Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/sj7wga20

A COMPARISON OF THE LEVELS OF SERUM HOMOCYSTEINE AND THEIR RELATIONSHIP TO SERUM VITAMIN B12 IN HEALTHY ADULTS OF SMOKERS AND NON SMOKERS IN UDAIPUR CITY

Dr. Rashmi[1*], Dr. Kiran Chandaliya[2], Dr. Rajul Lodha[3]

[1*]Assistant Professor , Department of Biochemistry, American International Institute of Medical Sciences , Udaipur, Rajasthan ,India
[2]Assistant Professor, Department of Medicine, American International Institute of Medical Sciences ,Udaipur, Rajasthan ,India E mail: kiran _intodia@yahoo.com
[3]Senior professor, Department of Biochemistry, RNT medical college, Udaipur, Rajasthan, India

***Corresponding Author:** Dr. Rashmi
* Assistant Professor , Department of Biochemistry, American International Institute of Medical Sciences , Udaipur, Rajasthan ,India

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.

Objectives: The objective of this study is the comparison of the levels of homocysteine and their relationship to Vitamin B12 in healthy adults of Smokers and Non Smokers.

Methodology: Total 300 patients were included in this study. The patients ranging within age 20-40 years, were further categorized according to use of tobacco smoking. Group A- This group consist of tobacco smoker patients between ages 20- 40 years. (n=150). Group B-This group consist of tobacco non-smoker patients between ages 20-40 years. (n=150). 10 ml blood was drawn through vein puncture. From all collected blood samples serum Vitamin B12 and Homocysteine levels measured. All collected data were analysed statistically to calculate p value to see the difference of significance.

Results: The Mean concentration of S.homocysteine(µmol/L) in smoker group was 27.32±17.41 and 6.66±3.09 in control group and the difference among them found to be highly significant. Vitamin B12(pg/ml) was 288.7±108.6 in smokers & 459.8±165.7 in non smokers .Mean Vitamin B12 level is found lower in smokers as compared to non -smokers and it is found to be statistically significant ($p<0.05$).

Conclusion: Based on the results, we came to the conclusion that smoking raises homocysteine levels and decreases vitamin B12 levels, which increases the risk of cardiovascular disease in long-term smokers.

.**Keywords:** Smoker, Homocystein, Vitamin B12 ,CVD

INTRODUCTION

Numerous consequences of tobacco smoking on the cardiovascular system put in to the pathogenesis of cardio vascular diseases. The consequences of the cigarette smoking, & second hand smoke disclosure have been researched the most, however many of the effects are shared by other kinds of tobacco utilization, together with smokeless tobacco. The relationship among cigarette use, and the risk of CVD was investigated in the very first Surgeon General's report in 1964, as well as in a number of following Surgeon General's reports until 2012[1,2]. Throughout this time span, our understanding of this association has expanded to include several particular cardiovascular diseases, diverse forms of tobacco exposure, and the physiological processes connecting these exposures and outcomes. Cigarette smoking boost coronary blood flow by up to 40% in healthy individuals, while smokers with coronary artery disease have decreased cardiac reserve, higher coronary vascular conflict proportionate to the severity of their coronary artery diseases. [3,4]

Cigarette smoking is known to be associated with a raised plasma homocysteine level[5]. Smokers also tend to have lower levels of the B-vitamins- folate, vitamin B6 and vitamin B12[6,7], all of which affect homocysteine levels by acting as co-factors (vitamins B6 and B12) or co-substrate (folate) for the enzymes controlling homocysteine metabolism[8]. Despite these observations, little information is available on the effect of homocysteine on the risk of cardiovascular disease in smokers, apart from a single report from our group.

Vitamin B12 helps get rid of cyanide from tobacco smoke, which is called exogenous cyanide (58). Cyanide is a major component of cigarette smoke; it hurts the nutritional status (132). Further, when tetrahydrofolic acid is mixed with cyanate, it makes a compound that is not biologically active (133). Lack of the hydroxocobalamin form of vitamin B12 is linked to optic neuritis in smokers. Therefor this study was carried out so see the correlation of effect of Smoking on Homocysteine and S.Vitamin B12 Level.

