Early Indicators Of Alterations In Hematological And Inflammatory Biomarkers Among Smoking And Smokeless Tobacco Users - Comparative Analysis

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Abstract

Background: *In India, 1 million tobacco-related deaths occur every year. Consumption of smoking and smokeless tobacco products was a more common practice. This study aimed to assess and compare hematological and inflammatory markers among smoking and smokeless tobacco users.*

Material and methods: *Ninety participants were recruited and divided into three groups: 30 smokers, 30 smokeless tobacco users, and age- and BMI-matched 30 healthy controls. The levels of hsCRP and complete blood count (CBC) were measured.*

Results: *Significant differences were observed in mean corpuscular volume (MCV) and neutrophil levels in both tobacco user groups compared to controls. Eosinophils were reduced in smokers but elevated in smokeless tobacco users, while basophils and monocytes were decreased in both groups. hsCRP levels were markedly elevated in both smokers and smokeless tobacco users, indicating systemic inflammation. The neutrophil-to-lymphocyte ratio (NLR) was significantly increased among smokers, suggesting a stronger systemic impact of smoking compared to smokeless tobacco use.*

Conclusion: *These findings emphasize the need for early monitoring of hematological and inflammatory biomarkers among tobacco users. Significant changes in MCV, neutrophils, eosinophils, basophils, monocytes, and elevated hsCRP levels and NLR suggest systemic inflammation and altered immune responses in tobacco users. While some hematological parameters remained unchanged, elevated inflammatory markers such as hsCRP and NLR in smokers indicate a stronger systemic impact of tobacco use. This study helps to guide targeted public health interventions and preventive strategies.*

Keywords: *Smokers, Smokeless tobacco users, High sensitive C-reactive protein, Neutrophil-Lymphocytes ratio. Systemic inflammation, hematological changes*

INTRODUCTION

Tobacco consumption is a serious health issue, such as cancer and respiratory and cardiovascular diseases, which can lead to death. According to the WHO, tobacco use worldwide is estimated at 1.3 billion and 7 million deaths occur. [1] In India, 330 million people consume tobacco in various forms. Khaini, Gutkha, betel quids with tobacco, and Zarda are the most popular smokeless tobacco products in India. Tobacco used for smoking includes bidi, cigarette, and hookah. Every year, one million tobacco-related deaths have been reported in India. [2] Early detection of inflammation helps to prevent disease progression. Tobacco contains many harmful chemicals that produce oxidative stress, tissue damage, and inflammation. [3] Toxic chemical products produce many reactive oxygen species (ROS), which are unstable oxygen molecules that damage cell membranes and DNA, contributing to inflammation. [4] In systemic inflammation, the release of cytokines (such as TNF-α, IL-1β, and IL-6) signals the liver to release acute-phase proteins, which spread through the bloodstream and send signals to immune cells to help fight infection and injury. [5] HsCRP is a highly sensitive biomarker to assess systemic inflammation.[6] Similarly, the neutrophil-to-lymphocyte ratio (NLR) has emerged as a simple and effective indicator of systemic inflammation in both smokers and SLT users.[7] Although several studies have reported hematological and inflammatory changes in tobacco users, comparative data between smoking and smokeless tobacco groups remains an uncovered research gap. Assessing and comparing these changes may offer insight into the early physiological alterations caused by different forms of tobacco exposure. This study aimed to assess and compare hematological and inflammatory markers among smoking and smokeless tobacco users, using non-tobacco users as controls. Understanding these early alterations may help guide the creation of awareness and prevent diseases.

MATERIALS AND METHODS

A cross-sectional study was carried out at Mahatma Gandhi Medical College and Research Institute in Puducherry after approval was granted by the Institutional Ethics Committee. A judgmental sampling technique was used to recruit volunteers from the Puducherry and Cuddalore regions. All participants were provided detailed information about the study objectives and procedure, and written informed consent was obtained before participation.

Ninety participants were recruited and divided into three groups. Inclusion criteria for Group I, 30 smokers reported daily or occasional use of any smoking tobacco product for more than a year. Group II, 30 smokeless tobacco (SLT) users who consumed SLT products daily or occasionally for one year based on WHO criteria. Group III, 30 Age- and BMI-matched healthy controls who had never used any form of tobacco product. All participants were aged between 25 and 45 years. Subjects with occupational exposure to dust or fumes, such as a history of working in textile mills, cement factories, coal industries, or similar environments. History of respiratory diseases, like asthma, chronic obstructive pulmonary disease (COPD), bronchitis, pneumonia, tuberculosis (TB), or COVID-19. Any other inflammation, infectious disease, or immunosuppressive treatment was excluded. Baseline demographic data and physical parameters were noted from all participants. Morning venous blood samples (5 ml) were collected under sterile conditions. The samples were then distributed into 2 mL of blood was transferred into EDTA (purple-top) tubes for CBC analysis, and 3 mL was transferred into plain (red-top) tubes for the estimation of hsCRP using ELISA method. The serum was isolated and stored at -20°C in the Biochemistry department until analysis after centrifuging the red-top tubes at 3000 rpm for 15 minutes. CBC was performed using a HORIBA Yumizen H2500 automated analyzer. HsCRP levels were estimated using an ELISA kit (Diagnostic Biochem Canada, Ref: CAN-CRP-4360), following the manufacturer's protocol. **Statistical analysis**

