

Study of Effect of Tobacco Smoking on Hemopoietic Markers at Tertiary Care Center of Udaipur, Rajasthan, IndiaRashmi¹, A K Verma², Akanksha Menaria³, Nirali Salgiya^{*4}¹Senior Demonstrator, Department of Biochemistry, RNT medical college, Udaipur, Rajasthan, India²Professor & Head, Department of Biochemistry, RNT medical college, Udaipur, Rajasthan, India³Biochemist, Department of Biochemistry, RNT medical college, Udaipur, Rajasthan, India⁴Associate Professor, Department of Biochemistry, RNT medical college, Udaipur, Rajasthan, India

Received: 15-10-2022 / Revised: 10-11-2022 / Accepted: 05-12-2022**Corresponding author: Nirali Salgiya****Conflict of interest: Nil**

Abstract**Background:** Smoking-induced hypoxia, inflammation, and oxidative stress result in impairment of hematological parameters. Contradictory evidence is found as far as the effect of smoking on blood cells and indices is concerned.**Objectives:** The objective of this study is to see the effect of use of tobacco smoking on serum vitamin-B12, S.Iron, S.TIBC and S. Ferritin level and compare it with non-smoker healthy adults.**Methodology:** Total 300 patients were include in this study, in which patients ranging within age 20-40 years, they 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 S. iron, S. TIBC, S. ferritine, S. vitamin B12 and Liver function tests were measured. All collected data were analysed statistically to calculate p value to see the difference of significance.**Results:** The Mean concentration of S. Iron (mg/dL) in smoker group was 162.12 ± 48.76 while that of Non-smoker control group 143.09 ± 47.86 and the difference among them found to be highly significant. The Mean concentration of S.TIBC (mg/dL) in smoker group was 314.08 ± 82.53 while that of Non-smoker control group 230.22 ± 79.17 and the difference among them found to be highly significant. The Mean concentration of S. Ferritin (micrograms/lit) in smoker group was 178.38 ± 50.02 while that of Non-smoker control group 66.73 ± 35.65 and the difference among them found to be highly significant. The Mean concentration of S. Vit. B12 (pg/mL) in smoker group was 288.78 ± 108.60 that is low as compared to non-smokers group. Non-smoker control group 459.89 ± 165.77 and the difference among them found to be highly significant**Conclusion:** The results of this study suggest that there were low serum vitamin B12 concentrations in smokers compared with non-smokers, which might contribute to the development of vascular and cardiovascular diseases. It may be concluded that there appears a link between smoking and alterations in hematological parameters.**Keywords:** Smoking, S. iron, S.TIBC, S. ferritine, S. vitamin B12s

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INTRODUCTION

Tobacco use is associated with accelerated mortality among adults especially in low- and middle-income countries, where the burden of tobacco-related illness and death is heaviest[1,2]. More than 1 million adults die each year in India due to tobacco use accounting for 9.5% of overall deaths[3]. India faces a dual burden of tobacco use in the form of smoking and smokeless tobacco. According to the Global Adult Tobacco Survey (GATS) conducted in 2016–17, the overall prevalence of smoking tobacco use is 10.38% and smokeless tobacco use is 21.38% in India. Of all adults, 28.6% currently consume tobacco either in smoke or smokeless form, including 42.4% of men and 14.2% of women. [4]

A cigarette contains about more than 4,000 compounds that can cause cellular-level damage in the human body [5]. Among these compounds, free radicals, nicotine, and carbon monoxide are believed to be responsible for the worst pharmacological effects of smoking. These compounds are often thought to be associated with cancer, lung diseases, and cardiovascular diseases[6]. However, compounds mentioned above play a role in the etiopathogenesis of many other diseases, as well. The formation of hematological components begins in the bone marrow and they continue to mature in the peripheral blood tissue. Therefore, they are affected by many harmful compounds that cause damage to the bone marrow and peripheral blood tissue. Tobacco contains mainly harmful compound is Nicotine that modulate their level of arousal and for mood control in daily life. There is a limited number of studies in the literature investigating the effects of smoking on hematological parameters. Some of these studies investigated a limited number of hematological parameters and some have contradictory results. Therefore, the purpose of this study is to perform a

comprehensive investigation of the effects of smoking on hematological parameters.