METHODS

This cross sectional study was conducted under the central laboratory of Biochemistry Department of RNT medical college & hospital, Udaipur. Samples were collected from general OPD and medical students in RNT medical hospital.

Study design and criteria- Total 300 patients were include in this study. The patients ranging within age 20-40 years, were further be categorized according to use of tobacco smoking.

Group A- This group consist of tobacco smoker patients (case) between ages 20- 40 years. (n=150).

Group B-This group consist of tobacco non-smoker patients (control) between ages 20-40 years. (n=150)

Smoking questionnaire: A questionnaire was conducted on every participant by face-to- face interview, to obtain their smoking status by asking whether they smoked or not. If the answer was "yes", further information was needed to provide on the duration of smoking and the number of cigarette smoked per day. "Smoking" was defined by answers of having smoked more than 100 cigarettes in participant's lifetime, almost having smoked at least 3 to 4 consecutive months or having smoked one year or more altogether.

The proposed study was done as per pre laid Performa. All participants were questioned and the information was noted on the printed Performa.

Inclusion criteria-

Patients aged between 20-40 years.

A detailed family history of enrolled candidates.

Tobacco smoker patients do not suffer from any other disease. The patients was diagnose based on clinical examination, laboratory investigations and other test.

In this study the BMI (BMI= Weight in Kg/ height in m^2), lifestyle, area, socioeconomic status and diet, religion and tobacco smoking habit of the enrolled participants was captured. The adults were considered for study after applying the exclusion criteria.

Exclusion criteria-

Pathophysiological status- Renal failure, congestive heart disease, chronic respiratory diseases, liver disease, malabsorption syndrome and nutritional anemia. Systemic disease like Hypertension and diabetes mellitus.

Supplementation of vitamins.

Modified physiological status- Pregnancy, psychological & mental disorders such as depression.

Sample collection- 10 ml Venous Blood sample was collected.

Samples were incubated & centrifuge at 3000 rpm for 15 min and serum was separated from all blood samples to analyse various Biochemical parameters Like S.Vitamin B 12 and S. Homocysteine.

Precautions were taken to avoid hemolysis of sample.

Homocysteine and Vitamin B12 was measured by Advia centure CP analyzer using principle of electro-chemiluminescence immunoassay(ECLIA)

Statistical analysis- The statistical analysis was performed using SPSS. All the participants were made aware about the main aim of the study and they were informed that the participation is voluntary. Written consent was taken before data collection.

RESULTS

Study includes total 300 adults of age group from 20-40 year and majority of the adults in smoker group is 21-25 year age.(Table 1)

Table 1: Age group (yrs) with Smokers Vs Non-Smokers

	Smokers		**Non -Smokers**		**P-value**
Age group (yrs)	**No.**	**%**	**No.**	**%**	
21-25	62.00	41.33%	40.00	26.66%	
26-30	38.00	25.33%	58.00	38.66%	
31-35	12.00	8%	26.00	17.33%	
36-40	38.00	25.33%	26.00	17.33%	**0.001**
Total	**150**	**50.00%**	**150**	**50.00%**	

Table 2: Educational status in Smokers vs Non-Smokers

	Smokers		**Non-Smokers**		**Total**	
Education Status	**No.**	**%**	**No.**	**%**	**No.**	**%**
Uneducated	14	9.33%	0	0.00%	14	4.67%
Primary	38	25.33%	20	13.33%	58	19.33%
High	30	20.00%	72	48.00%	102	34.00%
Under college	68	45.33%	58	38.67%	126	42.00%
Total	**150**	**100.00%**	**150**	**100.00%**	**300**	**100.00%**

P= <0.001 (HS)

The prevalence of smoking is high in educated person mostly college students as compared to

uneducated persons and the difference among them is found to be highly significant.

