



To Assess the Level of Vit B12, Vit C and Homocysteine in Chronic Smokers in Central India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Smoking is related to an increased risk of morbidity and mortality. Cigarette smoking has been related to higher levels of homocysteine in the blood. Both have been associated with a higher risk of cardiovascular disease. Smokers also have lower levels of vitamin B12, which affects homocysteine levels by serving as a cofactor or co substrate (folate) for the enzymes that regulate the metabolism of homocysteine.

Materials: This study was a case-control study, conducted in the Department of Biochemistry and medicine at DMMC & SMHRC, Nagpur in collaboration with ABVRH, Sawangi (Meghe). In the present study, the total number of subjects included was 200 having age Group between 30-60. The subjects were divided in two groups. Group 1: 100 subjects chronic smokers and Group 2: 100 subjects non- smokers.

Results: The homocysteine concentration significantly increased in group 1 (chronic smokers) 18.50 ± 8.40 as compared to group 02(non smokers) 8.30 ± 5.30 and p value $p < 0.005$. vitamin B12

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concentration significantly decreased in group 01 (chronic smokers) 340.80 ± 124.70 as compared to group 02 (non smokers) 485.45 ± 175.68 and p value $p < 0.005$. vitamin c concentration significantly decreased in group 1 (chronic smokers) 0.46 ± 0.07 as compared to group 02 (non smokers) 1.12 ± 0.15 and p value $p < 0.005$.

Conclusion: On the basis of findings we concluded that the smoking increases Homocysteine levels and lowers the level of Vit B12 and Vit C leading to increase cardiovascular disease risk among chronic smokers.

Keywords: Smoking; Homocysteine; Vit B12; Vit c; CVD.

1. INTRODUCTION

In developing countries, cardiovascular disease is one of the leading causes of death. For both men and women, cigarette smoking is a significant risk factor for cardiovascular disease. Smoking is responsible for up to half of all preventable deaths in the developed world, with cardiovascular disease accounting for the other half. Endothelial injury is considered to be a key trigger in the development of atherosclerosis [1]. In developing countries, cardiovascular disease is one of the leading causes of death. Homocysteine is a metabolite of the basic amino acid methionine that acts as an intermediate. In people with hereditary homocystinuria, the correlation between elevated circulating homocysteine levels and premature vascular thrombotic events is well founded [2].

Due to its irritant nature, too much homocysteine in the bloodstream can cause damage to arterial vessels, resulting in inflammation and plaque formation, which can lead to a blockage of blood flow to the heart. As a result, hyper homocysteinemia is now thought to be a separate risk factor for coronary artery disease (CAD). A study conducted in Norway found a relation between elevated plasma total homocysteine levels and a number of cardiovascular risk factors, especially smoking [3]. Total homocysteine levels have been reported as a major risk factor in the estimation of cardiovascular disease [4].

Smokers also have lower levels of vitamin B6 and B12, both of which affect homocysteine levels by acting as cofactors (vitamins B6 and B12) or co substrates (folate) for the enzymes that regulate homocysteine metabolism [4].

Vitamin C is a water-soluble free radical scavenger that can specifically scavenge O₂ and OH⁻ radicals and aid in the neutralisation of physiological oxidant stress from both exogenous and endogenous sources [5].

However, vitamin C oxidised during this phase. The ascorbyl radical is formed after an electron is lost, and it is relatively stable with a half life of 105 seconds and is relatively unreactive, which explains ascorbic acid's antioxidant role [6]. The rapid oxidation of ascorbic acid by free radicals may be the mechanism causing vitamin C deficiency in smokers [7].

The aim of this study is to investigate the effects of cigarette smoking on plasma Homocysteine , vitamin B12 and vitamin c concentrations in males Vidarbha region of central India.

2. MATERIAL AND METHODS

This study was a case-control study, conducted in the Department of biochemistry and medicine at DMMC & SMHRC, Nagpur in collaboration with ABVRH, Sawangi (Meghe). Study participant were selected who attended medicine OPD for medical reasons except mentioned below.

2.1 Study Population

In the present study, the total number of subjects included was 200 having age Group between 30-60. The subjects were divided in two groups.

- Group 1: 100 chronic smokers
- Group 2: 100 non- smokers.

2.2 Inclusion Criteria

subjects aged between 30-60 years, non smokers and smokers (cigarette smoking) of >10 years duration.

2.3 Exclusion Criteria

Alcoholics, Ex-smokers, Diabetes mellitus, Renal disease, Hypertension, Previous and family history of coronary heart disease, Acute infection
8. Chronic hepatic dysfunction, Nutritional derangements, Malignancies etc.