Data were analyzed using SPSS (version 21). The distribution of all variables was assessed using the ShapiroWilk test. For variables that followed a normal distribution, including physical parameters and select hematological indices, comparisons among the three groups were performed using one-way analysis of

variance (ANOVA), followed by Tukey's post-hoc test. The Kruskal-Wallis test was used to test variables that didn't meet normality criteria. The statistical significance of a p-value ≤0.05 was considered significant.

RESULTS

Table No. 1: Comparison of Physical Parameters among Smokers, Smokeless Tobacco Users, and Healthy Controls

Physical parameters	Group -I Smokers (n = 30) (Mean + SD)	Group -II Smokeless tobacco users (n = 30) (Mean + SD)	Group -III Control (n = 30) (Mean + SD)	F. Value	P. Value
Age (yrs)	40.6+9.2	38.3 + 7.1	39.1+ 8.2	0.713	0.492
Height (cm)	167+9.1	169.6+6.9	165.1+7.6	2.67	0.732
Weight (kg)	69.6+12.8	69.8+13.9	68.3+13.6	0.124	0.883
BMI	25.1+3.9	24.3+4.8	24.9+3.5	0.339	0.713

One–Way Anova test

Among the three study groups, there were no statistically significant differences in any of the physical parameters observed.

Table No.2: Comparison of hematological profile among smokers, smokeless tobacco users, and nontobacco users

CBC parameters	Group-I Smokers (n = 30) (Mean + SD)	Group -II Smokeless tobacco users(n=30) (Mean + SD)	Group -III Control (n = 30) (Mean + SD)	F.Value	P.Value
RBC (millions /cumm)	4.8+0.3	5.0 + 0.5	4.9 +0.3	1.6	0.20
Hb (g/dl)	14.6 +1.6	14.8 +1.2	14.7 +1.0	0.2	0.74
PCV (%)	43.9 +4.8	44.4 +3.7	43.8 + 2.9	1.52	0.860
MCV (fl)	91.8 +6.7	86.9 + 9.0	89.0 + 5.4a	3.9	0.02*
MCH (pg)	30.3 + 2.7	29.3 + 3.1	29.5 +1.3	1.4	0.24
Neutrophils %	54.7 +8.5	52.9 + 5.5	50.2 + 6.0a	4.4	0.01*
Lymphocytes %	32.5 +7	33.0 + 7.2	35.1 + 6.3	1.7	0.18
WBC (cells/cumm)	7745 +1571	7529 +1960	7967 +1631	0.6	0.51

Note: RBC - Red blood cell; Hb - Hemoglobin; PCV - packed cell volume; MCV - Mean Corpuscular Volume. MCH - mean corpuscular Hemoglobin; WBC- white blood cell count

One-way ANOVA - Post hoc Tukey HSD (P< 0.05* statistically significant, P<0.0001** - highly significant) a – significantly different from smokers groups, b – significantly different from smokeless groups

Complete blood count analysis revealed no significant changes in RBC, Hb, PCV, lymphocyte, and WBC counts, but a decrease in lymphocyte and WBC counts. The MCV was significantly different among the groups (P = 0.02). Neutrophil was significantly higher in both smoker and smokeless tobacco users when compared to controls (P = 0.01). The post-hoc Tukey's HSD test revealed a significant difference between smokers and controls. Neutrophil elevation indicates systemic inflammation in tobacco users.

Table No.3: Comparison of hematological profiles among the groups

CBC Parameters	Group-I Smokers (n=30) (Median, IQR)	Group-II Smokeless tobacco users (n =30) (Median, IQR)	Group -III Control (n = 30) (Median, IQR)	KW value	P.Value
MCHC (%)	33.4 (32.9,33.7)	33.5 (33,33.9)	33.6 (32.8,34)	1.1	0.55
PL(lakhs/cumm)	2.36(2.0,2.7)	2.39 (1.9,2.7)	2.54 (2.2,2.7)	2.1	0.33
Eosinophils (%)	2.75(1.5,4.0)	4.1(2.5,11)	3(2.0,50)	8.4	0.01*
Basophils (%)	0.8 (0.6,1.0)	0.6 (0.2,0.9)	1.0(0.4,1.0)	6.2	0.04*
Monocytes (%)	7.7(3.0,9.2)	6.7(5.6,8.3)	8.0(7.0,9.3)	5.7	0.05*

Kruskal-Wallis test: P< 0.05, * statistically significant, P.<0.0001** - highly significant MCHC, mean corpuscular hemoglobin concentration; PL, platelets.