Table 3: Smokers Vs Non-Smokers in Rural Vs Urban areas ,P=0.642 (NS)

	Smokers		**Non- Smokers**		**Total**	
Rural/ Urban	**No.**	**%**	**No.**	**%**	**No.**	**%**
rural	68	45.33%	64	42.67%	132	44.00%
urban	82	54.67%	86	57.33%	168	56.00%
Total	**150**	**100.00%**	**150**	**100.00%**	**300**	**100.00%**

According to locality, there is no such significant difference in rural and urban residents. (Table 3)

Table 4: Type of Smoking in Smokers

	Smokers	
Residence	**No.**	**%**
Occasional	56	37.33%
Regular	94	62.67%
Total	**150**	**100.00%**

Table 5: Association between Duration of Smoking in Smokers

Duration of Smoking (yrs)	**Smokers**	
	No.	**%**
0	0	0.00%
1-5	60.00	40.00%
6-10	62.00	41.33%
>10	28.00	18.67%
Total	**150.00**	**100.00%**

This Table 5 classifies the smokers as per duration of smoking. Maximum smokers (41.33%) are having smoking since 5 years while very few (18.67%) are having smoking habits for more than 10 years

Table 6: Comparison of Vitamin B12 in Smokers and Non smokers Group

	Smokers		**Non-Smokers**		**Total**		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**P value**
Vit.B12(pg/ml)	288.78	108.60	459.89	165.77	374.33	164.06	<0.001

Table 6 compares the vitamin B12 level in smokers as well as in non smokers. Mean Vitamin B12 level is found lower in smokers as compared to non -smokers and it is found to be statistically significant ($p<0.05$).

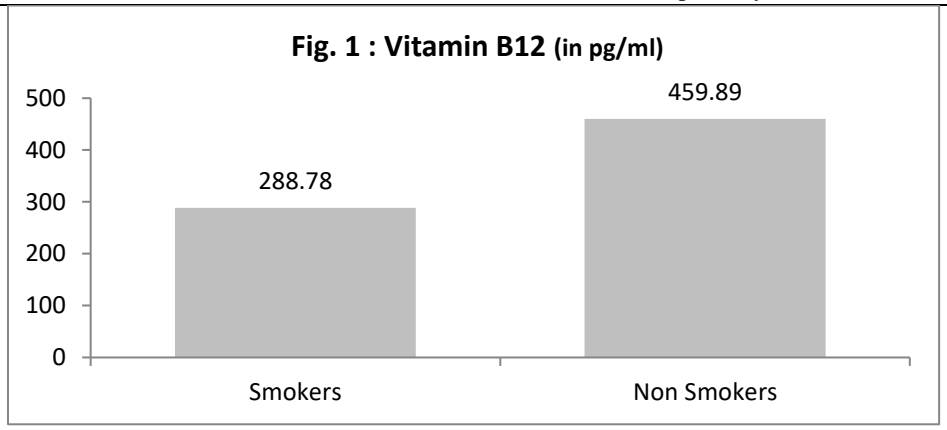

Figure 1. Comparison Of Vitamin B12 (pg/ml)in Smokers and Non Smokers

Table 7: Comparison of Homocysteine (HCY) (µmol/L) **in Smokers & Non Smoker**

Parameter	Smokers		Non Smokers		Total		P-Value
	Mean	SD	Mean	SD	Mean	SD	
Homocysteine (µmol/L)	27.32	17.41	6.66	3.06	16.99	16.21	<0.001

Table 7 compares the homocysteine level in smokers and non smokers. The Mean concentration of S.homocysteine (µmol/L) in smoker group was 27.32±17.41 and 6.66±3.09 in control group and the difference among them found to be highly significant. Mean homocysteine level in smokers is significantly higher in comparison with non smokers. The association between serum homocysteine level and smoking habits is found to be statistically significant($p<0.05$).

Table 8: Correlation between Vitamin B12 and Homocysteine (HCY) in Smokers

Smoker		**R- value**
Homocysteine	Vitamin B12	0.0292

Table 8 observed that correlation between vitamin b12 & homocysteine in smokers. The analysis observed a positive and significant correlation (r=0.292) between serum vitamin b12 and homocysteine in smoker cases. $p<0.01$

