects. Group A was again subdivided into two groups with 30 in each sub-group: group A<sub>1</sub> with smokers who had history of smoking 5-10 pack-years and group A<sub>2</sub> with smokers having >10 pack-years of smoking history. All the subjects were selected from the hospital staff members of Mitford hospital and Bangabandhu Sheikh Mujib Medical University (BSMMU) hospital, Dhaka, Bangladesh, and also some from personal contact.

Before collection of blood, detailed history about personal, family, dietary, medical and occupation of all the participants were taken and thorough physical examination of all participants were done and recorded in a data information sheet. Blood was collected from each participant for estimation of biochemical test. At first all the participants were tested for fasting blood glucose, serum creatinine and alanine aminotransferase (ALT) for exclusion of diabetes mellitus, kidney and liver diseases respectively. Then estimation of serum homocysteine and vitamin B<sub>12</sub> levels were done. All biochemical tests were performed by competitive immunoassay using direct chemiluminescent technology in the Clinical Biochemistry Laboratory of Department of Biochemistry and Molecular Biology of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh.

Data was presented as mean $\pm$ SD (standard deviation). The statistical analysis was done by using Statistical Package of Social Sciences (SPSS) version 22.0 for Windows. One-way ANOVA tests was performed for comparison among the groups and then Bonferroni tests were done to compare in between two groups. Unpaired Student's t-tests and Pearson's Correlation coefficient tests were done to compare the data as applicable. A p-value <0.05 was considered as level of significance.

The study was approved by the Ethical Review Committee of Sir Salimullah Medical College, Dhaka, Bangladesh.

## RESULTS

Table-I shows that the mean age of the smokers was 42.85 $\pm$ 9.59 years, while non-smokers' mean age was 42.00 $\pm$ 12.44 years. Regarding the mean body mass index (BMI), 23.05 $\pm$ 0.91 kg/m<sup>2</sup> and 22.67 $\pm$ 1.26 kg/m<sup>2</sup> were found among smokers and non-smokers respectively. No significant differences were observed in age and BMI between study group (smoker) and comparison group (non-smoker) (p>0.05). Hence, all the study subjects were age and BMI matched. Table-II shows the comparison of mean fasting blood glucose, serum creatinine and alanine aminotransferase (ALT) levels between smokers and non-smokers groups (p>0.05); all the values were within the normal reference range. Therefore, all the study subjects were found non-diabetic and with normal

renal and liver functions. Table-III shows the comparison of heart rate, systolic and diastolic blood pressure between smokers and non-smokers groups (p>0.05); all the values were within the normal reference range. Table-IV shows that the mean serum homocysteine levels were found 22.52 $\pm$ 3.80  $\mu$ mol/L and 11.08 $\pm$ 0.94  $\mu$ mol/L in smokers and non-smokers groups respectively (p<0.001). In contrast, serum vitamin B<sub>12</sub> levels were found 251.92 $\pm$ 26.46 pg/mL and 568.33 $\pm$ 53.26 pg/mL in smokers and non-smokers groups A respectively (p<0.001). Table-V shows that the mean serum homocysteine levels were 19.54 $\pm$ 2.67  $\mu$ mol/L, 25.49 $\pm$ 2.00  $\mu$ mol/L and 11.08 $\pm$ 0.94  $\mu$ mol/L in group A<sub>1</sub>, group A<sub>2</sub>, and B respectively. The differences among the groups were statistically significant (p<0.001). The mean serum vitamin B<sub>12</sub> levels were 268.83 $\pm$ 24.27 pg/mL, 235.00 $\pm$ 15.60 pg/mL and 568.33 $\pm$ 53.26 pg/mL in group A<sub>1</sub>, A<sub>2</sub> and B respectively. The differences among the groups were statistically significant (p<0.001). Table-VI shows that serum homocysteine level was positively correlated (r= +0.948) with pack-year of smoking duration in between two study groups (A<sub>1</sub> vs. A<sub>2</sub>). The relationship was statistically significant (p<0.001). However, serum vitamin B<sub>12</sub> was found negatively correlated (r= -0.844) with pack-year of smoking duration in between two study groups (A<sub>1</sub> vs. A<sub>2</sub>). The relationship was statistically significant (p<0.001).

**Table-I:** Age and BMI of the subjects in both groups (N=90)

Variables	Group A(smokers) (n=60)	Group B (non-smokers) (n=30)	p-value
Age (years)	42.85 $\pm$ 9.59 (25–60)	42.00 $\pm$ 12.44 (25–60)	0.721 <sup>NS</sup>
BMI (kg/m <sup>2</sup> )	23.05 $\pm$ 0.91 (20.06–24.60)	22.67 $\pm$ 1.26 (20.57–24.69)	0.113 <sup>NS</sup>

NS=not significant.

**Table-II:** Mean fasting blood glucose, serum creatinine and serum ALT levels in both groups (N=90)

Variables	Group A(smokers) (n=60)	Group B(non-smokers) (n=30)	p-value
Fasting blood glucose (mmol/L)	4.96 $\pm$ 0.52 (4.00–5.80)	4.87 $\pm$ 0.47 (4.00–5.60)	0.462 <sup>NS</sup>
Serum creatinine (mg/dL)	0.89 $\pm$ 0.08 (0.75–1.10)	0.87 $\pm$ 0.09 (0.72–1.10)	0.254 <sup>NS</sup>
ALT (U/L)	22.67 $\pm$ 6.96 (10–36)	21.50 $\pm$ 7.21 (10–36)	0.461 <sup>NS</sup>

NS=not significant.