2.4 Sample Collection

Blood was collected from each subject by venipuncture with standard blood collection technique in a plain vial for serum separation. Participant asked to come after overnight fast before blood collection. Quantity of blood is 5 ml. stored as per standard guidelines

2.5 Biochemical Analysis

- Estimation of homocysteine were analysed by enzymatic assay on fully autoanalyzer of Cobas Integra 400.
- Estimation of vitamin B12 concentrations were analysed by chemiluminescence (Elecsys) immunoassay.
- Estimation of vitamin c concentration by 2, 4 – dinitrophenyl hydrazine method.

3. RESULTS

Table 1: show the Homocysteine, vitamin B12 and vitamin c concentration in chronic smokers and non-smokers subject.. the homocysteine concentration significantly increased in group 1 (chronic smokers) 18.50±8.40 as compared to group 02(non-smokers) 8.30±5.30 and p value p<0.005.vitamin B12 concentration significantly decreased in group 01 (chronic smokers) 340.80±124.70 as compared to group 02 (non-smokers) 485.45±175.68 and p value p<0.005. vitamin c concentration significantly decreased in group 1 (chronic smokers) 0.46±0.07 as compared to group 02 (nonsmokers) 1.12±0.15 and p value p<0.005.

4. DISCUSSION

Smoking induced cancer and associated deaths are worlds one of major cause of preventable death [8]. therefore smoking cessation and prevention are essential public health priorities. According to the World Health Organisation, India's tobacco-related deaths could reach 1.5 million per year by 2020. Wide household surveys in recent years have revealed that in middle age, more than one-third of men and a

small percentage of women smoke cigarettes, with an estimated 120 million smokers in India [9,10].

Several mechanisms may explain the increased risk in smokers with elevated homocysteine levels in their blood. Smoking has many interconnected pathways that influence the vascular tree. Both nicotine and carbon monoxide cause tachycardia, hypertension, and vasoconstriction, as well as endothelial harm. Smoking affects platelet aggregation, plasma viscosity, and fibrinogen levels, among other vaso-occlusive factors [11]. The intracellular demethylation of methionine produces homocysteine, a thiol-containing amino acid. Complete homocysteine is the sum of all homocysteine types, including oxidized, protein-bound, and free homocysteine. The Homocysteine Enzymatic Assay is based on a novel enzyme cycling assay concept that tests the substrate conversion product rather than the substrate or homocysteine conversion products [12].

Vitamin C is the first powerful reductant in the aqueous process, readily reacting with cigarette smoke oxidants and offering substantial cell safety. Studies involving various forms of oxidative stress have shown that ascorbic acid effectively avoids observable oxidative damage under all types of oxidative stress, indicating that it may be useful in the prevention of diseases in which oxidative stress plays a causative or exacerbating function.

B12 deficiency can be indicated by a serum B12 level that is below the normal expected range. A B12 level in the low normal range, on the other hand, does not rule out B12 deficiency. Patients with symptoms should be tested for MMA, folic acid, and homocysteine. A competitive test principle is used in the vitamin B12 assay, which uses an intrinsic factor specific for vitamin B12. The added biotin-labeled vitamin B12 competes with the vitamin B12 in the study for binding sites on the ruthenium-labeled intrinsic factor complex [13].

Table 1. Show the Homocysteine, vitamin B12 and vitamin c concentration

Parameter	Group 01(chronic smokers)	Group 02 (non-smokers)	p-value
Homocysteine µmol/L	18.50±8.40	8.30±5.30	P<0.005
Vitamin B12 pg/ml	340.80±124.70	485.45±175.68	P<0.005
VITAMIN C (mg/dl)	0.46±0.07	1.12±0.15	P<0.005

Vitamin B12 concentration significantly decreased in group 01 (chronic smokers) 340.80 ± 124.70 as compared to group 02 (non-smokers) 485.45 ± 175.68 ($p < 0.05$) vitamin c concentration significantly decreased in group 1 (chronic smokers) 0.46 ± 0.07 as compared to group 02 (non-smokers) 1.12 ± 0.15 ($P < 0.05$).

Hordaland [14] and other study have shown increased homocysteine level in smokers as well as male subjects and older subjects and in present study show the homocysteine concentration significantly increased in group 1 (chronic smokers) 18.50 ± 8.40 as compared to group 02 (non-smokers) 8.30 ± 5.30 and ($P < 0.005$).

