

ASSESSMENT OF THE EFFECTS OF SMOKING AND CONSUMING GUTKA (SMOKELESS TOBACCO) ON SELECTED HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS: A STUDY ON HEALTHY ADULT MALES OF HAZARIBAG, JHARKHAND

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ABSTRACT

In this study, the differences in total erythrocyte count, total leukocyte count, packed cell volume, haemoglobin levels, differential leukocyte count, total serum cholesterol level and blood glucose levels were comparatively evaluated between control group (n=17), smokers (n=19) and gutka consumers (n=24). Thus the study included a total of 60 subjects. Healthy male students (age 20-30 years) of Hazaribag district, Jharkhand formed the study subjects. All the subjects had normal Body Mass Index and were from middle class family. Standard procedures were used to determine all the parameters under study. It was found that in comparison to control group both smokers and gutka consumers experienced the following : significant elevation (< 0.001) in total erythrocyte count, total leukocyte count, packed cell volume, haemoglobin level, neutrophil percentage, total serum cholesterol level and blood glucose level. Significant reduction in the percentage of monocyte (< 0.05) and lymphocyte (< 0.001). Majority of the variation of these parameters between smokers and gutka consumers were not significant (> 0.05). It may be concluded that smoking tobacco and consumption of gutka had deleterious effect on haematological parameters and biochemical parameters of the sample studied. The observed increased leukocyte count reflected that inflammatory responses had already started in the sample studied. Awareness is need to stop tobacco usage and safeguard health of youths.

Keywords: smokers, gutka, haematological parameters, biochemical parameters.

INTRODUCTION

Addiction to tobacco products has become common in Indian society. The addictive nature of tobacco is mainly due to the presence of nicotine (a major tobacco alkaloid) in it¹. Tobacco smoke produced by cigarette, bidi, etc contains harmful and carcinogenic substances viz., carbon monoxide, nicotine, free radicals and others^{2,3}. Smoking has many negative impact on health which includes cardiovascular complications, respiratory diseases and cancer^{4,5}. Diabetes has also been linked with smoking⁶. The harmful substances present in the tobacco smoke may lead to oxidative damage to the

lungs, especially by causing accumulation of neutrophil in the lungs^{7,8}. The effect of smoking on leukocyte count reflects inflammatory activity, exposures to oxidants or vulnerability of the host towards inflammatory conditions⁹. It has been known that to deal with the inflammatory response elicited by smoke of tobacco, protein degrading enzymes are released by the neutrophils which is more damaging for the normal cells in the vicinity¹⁰. Many earlier studies had shown ill effects of tobacco smoking on haematologic variables¹¹⁻¹⁷ and biochemical variables^{6,18}.

Smokeless tobacco products commonly used in India includes gutka, paan, khaini and jarda. Gutka mainly contains tobacco in powered form, along with areca nut, slaked lime and perfumery substances. Studies have linked consuming gutka with periodontal inflammation, oral cancer, etc. It has been concluded that damages caused by gutka may not be only limited to oral cavity. Paan or betel quid is a cocktail of areca nut, slaked lime, sweeteners. Sometimes tobacco is chewed with it. Khaini and jarda is mainly powered tobacco along with slaked lime^{19,20}. Although limited, reports are available that indicate adverse effect of different forms smokeless tobacco on hematologic and biochemical parameters^{21,22,23}. Such adverse effects of smokeless tobacco have been attributed by authors to be due to nicotine content of tobacco²³. In view of these reports, the present study comparatively assessed the effect of smoking and consuming gutka on selected blood hematology parameters and biochemical parameters, in contrast to control group in a sample of healthy young adult males.

MATERIALS AND METHOD

The study was conducted among 60 healthy male students (undergraduate/ post graduate /research scholars). Their age varied between 20-30 years. The sample included control group (n=17), smokers (n=19) and gutka consumers (n=24). All the subjects were selected from a few residential mess in Hazaribag district and belonged to middle class family. The study was conducted according to the Helsinki declaration. Purposive sampling were employed for selection of participants in the study; individuals who voluntarily agreed to give blood samples were only included in the study. Alcoholic smokers or gutka consumers were excluded from the study. Subjects of only normal nutritional status (Body Mass Index or BMI =18.5-24.9)²⁴ were included in the study. Control group – were formed with individuals who were non smokers and do not consume gutka. Smokers – were the subjects smoking cigarette / bidi regularly for at least 3 consecutive years. Gutka consumers – were the subjects consuming gutka regularly for at least 3 consecutive years. Informed consent were taken from the subjects.