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, in which patients ranging within age 20-40years, they 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.

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 m²), lifestyle, area, socioeconomic status and diet, religion and tobacco smoking habit of the enrolled participants was noted. The adults were obtain after applying the exclusion criteria.

Exclusion criteria- Pathophysiological status- Renal failure, congestive heart, heart disease, chronic respiratory diseases, liver disease, malabsorption syndrome and nutritional anemia's. Systemic disease 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. iron, S. TIBC, S. ferritine, S. vitamin B12 and Liver function test (LFT)

Precautions were taken to avoid hemolysis of sample.

S. Vitamin B12 and S. ferritine was measured by Cobs e 411 analyzer using principle of electro chemiluminescence immunoassay (ECLIA).

Biochemical parameter like S. iron, S. TIBC and liver function test was done on Simens RXL clinical chemistry analyzer.

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

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

Age group (yrs)	Smokers		Non -Smokers		P-value
	No.	%	No.	%	
21-25	62.00	41.33%	40.00	26.66%	0.001
26-30	38.00	25.33%	58.00	38.66%	
31-35	12.00	08%	26.00	17.33%	
36-40	38.00	25.33%	26.00	17.33%	
Total	150	50.00%	150	50.00%	

Table 2: Showing BMI in Smokers and Non-Smokers

BMI	Smokers		Non- Smokers		Total	
	No.	%	No.	%	No.	%
Normal	108	72.00%	100	66.67%	218	72.67%
Over Weight	28	18.67%	40	26.67%	68	22.67%
Obese	14	9.33%	10	6.67%	14	4.67%
Total	150	100%	150	100%	300	100%

P= 0.213 (NS)

Table 3: Educational status in Smokers vs Non-Smokers

Education Status	Smokers		Non- Smokers		Total	
	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 (Table 3) and the difference among them is found to be highly significant. According to locality, there is no such significant difference in rural and urban residents.(Table 4)

Table 4: Social Economic Status in Smokers Vs Non-Smokers

SES	Smokers		Non -Smokers		Total	
	No.	%	No.	%	No.	%
Lower	46	30.67%	38	25.33%	84	28.00%
Middle	78	52.00%	76	50.67%	154	51.33%
Higher	26	17.33%	36	24.00%	62	20.67%
Total	150	100.00%	150	100.00%	300	100.00%

According to socioeconomic status there is no such significant difference found between smokers and non-smokers.(Table 4)

Table 5: Marital Status in Smokers Vs Non-Smokers

Marital Status	Smokers		Non- Smokers		Total	
	No.	%	No.	%	No.	%
Married	76.00	50.67%	102.00	68.00%	178.00	59.33%
Unmarried	74.00	49.33%	48.00	32.00%	122.00	40.67%
Total	150.00	100.00%	150.00	100.00%	300.00	100.00%

Table 6: Smokers Vs Non-Smokers in Rural Vs Urban areas

Rural : Urban	Smokers		Non- Smokers		Total	
	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%