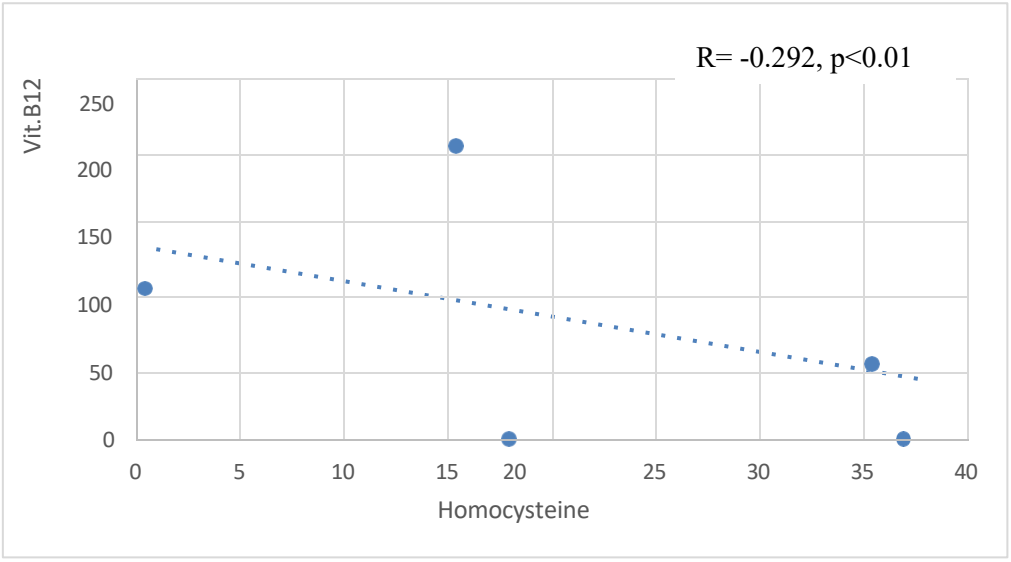

Fig 2: Showing correlation between vitamin B12 & homocysteine in smokers

DISCUSSION

Smoking is the biggest form of tobacco use in most of countries. Although has been demonstrated in other studies, and the present study also confirmed males. This study is an attempt to comprehensively assess the popularity, knowledge, thoughts, behavior, and interpersonal factors towards the use of smoked and smokeless forms of tobacco among the youth of Udaipur city, Rajasthan, India. The prevalence data on tobacco use among is important both to assess tobacco as a risk factor and to establish control measures for prevention of those diseases.

Some investigators consider increased homocysteine levels as an independent risk factor of cardiovascular disease, while its involvement in mechanisms of thrombosis has well been documented.[9]Moreover, other studies suggest that an elevated plasma total homocysteine concentration substantially increases the risk associated with some of the conventional cardiovascular risk factors. However, there are findings that do not confirm or recognize homocysteine importance in actually causing coronary artery disease, while recent studies have considered homocysteine more as a result than a cause of arteriosclerosis, especially due to the confounding effect of various nutrient and other lifestyle-related factors. [10]

Muhammad afzal et.al 2011 found that Study population consisted of thirty healthy individuals. Majority (50%) of individuals belonged to 46-55 years of age. Mean age was
51.1 years. There was male predominance. Out of total, 57% participants were smoker while 43% were non-smoker. There was statistically significant association of smoking with elevated serum Homocysteine levels ($p<0.05$) Smoking appeared to be strongly associated with elevated serum Homocysteine levels in healthy individuals. [11]

The dietary habits of the both subjects were considered. In which most of smokers were vegetarian, while the others were consuming mixed diet. In fact, majority of the patients were not consuming adequate amount of vegetables and fruits in their diet. Consumption of sufficient amounts of fruits and vegetables is recommended as part of a healthy diet. i.e., eating at least five servings of fruits and vegetables per day is recommended to reduce risks for cardiovascular diseases. In our study (Table 6) smokers (54.67%) are consuming unhealthy diet as compared to non smokers which is also evident from the study conducted by Gholamreza Heydari et al and they found smokers had consumed Fast food and while meat and less fruits and vegetables as compared to non smokers. [12]

Naeem afzal et al. 2010 concluded from our study that smoking is significantly associated with elevated homocysteine levels in healthy asymptomatic adults. [13]

In present we estimated Correlation between serum homocysteine and vitamin B12 in cigarette smoker. We observed a positive and significant correlation ($r=0.292$) between serum homocysteine and vitamin B12 in smoker cases is as per Figure 2 .It indicates homocysteine level increases 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. There is positive correlation between vitamin B12 and homocysteine , with increase in vitamin B12 level there is decrease in homocysteine level.

