

## Effect of Cigarette Smoking on Serum Homocysteine and Vitamin B12 Level in Male Population of Udaipur

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### Abstract

Cigarette smoking is known to be associated with increased plasma Homocysteine. Both known to be associated with increased risk of cardiovascular disease. This study investigated the effect of smoking on serum Homocysteine and vitamin B12 concentration. Serum Homocysteine and vitamin B12 levels were measured in 300 male subjects. Out of which 150 were smokers and 150 non-smokers (controls) with an age range of 50 to 60 years. Only chronic smokers who were smoking for more than 20 years were included in the study. The concentration of Homocysteine in smokers was  $17.51 \pm 7.44 \mu\text{mol/L}$  and in non-smokers was  $8.61 \pm 5.32 \mu\text{mol/L}$ . Instead the concentration of vitamin B12 in smokers was  $346.83 \pm 125.76 \text{ pg/ml}$  and in non-smokers was  $481.43 \pm 174.65 \text{ pg/ml}$ . There was significant increase in concentration of Homocysteine in serum of chronic smokers as compared to non-smokers while it was inverse in case of vitamin B12. The concentration of vitamin B12 was significantly decreased in serum of chronic smokers as compared to non-smokers. Thus this study concludes that smoking increases Homocysteine levels and decreases vitamin B12 levels leading to increase cardiovascular disease risk among smokers.

**Keywords:** Smoking; Homocysteine; Vitamin B12; Cardiovascular disease

### Introduction

Cardiovascular disease is among the leading cause of death in developed countries. Cigarette smoking is one of the major risk factors in cardiovascular diseases for both males and females. Up to 50% of the avoidable deaths in the industrialized world have been attributed to smoking, half of which show cardiovascular disease. Endothelial injury is considered to be a key initiating event in the pathogenesis of atherosclerosis [1]. It is possible that cigarette smoking may exert its effect through endothelial damage [2].

Cigarette smoking is known to be associated with a raised plasma Homocysteine level [3-8]. Smokers also tend to have lower levels of B-vitamins, folate, vitamin B6 and vitamin B12 [4,5,9], all of which affect Homocysteine levels by acting as cofactors (vitamin B6 and B12) or co-substrate (folate) for the enzymes controlling Homocysteine metabolism [10-15]. Elevated levels of total Homocysteine has emerged as an important risk factor in the assessment of cardiovascular disease [16-18]. Excess Homocysteine in the blood stream may cause injuries to arterial vessels due to its irritant nature, and result in inflammation and plaque formation, which may eventually cause blockage of blood flow to the heart. Therefore, Hyper Homocysteinemia has been considered as an independent risk factor of coronary artery disease (CAD). A positive association between elevated plasma total Homocysteine levels and a number of cardiovascular risk factors, smoking, particularly, was shown in study conducted in Norway [19]. It has already been reported about an amplifying effect of cigarette smoking on Homocysteine associated cardiovascular risk [20].

The aim of this study is to investigate the effect of cigarette smoking on plasma Homocysteine and vitamin B12 concentrations in males of Udaipur city.

### Materials and Methods

A total number of 300 subjects were evaluated at Arth Diagnostics Private Limited, Udaipur. 150 male chronic cigarette smokers who were smoking for more than 20 years, with an age range of 50 to 60 years, were included for this study after obtaining written informed consent (Group I). 150 male non-smokers, whom age and weight was approximately matched with the subjects in (Group I) were recruited as controls (Group II). Controls were clinically healthy and from a similar background to cases as possible.

The following criteria were used for exclusion:

1. Alcoholics
2. Ex-smokers
3. Diabetes mellitus
4. Renal disease
5. Hypertension
6. Previous and family history of coronary heart disease
7. Acute infection
8. Chronic hepatic dysfunction
9. Nutritional derangements
10. Malignancies

All patients were free of drugs which would influence the plasma Homocysteine levels including multivitamins. Venous blood samples were collected after 12 hours of an overnight fast into plain tubes. Serum was obtained by centrifugation and samples were immediately separated into aliquot and stored at  $-20^{\circ}\text{C}$  until analysed. Homocysteine levels were analysed by enzymatic assay on fully auto-analyser of Roche, Cobas Integra 400 and its reference range was  $4.44-13.56 \mu\text{mol/L}$  and vitamin B12 concentrations were analysed by

chemiluminescence (Elecsys) immunoassay of Roche, Cobas e411 and its reference range varied between 200-835 pg/ml for adults.