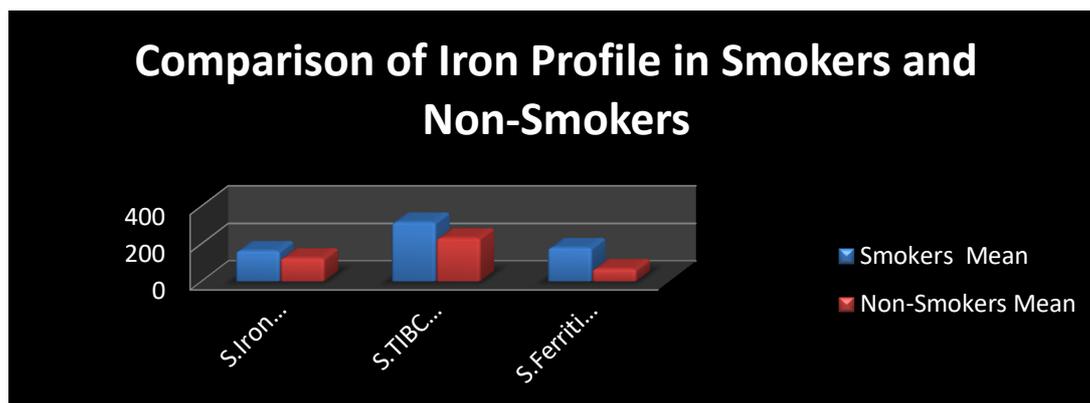
Table 7: Comparison of Iron Profile in Smokers Vs Non-Smokers

	Smokers		Non-Smokers		Total		P value
	Mean	SD	Mean	SD	Mean	SD	
S.Iron (mg/dL)	162.12	48.76	124.05	38.59	143.09	47.86	<0.001
S.TIBC (mg/dL)	314.08	82.53	230.22	79.17	272.15	91.00	<0.001
S.Ferritin (micrograms/lit)	178.38	50.02	66.73	35.65	122.56	70.76	<0.001

P=0.642 (NS)

The Mean concentration of S.Iron (mg/dL) in smoker group was 162.12 ±48.76 while that of Non-smoker control group

143.09 ±47.86 and the difference among them found to be highly significant

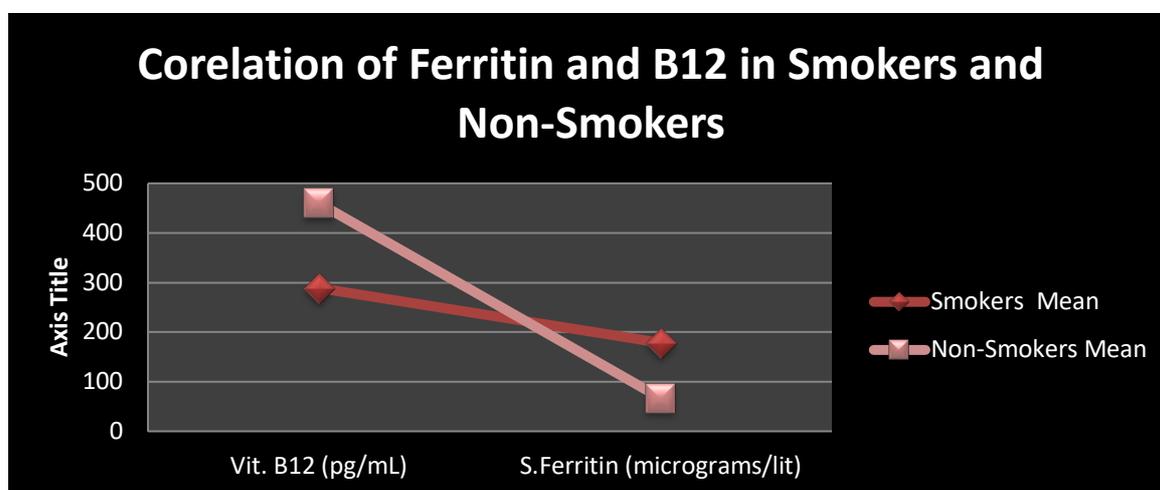


Graph 1: Showing Graphical presentation of Comparison of Iron Profile and B12 Smokers and Non-Smokers

The Mean concentration of S.TIBC (mg/dL) in smoker group was 314.08 ± 82.53 while that of Non-smoker control group 230.22 ± 79.17 and the difference among them found to be highly significant. The Mean concentration of S.Ferritin (micrograms/lit) in smoker group was 178.38 ± 50.02 while that of Non-smoker control group 66.73 ± 35.65 and the difference among them found to be highly significant.