In the resent study the level of vitamins were significantly decreased in group 01 (chronic smokers) 340.80 ± 124.70 as compared to group 02 (non-smokers) 485.45 ± 175.68 and p value $p < 0.005$. vitamin c concentration significantly decreased in group 1 (chronic smokers) 0.46 ± 0.07 as compared to group 02 (non-smokers) 1.12 ± 0.15 and p value $p < 0.005$. Pagan et. al. [15] Subar et. al [16] and Nagaraj, Satish Kumar D et al. [17] also shows the plasma concentration of Vitamins were significantly decreased in smokers than nonsmokers.

5. CONCLUSION

Cigarette smokers' serum homocysteine levels were found to be significantly higher than non-smokers'. When comparing chronic smokers to nonsmokers, serum vitamin C and vitamin B12 concentrations were found to be significantly lower. As a result, the synthesis of homocysteine is disrupted. As a result, smokers who have a high serum homocysteine level are at a much higher risk of cardiovascular disease and should be counselled to help them quit.

CONSENT AND ETHICAL APPROVAL

Written consent form was obtained from every subject involved in this study. Institutional Ethical clearance was obtained for the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. De Jongh S, Lilien MR, Bakker HD, Hutten BA, Kastelein JJ, Stroes ES. Family history

- of cardiovascular events and endothelial dysfunction in children with familial hypercholesterolemia. *Atherosclerosis*. 2002;163(1):193-197.
2. Dhouha Haj Mouhamed et al. Effect of cigarette smoking on plasma homocysteine concentrations *Clin Chem Lab Med*. 2011;49(3):479–483.
 3. Wilcken DE. Homocysteine, smoking and vascular disease. *Eur Heart J*. 2002;23(20):1559-60.
 4. Singh D .Effect of Cigarette Smoking on Serum Homocysteine and Vitamin B12 Level in Male Population of Udaipur. *Biochem Anal Biochem* 5:282.10.4172/2161-1009.1000282
 5. Rai RR, Phadke MS. Plasma oxidant-antioxidant status in different respiratory disorders. *Indian J Clin Biochem*. 2006;21(2):161-164
 6. Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee Je-Hyuk et al. Vitamin C as an Antioxidant: Evaluation of its role in disease prevention. *J Am Coll Nutr*. 2003;22(1):18-35
 7. Nagaraj, Satish Kumar D, Prashant V Paunipagar. Study of serum malondialdehyde and vitamin c in smokers. *Journal of Scientific and Innovative Research* 2014;3(6):569-571
 8. Jha P, Jacob B, Gajalakshmi V, Gupta PC, Dhingra N, Kumar R, Sinha DN, Dikshit RP, Parida DK, Kamadod R, Boreham J, Peto R; RGI-CGHR Investigators. *N. Engl. J. Med*. 2008;358;1137-1147.
 9. Gupta R, Prakash H, Gupta VP, Gupta KDJ. *Clinl. Epidemiol*. 1997;50:203-209.
 10. Rani, M., Bonu, S., Jha, P., Nguyen, S.N., Jamjoum, L. *Tobacco Control*. 2003;12:e4- e4.
 11. MacKenzie TD, Bartecchi CE, Schrier RW. The human costs of tobacco use (2). *N Engl J Med*. 1994;7; 330(14):975-80.
 12. Lykkesfeldt J, Loft S, Nielsen JB, Poulsen HE. Ascorbic acid and dehydroascorbic acid as biomarkers of oxidative stress caused by smoking. *Am J Clin Nutr*. 1997;65:959–63.
 13. Singh D. Effect of Cigarette Smoking on Serum Homocysteine and Vitamin B12 Level in Male Population of Udaipur. *Biochem Anal Biochem*. 2016;5:282.
 14. Nygård O, Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug JE, Ueland M, Kvaløe G. Total plasma homocysteine and cardiovascular risk

- profile. The Hordaland Homocysteine Study. JAMA. 1995;15;274(19):1526-33.
15. Pagán K, Hou J, Goldenberg RL, Cliver SP, Tamura T. Effect of smoking on serum concentrations of total homocysteine and B vitamins in mid-pregnancy. Clin Chim Acta. 2001;306(1-2):103-9.
16. Subar AF, Harlan LC, Mattson ME. Food and nutrient intake differences between smokers and non-smokers in the US. Am J Public Health. 1990;80(11):1323-9.
17. Nagaraj, Satish Kumar D et al. Study of serum malondialdehyde and vitamin c in smokers. Journal of Scientific and Innovative Research. 2014;3(6):569-571

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