Vitamin B12 is an essential cofactor for two enzymes involved in one-carbon metabolism: methylmalonyl-CoA-mutase (reduced function of this enzyme results in increased serum methylmalonic acid (MMA) levels) and methionine synthetase (this enzyme catalyzes the remethylation of Homocysteine to methionine). A serum B12 level below the normal expected range may indicate B12 deficiency. However, a B12 level within the low normal range does not exclude B12 deficiency; symptomatic patients need to be further evaluated with MMA, folic acid, and Homocysteine. The Elecsys vitamin B12 assay employs a competitive test principle using intrinsic factor specific for vitamin B12. Vitamin B12 in the sample competes with the added vitamin B12 labelled with biotin for the binding sites on the ruthenium-labelled intrinsic factor complex.

Homocysteine is a thiol containing amino acid produced by the intracellular demethylation of methionine. Total Homocysteine represents the sum of all forms of Homocysteine including forms of oxidized, protein-bound and free. Homocysteine Enzymatic Assay is based on a novel enzyme cycling assay principle that assesses the co-substrate conversion product instead of assessing co-substrate or Homocysteine conversion products of Homocysteine.

## Results

As shown in Table 1, serum Homocysteine concentrations were significantly higher in smokers than in non-smokers. Also, there was significant variation in the levels of vitamin B12 in both the groups as shown in Table 2. Vitamin B12 concentrations were significantly lower in smokers as compared to non-smokers.

	Group I	Group II	p-value
	Smokers (mean $\pm$ SD)	Non Smokers (mean $\pm$ SD)	
Homocysteine $\mu$ mol/L	17.51 $\pm$ 7.44	8.61 $\pm$ 5.32	<0.05

Table 1: Serum homocysteine concentrations.

	Group I	Group II	p-value
	Smokers (Mean $\pm$ SD)	Non Smokers (Mean $\pm$ SD)	
Vitamin B12 pg/ml	346.83 $\pm$ 125.76	481.43 $\pm$ 174.65	<0.05

Table 2: Serum Vitamin B12 concentrations.

## Discussion

In this study data revealed that in cigarette smokers, serum Homocysteine levels was almost double i.e., 103.3% higher than non-smokers. Several mechanisms might explain the increased risk in smokers with raised serum Homocysteine. Smoking affects the vascular tree via several different interactive mechanisms [21]. Nicotine and carbon monoxide separately produce tachycardia, hypertension and vasoconstriction and both produce direct endothelial damage. Smoking also affects vaso-occlusive factors such as platelet aggregation, plasma viscosity and fibrinogen levels [21]. Hyperhomocysteinaemia has been associated with impaired endothelial function and abnormal flow mediated vasodilation has been demonstrated with mild hyperhomocysteinaemia [22-24]. It may also damage the vascular tree via platelet activation, lipid peroxidation, enhanced tissue factor activation, reduced Von Willebrand factor; increased fibrinogen levels and smooth muscle proliferation [25]. The Hordaland [6-8] and other studies [3-5] have shown higher homocysteine levels in smokers, as well as in males and older subjects.

Data also showed serum vitamin B12 was 27.9% lower in smokers as compared to non-smokers. The results obtained in our study are in accordance with the results of those from Pagan et al [4] who found plasma vitamin B12 concentrations to be significantly lower in smokers than in non-smokers. Thus smoking affects the nutritional

status of smokers. Subar et al. [26] have studied food and nutrient intake differences between smokers and non-smokers based on data from the Second National Health and Nutrition Survey (NHANES II) and found that smokers have a lower intake of most vitamins and were less likely to have consumed fruit, vegetables, vitamin and mineral supplements. In the Caerphilly Heart Disease Study [27] total nutrient intake was also found to be lower in smokers than in non-smokers.

## Conclusion

Serum Homocysteine concentration was found to be significantly high in cigarette smokers as compared to non-smokers. In contrast, vitamin B12 levels were low in smokers which lead to derangement of Homocysteine metabolism. Thus, smokers with high serum Homocysteine concentration are at greatly increased risk of cardiovascular disease and should be counselled to help them cease smoking. In addition, supplementation of multivitamins may be appropriate for such chronic smokers.

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