Table 8: Comparison of Vitamin B12 in Smokers Vs Non-Smokers

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



Graph 2: Showing Correlation of Ferritin and B12 in Smokers and Non-Smokers

The Mean concentration of S. Vit. B12 (pg/mL) in smoker group was 288.78 ± 108.60 that is low as compared to non-smokers group. Non-smoker control group 459.89 ± 165.77 and the difference among them found to be highly significant.

DISCUSSION

Smoking is strongly and independently associated with cardiovascular disease and

is the biggest single avoidable health habit contributing to chronic disease in the Western world[7]. Up to 50% of avoidable deaths in the industrialized world have been attributed to smoking, half of which are cardiovascular. Cigarette smoking is known to be associated with a raised plasma homocysteine level[8,9]. Smokers also tend to have lower levels of the B-vitamins, folate, vitamin B6 and vitamin

B12[10], 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[11]. 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.

In the present study, despite lower levels of B vitamins in smokers, adjustment for nutrient levels had an insignificant effect on risk estimates, highlighting the robustness of smoking as a cardiovascular risk factor. It should, however, be considered that smoking may produce vitamin deficiency in individual tissues; Piyathilake et al. have demonstrated reduced red cell and buccal mucosal B12 and folate levels in current smokers[12], with evidence of cell damage in these tissues. Such an effect contributing to overall cardiovascular risk in smokers would not be reduced by adjustment for plasma nutrients and must be considered. Shengfang Chen¹, 2015 founded that the serum tHcy level in current smokers had a rising trend. To investigate how smoking affects the Hcy level, the VB12 were analyzed. The results showed that the serum folate level in former smokers and current smokers were both lower than that in the never smokers, and the lowest level was of current smokers. Folic acid plays a critical role in the synthesis of DNA, methylation and cell repair, and is the major methyl donor in the methylation of Hcy together with VB12. Lots of investigations have confirmed that the serum folate is negatively correlated with the serum tHcy level [13]. And smoking was independently associated with the elevated tHcy level in this study.

Dastur et al[14]. 2010 founded serum vitamin B12 in vegetarians was significantly lower than in nonvegetarians regardless of their smoking habits. On the other hand, folate was significantly higher in vegetarians (mean 6.60 ng/mL) than in

nonvegetarian (mean 4.79 ng/mL) reflecting the greater content of folate in vegetarian diet. The significantly lower serum vitamin B12 values in vegetarians than in nonvegetarians are probably a reflection of the lower intake of the vitamin in their diet particularly meat products. Vegetarians who take adequate amounts of milk and nuts, which are also rich in B12, can maintain adequate serum levels. Results of our study are similar in regards of vegetarians and nonvegetarians to this study. Herrmann et al (2003)¹¹ also reported low vitamin B12 in vegetarian compared to nonvegetarian similar to our study. [15]

Erdemir et al 2006 studied relationship between smoking and folic acid, vitamin B12 and some hematological variables in patients with chronic periodontal disease. They reported results similar to our study of lower serum folic acid concentration in smokers as compared to nonsmokers ($p < 0.05$) [16]

A number of mechanisms may underlie the negative associations of smoking exposure with folate and vitamin B12 levels and the positive associations with homocysteine levels. Firstly, several components of cigarette smoke, such as organic nitrites, nitrous oxide, cyanates, and isocyanates, can increase oxidative stress and interact with folate and vitamin B12, causing these micronutrients to become inactive (Northrop-Clewes & Thurnham, 2007). This leads to lower levels of folate and vitamin B12 and higher levels of homocysteine. [17]

Secondly, nicotine use can lead to a change in basal metabolic rate by increasing serum levels of catecholamines (Walker et al., 1999) [18]. This leads to higher nutritional demands in smokers.