Laboratory tests

Venous blood (approximately 5 ml) were collected in the morning hours from each subjects for hematological and biochemical investigations.

Hematological investigations: The total erythrocyte count, total leukocyte count were

determined by haemocytometer. Haemoglobin was estimated by Sahli's hemoglobinometer. Packed cell volume was determined by Wintrobe's Method. Tally chart method was used for differential leukocyte count.

Biochemical investigations: Total serum cholesterol and fasting blood glucose level was estimated employing a kit method on a semiautoanalyzer (Microlab 300).

Statistical analysis of the data: Mean and standard deviation (SD) were calculated for all the hematologic and biochemical parameters. One way ANOVA were applied to test the significance of variance of the parameters under study between control group, smokers and gutka consumers. Post- hoc Tukey's pairwise comparison were also used.

RESULTS

The mean and standard deviation of various hematological parameters of control group, smokers and gutka consumers are presented in Table 1. It was prominent from the data that the hematological profile of smokers and gutka consumers differed from control group in the following ways: both of them had significantly higher total erythrocyte count, total leukocyte count, packed cell volume and Haemoglobin levels. The results of differential count showed that they even had significantly higher percentage of neutrophil in their blood. Moreover, monocyte percentage and lymphocyte percentage in their blood were significantly lower. It was noted that in majority of the cases smokers and gutka consumers did not significantly differ among themselves in these hematological parameters.

The mean and standard deviation of the determined biochemical parameters of control group, smokers and gutka consumers are presented in Table 2. It was evident from the data that in contrast to control group, smokers and gutka consumers had significantly higher levels of total serum cholesterol and higher fasting blood glucose levels. The two biochemical parameter did not significantly differ between smokers and gutka consumers.

DISCUSSION

The present study was conducted to assess the impact of smoking and gutka consumption of some hematological and biochemical parameters in a sample of healthy young adult males. The study showed that both hematological and biochemical parameters are susceptible to adverse effects of smoking and gutka consumption.

The increased total erythrocyte count, packed cell volume (PCV) and haemoglobin (Hb) levels in smokers, in contrast to control group in the present study confirms the results obtained by several earlier studies¹¹⁻¹⁷. These effects are possibly secondary to hypoxic stimuli exerted by smoking.

High levels of carbon Monoxide (CO) are present in tobacco smoke. Tobacco smokers may possess continuous increased levels of carboxy-Hb in blood²⁵. CO lowers the affinity of haemoglobin for oxygen by binding with Hb to form carboxy-Hb. This affects the capacity of Hb to carry and deliver oxygen to different tissues of the body²⁶. Changes in arterial oxygen tension are known to affect erythropoietin production. In hypoxic conditions erythropoietin production is enhanced as a result more erythrocytes are produced by erythropoiesis. Thus the increase in packed cell volume and Hb levels following such increase in erythrocyte production are quite obvious and expected. Evidences suggest that CO due to excessive smoking may lead to hypoxic polycythemia^{27, 28}.

In the present study, the total erythrocyte count, packed cell volume and haemoglobin levels of gutka consumers showed similar increase as seen in smokers. Animal model studies have even found increase in PCV in gutka treated mice²⁹. Earlier studies have also shown that Hb level may increase in Gutka users³⁰. In the present study, the increased total erythrocyte count of gutka users seems to reflect that consuming gutka may also stimulate erythropoiesis. An earlier study on mice treated with gutka observed insignificant rise in total erythrocyte count²⁹. According to some authors, insufficient pulmonary function in gutka consumers may impart a necessity of stimulating erythropoiesis for fulfilling the oxygen demands of the body²⁹. Likewise, adverse effects on lung function due to smoking may have also contributed to the enhanced erythrocyte production in smokers of the present study.