Among the parameters analyzed, eosinophil (P = 0.01), basophil (P = 0.04), and monocyte (P = 0.05) counts revealed statistically significant distinctions between the groups. No significant differences were observed in MCHC (%) (p = 0.55) and PL (p = 0.33).

Table No.4: Assessment of HsCRP and NLR Among the Groups

Inflammatory Markers	Group – I Smokers (n=30) (Median, IQR)	Group – II Smokeless tobacco users (n=30) (Median, IQR)	Group – III Control(n=30) (Median, IQR)	Kruskal Wallis test value	P.Value
HsCRP(mg/dl)	3.2(1.3,4.6)	3.1(1.3,3.7)	0.8(0.4,1.2)	5.7	0.049*
NLR%	1.8(1.2,2.1)	1.5(1.3,1.8)	1.4(1.2,1.8)	6.1	0.047*

Significant differences were observed in the inflammatory markers among the groups. HsCRP levels were elevated in smokers and smokeless users compared with controls (p = 0.04). NLR (%) was also significantly higher in smokers compared to smokeless users and controls (p = 0.047). An increase in both these parameters indicates that systemic inflammation was observed in both smoking and smokeless tobacco users.

DISCUSSION:

This study aimed to evaluate physiological alterations in hematological parameters among both smoking and smokeless tobacco (SLT) users. The MCV and neutrophil levels showed significant differences in both tobacco user groups when compared to healthy controls. These findings are consistent with those of Malenica et al., who also reported significant changes in the MCV, suggesting potential macrocytic alterations related to chronic tobacco exposure.[8] Other hematological indices, such as RBC count, Hb, PCV, MCH, lymphocyte percentage, and total WBC count, did not show statistically significant changes in our study. In contrast, Elisia et al. observed significant elevations in WBC, RBC, Hb, and PCV among tobacco users, which was attributed to compensatory erythropoiesis and leukocytosis triggered by chronic hypoxia. [9]

Similarly, Shukla et al. and Shaik et al. found that SLT users had notable hematological alterations, including reduced Hb and RBC levels and increased WBC counts.[10,11] These discrepancies could be attributed to differences in the study population, duration of tobacco use, and regional variation in the composition of tobacco products. The role of nicotine, which stimulates catecholamine release from the adrenal medulla, may further explain the leukocytosis observed in some studies owing to enhanced systemic inflammation and tissue stress.

Although the mean corpuscular hemoglobin concentration (MCHC) and platelet levels (PL) did not differ significantly among the groups, significant changes were observed in eosinophils, basophils, and monocytes. Eosinophils were reduced in smokers but elevated in SLT users, whereas basophils and monocytes were decreased in both groups. These findings are partially aligned with those of Benowitz et al and Recto et al., who reported increased eosinophil counts and reduced basophils.[12,13] Interestingly, Çekici et al. documented elevated monocyte levels, highlighting tobacco-induced systemic inflammation, which is known to increase granulocytic and monocytic activities.[14] These differences may reflect the varying immunological responses to smoking and SLT exposure. Importantly, hsCRP levels were markedly elevated in both smokers and SLT users, indicating a inflammatory state. The hsCRP is a well-established biomarker for systemic inflammation. Previous studies by Tibuakuu et al. and Kianoush et al. support our findings, with smoking linked to the level of cytokines, such as interleukin-6 and tumor necrosis factor-alpha, has been elevated which stimulate hepatic production of hsCRP.[14,15] Christofaro et al., Siddiqi et al., and Memon et al. also reported increased hsCRP levels among SLT users, reinforcing the inflammatory impact of tobacco use regardless of the route of administration.[16-18] Regarding the neutrophil-to-lymphocyte ratio (NLR), a recognized inflammatory marker, we observed a significant increase among smokers, whereas SLT users showed no marked change. This is in agreement with the findings of Zutshi et al., who also reported minimal changes in NLR among SLT users. [19,20] The elevated NLR in smokers may reflect an acute systemic inflammatory response associated with consumption of toxicants, while more localized exposure to SLT may produce a less pronounced systemic effect.

Limitations: Due to bias, we excluded the female subjects.

Future scope of the study: The withdrawal effect and reversibility of blood parameters need to be studied.

CONCLUSION

These findings emphasize the need for early monitoring of hematological and inflammatory biomarkers among tobacco users. Significant changes in MCV, neutrophils, eosinophils, basophils, monocytes, and elevated hsCRP levels and NLR suggest systemic inflammation and altered immune responses in tobacco users. While some hematological parameters remained unchanged, elevated inflammatory markers such as hsCRP and NLR in smokers indicate a stronger systemic impact of tobacco use. This study helps to guide targeted public health interventions and preventive strategies.

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