

Serum Bilirubin and Uric Acid Levels in Pan Masala Tobacco Users as Compared to Non-Users

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ABSTRACT

INTRODUCTION: Pan Masala Tobacco (PMT), consumed in smoke and smokeless form, contains various carcinogenic chemicals and free radicals. Bilirubin and uric acid are said to have both pro- and anti-oxidant activities. In this context, we aimed to compare the serum levels of bilirubin and uric acid between PMT users and non-users. We also wanted to observe the pattern of PMT consumption among the users. **MATERIALS AND METHODS:** A total of 204 participants were enrolled in this community-based comparative cross-sectional study. The study was conducted in the Ranigaun community, Rupandehi, Nepal, from March 2020 to December 2020 AD for ten months. Serum bilirubin (total, conjugated and unconjugated) and uric acid levels of both PMT users (n = 103) and non-users (n = 101) were measured. Mann-Whitney U test and Chi-squared analysis were performed. A p-value of < 0.05 was considered statistically significant. **RESULTS** The majority of PMT users were male (n= 90; 87.3%). Most of them consumed smokeless forms of PMT (n = 64; 62.1%), with tobacco being the most preferred product (n = 42; 40.8%). The average age of PMT users was significantly higher (44 years) than the non-users (38 years). The serum uric acid and bilirubin levels were not significantly different among the PMT users and non-users. **CONCLUSIONS:** Male consumed more PMT than females. Smokeless tobacco was the most preferred product in the studied area. Serum bilirubin and uric acid levels did not vary significantly between PMT users and non-users.

Keywords: Bilirubin, Oxidative stress, Pan Masala Tobacco, Uric acid



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INTRODUCTION

Tobacco and related products, collectively called Pan Masala Tobacco (PMT), are the leading cause of preventable death worldwide. With 1.3 billion global tobacco users, tobacco consumption is rightfully considered an epidemic [1]. About 30.8% of the adult population in Nepal, especially males, consumes PMT claiming more than 27,000 lives annually [2-4]. PMTs are consumed in both smoke and smokeless form [5]. They contain more than 4,000 chemicals like nicotine, carbon monoxide, and free radicals leading to addiction, carcinogenesis, and generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). It generates considerable oxidative stress and also interferes anti-oxidant defence mechanism [6-10]. Bilirubin and uric acid are products of heme and purine catabolism, respectively. They are considered to

have potent anti-oxidant and pro-oxidant activities [11-17]. Numerous investigations have addressed the effect of PMT consumption on serum bilirubin and uric acid levels. However, a debate over an association between PMT users and their serum levels remains. Various publications have shown increased bilirubin and uric acid levels upon PMT consumption, whereas others have reported decreased levels or no association of PMT consumption on these parameters. [2, 8, 18-21]. Serum bilirubin and uric acid can be easily measured in the laboratory, is cost-effective, and may help estimate oxidative stress. We aimed to estimate bilirubin and uric acid levels in PMT users and compare them with healthy controls. We also intended to evaluate the pattern of PMT consumption among users.

MATERIALS AND METHODS

Study design and setting

This community-based comparative cross-sectional study was conducted for ten months, from March 2020 to December 2020 in Ranigaun, Bhairahawa, Rupandehi, province No.5, Nepal.

Participants, sample size and sampling technique

Study populations were selected from the local community dwellers of Ranigaun, Bhairahawa. The sample size was calculated based on the prevalence of PMT consumption in Western Nepal ($P = 21.32\%$) [2]. The following formula was used for calculation: Sample size (N) = Z^2PQ/d^2 , where $Z = 1.96$, $P =$ prevalence (21.32%), $Q = 100-P$, $d =$ allowable error (8%). The calculated sample size was $100.68 \approx 101$ (for each group). Based on the above calculation, a total of 204 participants were included in the study, where 103 were PMT users and 101 were non-users.