The increased total leukocyte count observed in smokers is similar to earlier studies^{11,15,31,32}. Based on available literature authors³¹ summarises the plausible reasons behind such increase in total leukocyte count. They highlight that damaging of tissues, inflammation of bronchioles due to chronic smoking may lead to such increase in total leukocyte count. Additively, nicotine present in tobacco may also influence suprarenal glands causing it to secrete more catecholamine which may affect leucocytosis. Furthermore they asserts that cytokine related to inflammatory responses liberated from respiratory epithelium due to

smoking may also influence the number of leukocytes present in blood.

It may be speculated that damages to tissues and inflammation²⁹ might have also operated behind an increase in total leukocyte count in gutka users of the present study. Smokless tobacco may act as a carcinogen on chronic usage³³.

The observed increase in neutrophil percentage and decrease in lymphocyte percentage in smokers in the present study is in accordance to previous studies^{34,35,36}. Varried reports of increase in one or more types of leukocyte are available in literature^{31,37,38}. The reason behind such variations warrants exploration³⁹. Neutrophils are known to produce cytotoxic substances which adversely affects lung function^{40,41,42}. Therefore, the increased number of neutrophils in smokers of the present study may result in a compromised pulmonary function⁴³. The increased neutrophil percentage found in smokers and gutka consumers of the present study may be associated with ongoing inflammation of tissues.

In the present study the decrease in lymphocytes observed among smokers was not surprising. Systemic stress potentiates the activity of sympathetic nervous system. This raises the cortisol secretion which has been found to be associated with a decrease in blood lymphocyte percentage^{44,45}. Nicotine is also known to be stimulatory to the sympathetic nervous system⁴⁶. Thus it may be speculated smoking or using other tobacco products may result in similar lowering of lymphocyte percentage. An earlier study had concluded that lymphocytes, especially cytotoxic T cells or CD8+ T cells may get lowered in smokers⁴⁷. Variations in these T lymphocytes may make the smokers vulnerable to develop neoplastic growths and infections³¹.

The present findings on percentage of neutrophil and lymphocyte of gutka consumers corresponded with an earlier study on animal model, which similar to the present study documented increased percentage of neutrophil and decreased percentage of lymphocytes²⁹. Like smoking, the inflammatory effects smokeless tobacco on lungs are well explored⁴⁸. This present study also found that both smokers and gutka users had lower levels of monocytes in blood than control group. Similar effects of gutka³⁰ and smoking³⁷ on monocytes are documented in literature. Mild adverse effect on lungs may be the result of such variations of monocytes³⁷. The findings of the present study prominently showed that the negative effect of gutka on blood hematology is no less adverse than smoking.

The negative effects of smoking on lipid profile is a well documented¹⁸. A recent study on gutka consumers observed similar negative effects on serum cholesterol³⁰. In the present study, as expected the total serum cholesterol levels of both smokers and gutka consumers were well above the control group. This finding of the present study reflects affecting of fat metabolism by smoking and gutka consumption. It implied that these forms of tobacco may promote increment in lipid content of the blood. The findings also reflect an increased risk of developing coronary artery disease/ or other cardiovascular complications in smokers and gutka users^{49,50}. Recurrent administration of epinephrine has been found to raise blood cholesterol levels. Thus there is possibility that chronic tobacco users due to continued nicotine stimulated catecholamine secretion may have raised blood cholesterol level⁵¹. In mice it has been documented that epinephrine may be stimulatory to HMG-CoA reductase (a rate-limiting enzyme in cholesterol Biosynthesis)⁵².

In the present study significant increase blood glucose level in smokers and gutka consumers were observed. Glycated haemoglobin (HbA1c), is an indicator of chronic blood sugar homeostasis. It gives an idea about concentration of blood glucose that the subjects are having for a continued period of time⁶. Earlier studies had found it to be more in smokers than non smokers which reflected that smokers may have a raised blood glucose concentration and hence they are vulnerable to develop diabetes mellitus⁶. Rise in mean values of glucose levels in gutka consumers have also been noted by an earlier study³⁰. It has been proposed that nicotine present in tobacco may hamper the maintenance of blood glucose level by insulin. Nicotine possibly lowers insulin sensitivity⁵³. Moreover, adiponectin (a hormone secreted by adipocytes) gets lowerd in smokers due to nicotine component of tobacco⁵⁴. In addition to other functions, adiponectin is involved in the maintenance of blood glucose levels⁵⁵. Lowering of adiponectins had been linked to the possibility of developing type 2 diabetes⁵⁶.