Sunil Kumar Raina et al 2015, who had conducted their study in North West India, also found inverse correlation between homocysteine and vitamin B12. [14]

In present study (Table 7) we observed : the mean statistical concentration of S. Vit. B12 (pg/mL) in smoker group was 288.78 ±108.60 that is low as compared to non-smokers group. Which is 459.89 ±165.77 . The difference among them is found to be highly significant.

Similarly Piyathilake et al. [15] 1986 have demonstrated reduced red cell and buccal mucosal vitamin B12 and folate levels in current smokers. There is evidence of cell damage in these tissues also .Such an effect contributing to overall cardiovascular risk in smokers would not be reduced by alteration for plasma nutrients. The serum vitamin B12 level is highest in middle age group and decreases as age advances and lowest in elderly age group. Similar studies carried out by Stover PJ.et al. 2010[16] .

CONCLUSION

Based on the results, we came to the conclusion that smoking raises homocysteine levels and decreases vitamin B12 levels, which increases the risk of cardiovascular disease in long-term smokers.

ACKNOWLEDGEMENTS

We acknowledge the whole department of biochemistry of our institute.

DECLARATIONS

Funding: NIL
Conflict of interest: None
Ethical approval: The study was done after permission of institution Ethics committee.

REFERENCES

1. Wilson PWF. Established risk factors and coronary artery disease.The Framingham study. Am J Hypertens 1994; 7: S7–S12.
2. Jacobs, A. and Worwood, M. Ferritin in serum. Clinical and biochemical implications. N.Engi. J. Med., 1975;292: 951.
3. Gupta R, Prakash H, Majumdar S, Sharma S, Gupta VP. Prevalence of coronary artery disease and coronary risk factors in an urban population of Rajasthan. Indian Heart J 1995; 47: 331–338..
4. B12, folic acid and haematological indices in healthy adults. Public Health Nutr 2003;6(7):67 81Chen1, Ping Wu1, Lin Zhou2,2015
5. Refsum H, Ueland PM. Homocysteine and cardiovascular disease. Annu Rev Medicine 1998; 49: 31–62.
6. Clarke R et al. Screening for vitamin B-12 and folate deficiency in older persons Americanjournal of clinical nutrition, 2003, 77:1241–7
7. Association of Smoking with Serum Homocysteine Level in Healthy Adults: A CrossSectional study muhammad naeem afzal 1, mehr-ul-nisa2,2010
8. He Y, Jiang B, Wan ZH, Zheng QS and Li LS. 2007. Study on the relationship between passive smoking and blood lipids, fibrinogen and viscosity among women who never smoke. Zhonghua Liu Xing Bing Xue Za Zhi., **28**:1167-1170
9. He R, Lopez D, Boreham J et al. Mortality From Smoking In Developed Countries 1950–2000. Oxford: Oxford University Press, 1994.
10. Wilcken,, Azinheira J, Reis HP, Pina JE, Correia JM, Luis AS. Influence of smoking on homocysteinemia at baseline and after methionine load. Rev Port Cardiol 2000; 19: 471–4.
11. Muhammad afzal , 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: 103–9.
12. Gholamreza Heydari ,W.Biomarkers of cobalamin (vitamin B12) defect and its application. J Nutr Health Aging 2011 Mar;15(3):227-313.
13. Naeem afzal. Increased homocysteine associated with smoking chronic inflammation and aging may reflect acute-phase induction of pyridoxal phosphatase activity. Med Hypotheses 2000; 55: 289–93.
14. Sunil Kumar Raina, Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug JE, Ueland M and Kvale G. 1995. Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. JAMA., 274: 1526–1533.
15. Piyathilake L , Robinson K, Clarke R, Refsum HM, Ueland P, et al. (1993) Vitamin B6 and folate Homocysteine concentrations in coronary artery disease (Abstr). Ir J Med Sci 162: 197A.
16. Stover PJ , Pearce, M. S. ,& Parker, L. (2005). The influence of erythrocyte folate and serum vitamin B12 status on birth weight. The British Journal of Nutrition ,2005 Vol 93(5), 593–599