The participants consuming tobacco in any form (smoke or smokeless) were considered PMT users, and the apparently healthy population who did not use PMT products was considered as non-users. Individuals with diseases or conditions that altered serum levels of bilirubin and uric acids, such as jaundice, liver diseases, gout, and renal diseases, were excluded from the study. Similarly, individuals on medications altering these parameters, such as morphine, oral contraceptives, rifampicin, pyrazinamide, steroids, and penicillin, were also excluded. Purposive sampling technique was used to select the study participants.

Data collection procedure and study variables

A study proforma was filled out via face-to-face interview. The proforma included the socio-demographic details of the participants and the details of PMT consumption in the case of users. Subject proforma was encoded for confidentiality. 3 ml of blood was collected in plain vials under aseptic conditions, and serum was separated by centrifugation at 3,000 rpm for ten minutes. The laboratory analyses were performed in the biochemistry lab of Universal College of Medical Sciences, Rupandehi. Serum bilirubin and uric acid levels were estimated using modified Jendrassik and Groff method and uricase method respectively by an automated analyzer (Humalyzer 600, Germany). Total, conjugated, and unconjugated bilirubin levels were estimated. Following reference range (all values in mg/dl) was considered as per the manufacturer's instructions: total bilirubin: 0.2-1; direct bilirubin: 0-0.2; indirect bilirubin:

0.2-0.8; uric acid (male): 4.4-7.6; and uric acid (female): 2.3-6.6.

Statistical analysis and data management

The data were entered in Microsoft Excel and analyzed by Statistical Package for Social Sciences (SPSS version 16). The qualitative data were expressed as frequency and percentage. The Shapiro-Wilk test was performed to evaluate the normality of the quantitative variables. Since the data were non-normal, non-parametric tests were employed to analyze and were expressed in their median and inter-quartile range. Specifically, the Mann-Whitney U test and chi-square analysis were performed. Sex- and Age group-wise analyses of the laboratory parameters between PMT users and non-users were also performed. A p-value of < 0.05 was considered statistically significant.

Ethical considerations

Ethical approval for the study was obtained from the institutional review committee of Universal College of Medical Sciences and Teaching Hospital, Bhairahawa (IRC no: UCMS/IRC/015/20). Both verbal and written consent was taken from the participants before the study.

RESULTS

Of the total 204 participants, 103 were PMT users. More than half ($n = 122$; 59.8%) of the total participants were males. The majority were males among the PMT users as well ($n = 90$; 87.4%). The sex-wise distribution of the study participants is shown in Figure 1 and Table 1.

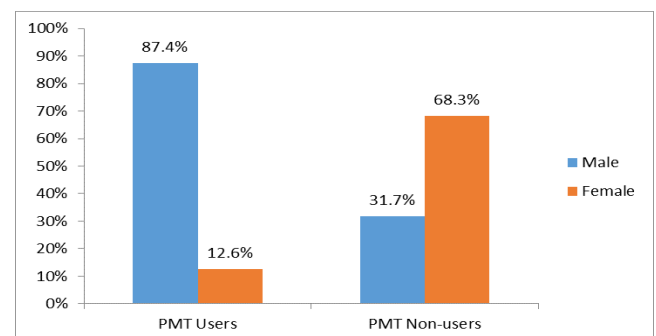


Figure 1 | Sex-wise distribution of the study groups

Most participants in both groups were 31-50 years of age (42.7 % in users and 46.5 % in non-users). However, the median age of the PMT users (44 years) was significantly higher than the non-users (38 years), as shown in Table 1. Most of the PMT users used smokeless products ($n=64$; 62.1%) followed by smoked ($n=22$; 21.4%). Less than one-fifth of the PMT users ($n=17$; 16.5%) consumed both smoked and smokeless

products. The overall PMT consumption pattern (Figure 2). A comparison of the laboratory parameters between PMT users and non-users is shown in table 1. The median total, indirect and direct serum bilirubin levels in both PMT users and non-users were 0.5 mg/dl, 0.3 mg/dl, and 0.2 mg/dl respectively ($p > 0.05$ for each). The median serum uric acid level in PMT users (4.2 mg/dl) was slightly higher than non-users

(4.0 mg/dl), although the difference was not statistically significant ($p = 0.834$). We also analyzed the laboratory parameters for each sex and age group as they varied significantly between the PMT users and non-users. The sub-group analysis also showed no significant differences in these parameters between users and non-users (Table 2).