**Table-III:** Mean pulse rate and blood pressure of the subjects in both groups (N=90)

Variables	Group A(smokers) (n=60)	Group B(non-smokers) (n=30)	p-value
Heart rate (beats/min)	77.70±6.30 (68-90)	78.27±6.12 (68-90)	0.686 <sup>NS</sup>
Systolic BP (mm of Hg)	117.33±9.09 (100-130)	117.00±9.15 (100-130)	0.870 <sup>NS</sup>
Diastolic BP (mm of Hg)	75.08±7.04 (60-85)	74.83±7.37 (60-85)	0.876 <sup>NS</sup>

NS=not significant.

**Table-IV:** Mean serum homocysteine and serum vitamin B<sub>12</sub> levels in both groups (N=90)

Variables	Group A(smokers) (n=60)	Group B(non-smokers) (n=30)	p-value
Serum homocysteine (μmol/L)	22.52±3.80 (15.20-28.80)	11.08±0.94 (9.80-13.36)	<0.001 <sup>***</sup>
Serum vitamin B <sub>12</sub> (pg/mL)	251.92±26.46 (206-315)	568.33±53.26 (480-670)	<0.001 <sup>***</sup>

<sup>\*\*\*</sup>=highly significant.**Table-V:** Mean serum homocysteine and serum vitamin B<sub>12</sub> levels in different groups (N=90)

Variables	Group A <sub>1</sub> (n=30)	Group A <sub>2</sub> (n=30)	Group B (n=30)
Serum homocysteine (μmol/L)	19.54±2.67 (15.20-22.50)	25.49 ± 2.00 (21.90-28.80)	11.08 ± 0.94 (9.80-13.36)
Serum vitamin B <sub>12</sub> (pg/mL)	268.83±24.27 (239-315)	235.00±15.60 (206-260)	568.33±53.26 (480-670)
Multiple comparison	Serum homocysteine	Serum vitamin B <sub>12</sub>	
	p-value	p-value	
A <sub>1</sub> vs A <sub>2</sub> vs B	0.000 <sup>***</sup>	0.000 <sup>***</sup>	
A <sub>1</sub> vs A <sub>2</sub>	0.000 <sup>***</sup>	0.001 <sup>**</sup>	
A <sub>1</sub> vs B	0.000 <sup>***</sup>	0.000 <sup>***</sup>	
A <sub>2</sub> vs B	0.000 <sup>***</sup>	0.000 <sup>***</sup>	

<sup>\*\*\*</sup>=highly significant.**Table-VI:** Correlation of serum homocysteine and serum vitamin B<sub>12</sub> levels with pack-year of smoking in study group (n=60)

Comparison between A <sub>1</sub> and A <sub>2</sub>	r value	p-value
Serum homocysteine (μmol/L)	+0.948	<0.001 <sup>***</sup>
Serum vitamin B <sub>12</sub> (pg/mL)	-0.844	<0.001 <sup>***</sup>

<sup>\*\*\*</sup>=highly significant.

## DISCUSSION

In our study, the mean serum homocysteine levels were found significantly higher among smokers compared to non-smokers. This finding is in agreement with the several previous studies done in different countries.<sup>16-20</sup> Moreover, when the smokers were further compared based on the duration of smoking, higher level of serum homocysteine was more marked in the smokers with more than 10 pack-



year of smoking history. This finding was in consistent with a previous study done in Tunisia.<sup>9</sup> In contrast, a study done in Iran showed no significant change in serum homocysteine levels in smokers.<sup>21</sup> Another study done in Saudi Arabia reported that homocysteine levels were similar between hypertensive and nonhypertensive smokers, but they were significantly elevated in hypertensive nonsmokers and lower in smokers with family history of diabetes.<sup>22</sup> Those findings are diametrically opposite to our study findings.

In the present study, the mean serum vitamin B<sub>12</sub> levels were significantly lower among smokers compared to non-smokers. Similar findings were reported in previous studies.<sup>10,23</sup> Moreover, when the smokers were further compared based on the duration of smoking, lower levels of serum vitamin B<sub>12</sub> were more profound in the smokers group having more than 10 pack-year of smoking history. Our finding is consistent with a previous study done in India.<sup>24</sup> In contrast, another study found that the mean serum vitamin B<sub>12</sub> level was significantly higher among smokers compared to non-smokers.<sup>24</sup> However, a study done in reported that vitamin B<sub>12</sub> levels were not affected by smoking status.<sup>25</sup>

In this study, serum homocysteine level was positively correlated with pack-year of smoking duration among the smokers ( $p < 0.001$ ). Our finding is in congruence with a previous study finding from China.<sup>14</sup> On the contrary, another study done in the United States showed a negative and insignificant correlation between serum homocysteine levels and smoking duration among smokers.<sup>26</sup> Meanwhile, we observed that serum vitamin B<sub>12</sub> level was negatively correlated with pack-year of smoking duration among the smokers ( $p < 0.001$ ). Similar observation was reported by another study done in India.<sup>24</sup> However, another study done in Turkey reported that there was no significant correlation of vitamin B<sub>12</sub> level with either number of cigarettes smoked per day or duration of smoking.<sup>27</sup>

## CONCLUSION

To summarize, serum homocysteine level is significantly higher whereas serum vitamin B<sub>12</sub> levels is significantly lower among smokers which are more profound in the smokers with >10 pack-years of smoking history. Therefore, study on the serum homocysteine and vitamin B<sub>12</sub> level might be

beneficial for prediction of future cardiovascular risk in male smokers. So, estimation of these parameters in smokers may provide further information for medical care and will minimize the smoking related other complications.

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