

## ORIGINAL ARTICLE

## Folic Acid and Vitamins B12 Levels and Their Correlation in Cigarette Smoker with Hyperhomocysteinemia

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**Abstract:** To estimate the levels of folic acid and vitamin B12 and their relationship in cigarette smoker with hyperhomocysteinemia. 30 male cigarette smokers in the age group of 30 to 60 years were studied for estimation of serum folic acid, vitamin B12 and total homocysteine (tHcy) over a period of 6 months. These tests were determined by means of Immulite 1000 analyzer. The statistical analysis of smoker group compared with normal control group, showed significant ( $p < 0.001$ ) decreases in serum folic acid, vitamin B12 levels whereas increased concentration of total homocysteine in all 30 smoker subject indicate hyperhomocysteinemia. A positive and significant correlation was observed between folic acid and vitamin B12 in smoker. The present study found a significant association between serum folic acid, vitamin B12 levels and their deficiency in smoker with hyperhomocysteinemia indicate cardiovascular risk in cigarette smoker with hyperhomocysteinemia.

**Key Words:** Folic acid, vitamin B12, hyperhomocysteinemia, cigarette smoker.

### Introduction

In the Western world, smoking is strongly associated with cardiac complications and is the biggest single avoidable health style contributing to chronic health problems. [1]. Up to 50% of avoidable deaths in the industrialized world have been attributed to smoking, half of which are cardiovascular [2-3]. Hypercholesterolemia and smoking have been recognized as major risk factors for cardiovascular diseases. A new class of emerging risk factor for cardiovascular diseases is increased homocysteine levels in serum. Homocysteine is a sulphur containing amino acid that results as an intermediary product of the methionine metabolic pathway. Life style and genetic defects such as folic acid and vitamin B12 deficiencies can play a role in increase hyperhomocysteinemia [4].

Hyperhomocysteinemia is a new cardiovascular risk factor that could be modified by reducing homocysteine intake or by supplementing the diet with vitamin B12 [5]. Cigarette smoking is known to be associated with a raised homocysteine levels [6-9]. Smokers also tend to have lower levels of the folic acid and vitamin B12 [7-8,10], all of which affect homocysteine levels by acting as co-enzyme i.e. vitamins B12 or co-substrate i.e. folic acid for the enzymes controlling homocysteine metabolism [11-14]. We studied the concentrations of serum folic acid, vitamin B12 and tHcy concentrations in cigarette smoker.

## Materials and Methods

A total of 30 male cigarette smoker of moderate socio-economic status from region of Mumbai were selected for this study. Thirty non-smokers was someone who had never smoked or who had not smoked for more than 10 years, normal renal and liver functions and belonging to the same socio-economic background were also included as a control group. The age of both smoker and non-smoker volunteers was between 30 to 60 years and had never use of medications such as therapy involving S-adenosyl methionine, carbamazepine, phenytoin, 6-azauridine, anthopterin, antifolates, anticonvulsant agents, tamoxifen, and theophylline. Subjects with diabetes mellitus, cancer, anemia, and systemic illness were excluded from this study.

*Blood sample collection:* Venous blood samples were collected in test tube with aseptic precautions. After 2 hours of collections sample was centrifuged at 3000 rpm for 5 minutes. Serum was separated and collected in polythene tube with cork. The sera with no sign of hemolysis used for the analysis of folic acid, vitamin B12 and total circulating homocysteine (tHcy).

*Biochemical Analysis:* Serum folic acid estimated by the method of boil, competitive, liquid-phase, ligand-labeled, protein binding chemiluminescent assay with in situ immobilization, and with an anti-ligand detection system. The solid phase, a polystyrene bead enclosed within an Immulite test unit, is coated with murine monoclonal antibody specific for folic acid binding protein [15]. Serum vitamin B12 concentration was evaluated by solid phase, competitive chemiluminescent assay method [16]. Serum tHcy concentration was measured by competitive chemiluminescent enzyme immunoassay method [17]. We used fully automated enzyme amplified chemiluminescent immunoassay based Immulite 1000 analyzer. Measurement of these blood parameters by using commercial kits from Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA.

*Statistical Analysis:* Numerical variables were reported in terms of mean and standard deviation. Statistical analysis of results was done by normal 'z' test. In this analysis, variables showing p value less than 0.05 and 0.001 were considered to be statistically significant and highly significant respectively. Pearson correlation test was used to test.