Table 1 | Comparison of study parameters between PMT users and non-users

Parameters	Total study population (n = 204)	Study Group		p-value ^a
		PMT users (n = 103)	PMT non- users (n = 101)	
Age (years)	40 (28-53)	44 (32-59)	38 (27.5-48)	0.020
TB (mg/dl)	0.5 (0.4-0.75)	0.5 (0.4-0.75)	0.5 (0.4-0.75)	0.766
DB (mg/dl)	0.2 (0.15-0.3)	0.2 (0.15-0.3)	0.2 (0.15-0.3)	0.820
IB (mg/dl)	0.3(0.2-0.45)	0.3(0.2-0.45)	0.3 (0.2-0.45)	0.878
UA (mg/dl)	4.0 (3.5-4.7)	4.2 (3.5-4.7)	4.0 (3.6-4.8)	0.834
Sex	Male	122 (59.8%)	90 (73.77%)	<0.001
	Female	82 (40.2%)	13(15.5%)	

Abbreviations: TB-Total bilirubin; DB-Direct bilirubin; IB- Indirect bilirubin; UA-Uric Acid, PMT- Pan masala tobacco. Data for numerical variables (all except sex) expressed in median and inter-quartile range. Sex represented as frequency & percentage. A- p-values for numerical parameters obtained from Mann-Whitney U test; p-value for sex obtained from Pearson's Chi-square analysis. $p < 0.05$ considered statistically significant.

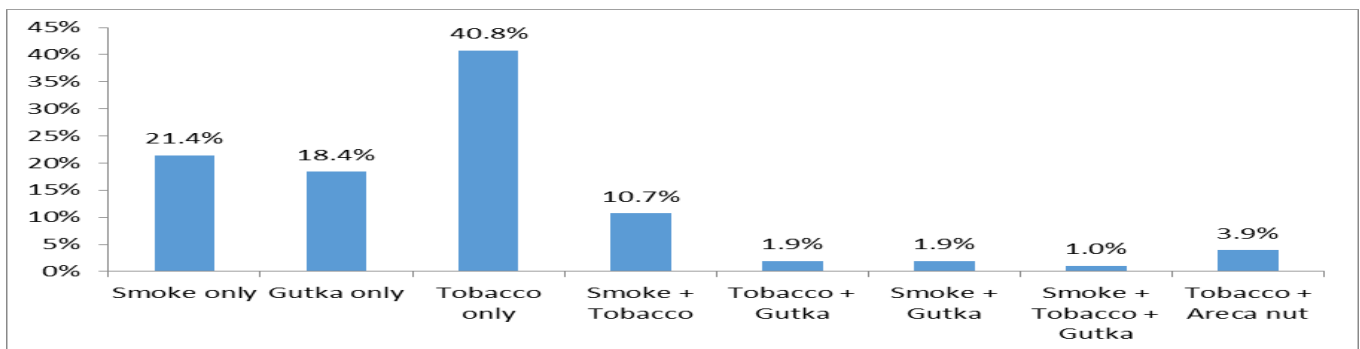


Figure 2: PMT consumption pattern of the users (n = 103)

Table 2 | Age-group and sex-wise comparison of lab parameters between the study groups