Table 1: Mean and Standard deviation (SD) of hematological parameters of control group, smokers and gutka consumers

Parameter	Control Group (n=17)	Cigarette/ Bedi Smokers (n=19)	Gutka Consumers (n=24)	One way ANOVA P value
Total Erythrocyte count (Cells/cu.mm)	4.83 ± 0.39 a	6.71 ± 0.64 b	5.76 ± 0.93 c	< 0.001
Total Leukocyte count (Cells/cu.mm)	5387.8 ± 609.4 a	6865.4 ± 780.5 b	6370.3 ± 673.9 b	< 0.001
Packed Cell Volume (%)	41.2 ± 3.82 a	47.8 ± 5.11 b	46.9 ± 6.24 b	< 0.001
Haemoglobin (g/dl)	13.2 ± 0.31 a	15.9 ± 1.48 b	15.1 ± 0.80 c	< 0.001
Differential count (DC)				
Neutrophil (%)	58.0 ± 2.76 a	64.3 ± 5.59 b	66.1 ± 4.33 b	< 0.001
Eosinophil (%)	1.88 ± 0.99	2.0 ± 1.25	2.25 ± 1.39	> 0.05 NS
Basophil (%)	0.6 ± 1.0	0.6 ± 0.7	0.9 ± 1.3	> 0.05 NS
Monocyte (%)	2.88 ± 1.22 a	2.21 ± 1.58 a b	1.71 ± 1.39 b	< 0.05
Lymphocyte (%)	36.6 ± 2.15 a	30.9 ± 6.14 b	29.0 ± 5.12 b	< 0.001

Data presented are mean ± SD.

Means bearing different letters in same row are significantly different (p< 0.05) using post-hoc Tukey' s pairwise comparision Unless otherwise indicated by NS the p values are significant

Table 2: Mean and Standard deviation (SD) of biochemical parameters of control group, smokers and gutka consumers

Parameter	Control group (n=17)	Cigarette/ bedi Smokers (n=19)	Gutkha (n=24)	One way ANOVA p value
Cholesterol (mg/dl)	157.1 ± 15.3 a	176.9 ± 14.8 b	172.8 ± 20.9 b	< 0.001
Blood glucose (mg/dl)	83.6 ± 3.26 a	90.1 ± 4.32 b	93.1 ± 6.80 b	< 0.001

Data presented are mean ± SD.

Means bearing different letters in same row are significantly different (p< 0.05) using post-hoc Tukey' s pairwise comparision. Unless otherwise indicated by NS the p values are significant

CONCLUSION

The present study showed that, adverse consequences in hematological parameters (viz . increase in total erythrocyte count, total leukocyte count, PCV, Hb levels, neutrophil

percentage and decrease in the percentage of monocytes and lymphocytes) and biochemical parameters (eg increase in blood cholesterol levels, blood glucose levels) may be the possible outcome of smoking or consuming gutka.

Furthermore, no statistical differences were evident in these ill effects between smokers and gutka consumers. This indicated that consuming smokeless tobacco instead of smoking tobacco imparts no less harmful effects on hematological and biochemical parameters. These negative effects of tobacco use may increase the possibility of developing other health related complications like respiratory diseases, cardiovascular diseases, diabetes, etc. In the present study, it seemed that increased number of neutrophils in blood had resulted in an increase in total leukocyte count in both smokers and gutka consumers. The increased neutrophil percentage reflected inflammatory activity in the sample studied. Owing to the small sample size of the present study, the findings may not be extrapolated to the entire population of young adult males of the region studied. Similar study on a large sample is recommended before forming a definite opinion or judging of the clinical significance of the present findings. The present study also provided evidences in favour of the view of earlier studies that in case of individuals using tobacco, the assessment of hematological parameters⁵⁷ or screening of anemia⁵⁸ should be dealt with caution. This is because altered haematologic profile due to tobacco use by the subject may lead to misleading interpretations. Therefore smoking or use of other tobacco products by the subjects should be taken into consideration when evaluating hematological profile to avoid possible misleading interpretations. Awareness campaign among youths regarding the deleterious effect of tobacco may lower the trend of smoking/ consuming gutka /using tobacco products.

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