## Results

Table-1 showed significant change in serum profile when control group was compared with smoker group. As can be seen, significant decreases ( $p < 0.001$ ) were observed in serum folic acid and vitamin B12 whereas, tHcy levels showed significant increase ( $p < 0.001$ ) in cigarette smoker as compared to control group.

Table 1: Serum folic acid, vitamin B12 and total homocysteine in controls and cigarette smoker.

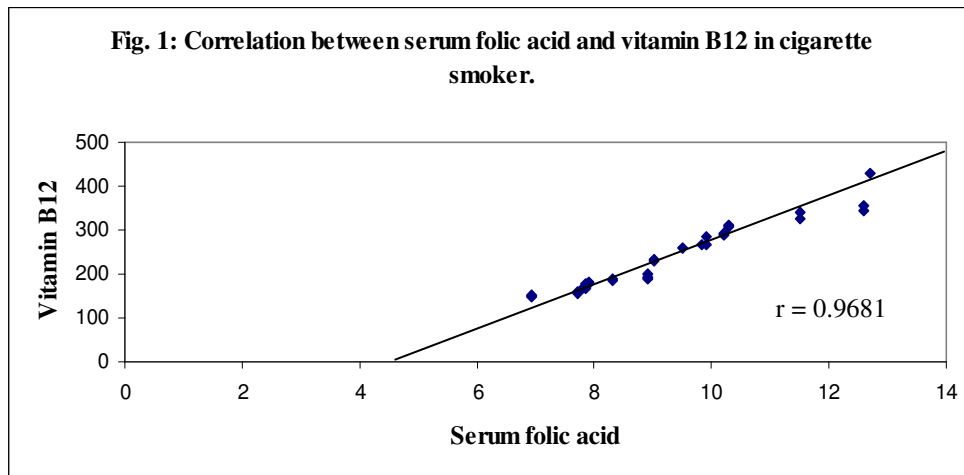
Sr. No.	Biochemical Parameters	Control (n=30)	Smoker (n=30)
1.	folic acid, (ng/mL)	12.2±2.10	9.29±1.65**
2.	Vitamin B12 (pg/mL)	409±112	238±74.9**
3.	tHcy (µmol/L)	6.88±1.15	28.6±7.95**

The results were compared between control and smoker group. The values are presented in mean ± S.D. \*\*P < 0.001.

**Discussion**

Smoking is known to be positively related with an increased homocysteine concentration in serum. Both are associated with an increased risk of cardiovascular disease. The tHcy level more than 15 µmol/L termed as hyperhomocysteinemia [18]. In our study group, hyperhomocysteinemia observed in all smoker subjects.

In the present study, indicate reduced levels of folic acid and vitamin B12 in smokers, adjustment for nutrient levels had an insignificant effect on risk estimates, highlighting the robustness of smoking as a cardiovascular risk factor. Smoking may produce vitamin deficiency in individual tissues; reduced red cell and buccal mucosal B12 and folic acid levels in smokers observed, with evidence of cell damage in these tissues reported by Piyathilake et al [19] and [20]. Such an effect contributing to overall increased risk of cardiovascular diseases in smokers would not be reduced by adjustment for plasma nutrients and must be considered.



Hyperhomocysteinemia observed in all 30 smoker subjects in our study. Various authors have been performed to demonstrate association between homocysteine levels and cardiovascular diseases [21-22]. Many of them have found a significant association [23-24] but others have failed to show this association [25-26]. Differences in the characteristics of the study population such as nutritional habits,

the use of vitamin supplements or ethnic differences could explain the contradictory results [27].

We observed a positive and significant correlation ( $r=0.9681$ ) between serum folic acid and vitamin B12 in smoker cases as per Fig 1, indicates folic acid level decreases with decrease in vitamin B12 level. Nicotine is associated with a lower nutritional intake and thus with reduced blood levels of folic acid and vitamin B12 [28-29]. A good correlation between serum folic acid and vitamin B12 levels in our study support this view.

From this study it can be concluded that, smokers with hyperhomocysteinemia are at greatly increased risk of cardiovascular disease and atherosclerosis should therefore be offered intensive advice to help them cease smoking. This case control study suggests that smokers with have reduced levels of folic acid and vitamin B12 with hyperhomocysteinemia that modulate homocysteine metabolism. They also while this finding may reflect a direct effect of smoking or reduced B-vitamin intake, supplementation of these nutrients may be appropriate in smokers with hyperhomocysteinemia.

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