Laboratory Parameters		Sex		Age- group (years)		
		Male (n=122)	Female (n=82)	≤30 years (n=58)	31-50 years (n=91)	>50 years (n=55)
Total Bilirubin (mg/dl)	PMT users	0.6 (0.4-0.75)	0.4 (0.3-0.75)	0.6 (0.35-0.77)	0.6 (0.4-0.75)	0.45 (0.37-0.6)
	PMT non-users	0.55 (0.40-0.79)	0.5 (0.4-0.6)	0.5(0.4-0.72)	0.5 (0.4-0.6)	0.6 (0.4-0.85)
	P-value ^a	0.726	0.468	0.527	0.508	0.358
Conjugated Bilirubin (mg/dl)	PMT users	0.2 (0.15-0.3)	0.15(0.15-0.25)	0.3 (0.15-0.3)	0.2 (0.15-0.3)	0.15 (0.15-3)
	PMT non-users	0.2 (0.15-0.3)	0.2 (0.15-0.3)	0.2 (0.15-0.3)	0.2 (0.15-0.3)	0.2 (0.2-0.3)
	P-value ^a	0.728	0.259	0.179	0.402	0.065
Unconjugated Bilirubin (mg/dl)	PMT users	0.3 (0.2-0.45)	0.2 (0.15-0.45)	0.3 (0.15-0.52)	0.3 (0.2-0.45)	0.3 (0.18--0.45)
	PMT non-users	0.3 (0.23-0.45)	0.3 (0.2-0.4)	0.3 (0.3-0.4)	0.3 (0.2-0.45)	0.3 (0.2-0.6)
	P-value ^a	0.575	0.597	0.994	0.671	0.706
Uric Acid (mg/dl)	PMT users	4.15 (3.5-4.7)	4.2 (3.37-5.62)	3.7 (3.26-4.34)	4.3 (3.5-4.95)	4.2 (3.57-5.22)
	PMT non-users	4.4 (3.7-5.19)	3.9 (3.5-4.5)	4.0 (3.55-4.3)	4.0 (3.5-4.8)	4.0 (3.6-5.03)
	P-value ^a	0.137	0.509	0.358	0.567	0.965

a-p-values from Mann-Whitney U test. $P < 0.05$ considered statistically significant. Laboratory values are expressed in median (Inter-quartile range).

DISCUSSION

In this study, most of the PMT consumers were males (n=90; 87.4%). Out of the 82 females, only 13 (15.85%) were PMT users. It is consistent with findings from other studies done in Nepal and India [4, 22]. The probable reasons for higher prevalence might be due to the underrepresentation of female participants associated with social taboos and lesser influence of social circles, which too can be traced to ethno-socio-cultural roots. The median age of the users in our study was significantly higher than that of non-users (44 years vs. 38 years; $p = 0.020$). Another study from Nepal also showed a similar age discrepancy [2].

Overall, smokeless variants were consumed by majority (62.1% vs. 21.35%) than smoked forms. In the smokeless form, tobacco was preferred to "Gutka" by a majority of 40.8% to 18.4%. Only 17 (16.5%) users preferred used both smoke and smokeless forms. However, among the 13 female users, the majority (76.9%) were smokers. Other studies have also reported similar observations. The increased use of smokeless tobacco may be attributed to government legislation that imposes higher tax on smoking forms than smokeless, and also due to a lack of effective regulation system regarding such products [4, 23].

We found no significant differences in the bilirubin levels (total, conjugated, and unconjugated) between the PMT users and non-users. Previously published reports from Nepal show varied results, ranging from

similar bilirubin levels between groups [8], to increased bilirubin levels in PMT users [2]. Other studies from Poland and Korea showed decreased bilirubin levels among smokers compared to non-smokers [18, 24]. Uric acid is present in higher concentrations in mammals than bilirubin [16, 17].

We found no significant difference in serum levels of uric acid between the PMT users and non-users. Shrestha et al. also reported no significant difference in uric acid levels between PMT users and non-users [2]. However, another study from eastern Nepal showed significantly lower uric acid levels in PMT users [8]. Studies on smokers and non-smokers also revealed lower uric acid levels in smokers than non-smokers [20,21].

Our study did not evaluate other measures of oxidative stress and anti-oxidant activities like malonaldehyde, superoxide dismutase, and catalase activities due to budget constraints. Further large-scale studies with the incorporation of the above-mentioned parameters are recommended.

CONCLUSIONS

Most of the PMT users were males. The prevalence of smokeless tobacco consumption was much higher than in smoked forms. Tobacco was the most preferred smokeless form. Serum bilirubin and uric acid levels did not vary significantly between PMT users and non-users.

ADDITIONAL INFORMATION AND DECLARATIONS

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data collection, revision and editing. All authors have read and agreed with the contents of the final manuscript towards publication.

Data Availability: Data will be available upon request to corresponding authors after valid reason.

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