A third pathway that can cause lower micronutrient levels in smokers is a difference in diet between smokers and nonsmokers (Dallongeville, Marecaux, Fruchart, & Amouyel, 1998) [19]. It is not

completely clear what causes the difference in dietary preferences of smokers as compared with nonsmokers. Hypotheses include a reduction of monoamine oxidase in smokers, leading to changes in mood and appetite, a direct influence of nicotine on taste receptors, and a generally unhealthier lifestyle. Differences in dietary habits may have larger effects in pregnancy, when the nutritional demands are different (King, 2000; Picciano, 2003) [20].

CONCLUSION

The results of this study suggest that there were low serum vitamin B12 concentrations in smokers compared with non-smokers, which might contribute to the development of vascular and cardiovascular diseases. It may be concluded that there appears a link between smoking and alterations in hematological parameters.

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REFERENCES

1. Scott CW, Bernstein SL, Coble YD et al. The Worldwide Smoking Epidemic: Council Reports. JAMA 1990; 24: 3312–3318.
2. Bartecchi CE, Mackenzie TD, Schrier RW. The human costs of tobacco use. N Engl J Med 1994; 330: (pt1) 907–912.
3. Peto R, Lopez D, Boreham J et al. Mortality from Smoking in Developed Countries 1950–2000. Oxford: Oxford University Press, 1994.
4. Garcia G, Trejos J, Restrepo B, Landázuri P. Homocysteine, Folate and Vitamin B12 in Colombian Patients with Coronary Disease Arq Bras Cardiol 2007; 89(2): 71-76
5. Schnyder G, Roffi M, Flammer Y. Effect of homocysteine – lowering therapy with folic acid, vitamin B(12), and Vitamin B(6) on clinical outcome after percutaneous coronary.
6. Intervention: The Swiss Herat study a randomized controlled trial. JAMA 2002; 288: 973-979.
7. Reis RP, 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: 471–474.
8. Pagan K, Hou J, Goldenberg RL, Cliver SP, Tamura T. Effect of smoking on serum
9. concentrations of total homocysteine and B vitamins in mid-pregnancy. Clin Chim Acta World Health Assembly. Global Strategy for the Prevention and Control of Noncommunicable Diseases. WHA A53/14. Geneva, World Health Organization, 2000 (http://apps.who.int/gb/archive/pdf_files/WHA53/ea14.pdf, accessed 23 September 2022).
10. Piyathilake P, finkelstein J.D. and martin J.J, 1986 methionine metabolism in mammals. N. Engi. J. Med., 1975;292: 951.
11. Dastur et al., Cook, J.D. and Finch, C.A. A clinical evaluation of serum ferritin as an index of iron stores. N. Engi. J. Med., 1974; 290: 1213.
12. Daher, R.; van Lente, F. Relationship of increased homocysteine with copper, iron, and zinc concentrations in serum. Irish J. Med. Sci. 1995, 164 (Suppl. 15), 21
13. Herrmann J, Rosenberg IH, Rogers G, et al. Serum total homocysteine concentrations in adolescent and adult Americans: Results from the third National Health and Nutrition Examination Survey. Am J CZinNutr. 1999;6(9):482-489.
14. Erdemir J Balarajan R, Bulusu L. Prevalence of cobalamin deficiency in the Framingham elderly population. American journal of clinical nutrition, 1994
15. Northrop-Clewes & Thurnham, Cook, J.D. and Finch, C.A. Screening for vitamin B-12 and folate deficiency in older persons American journal of

- clinical nutrition, 2003; 77:1241–7
16. Walker B, Refsum H, Ueland PM., 1999. Association of Smoking with Serum Homocysteine Level in Healthy Adults: A Cross-Sectional study muhammad naeem afzal 1, mehr-ul-nisa. 2010;2.
17. Dallongeville J., Marecaux N., Fruchart J. C., & Amouyel P. Cigarette smoking is associated with unhealthy patterns of nutrient intake: A meta-analysis. *The Journal of Nutrition*, 1998;128(9): 1450–1457
18. King J. C. Physiology of smoker and nutrient metabolism. *The American Journal of Clinical Nutrition*, 2000;71(5 Suppl): 1218S–1225S.