The Functions Of SLT And Smoking Tobacco Consumers Contribute To The Oxidative Stress In Plasma Of Humans

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Abstract

Smokeless tobacco product (SLT) and Smoking products are available in different brands like Rajnignadha, Vimal, Hira, and RMD (Rasikala Manikchand Dhariwal) cigar manufactured by various smokeless tobacco and smoking tobacco industries. The impact of the use of SLT users and Cigar users on plasma biochemical profile especially the levels of glucose, lipids and lipoprotein patterns, proteins, free amino acids and iron. SLT/smoking tobacco (cigar)-exposure caused permissive but significant effect role by elevating the plasma glucose levels by 12% and 15% in group II and III human exposed subjects respectively when compared to control group I subjects who do not use any SLT/smoking tobacco (cigar) products, there is a net drop in plasma iron, glycolipids with a significant increase in free amino acids. There was no change in HDL-C levels among the three groups. Triglycerides and VLDL-C were significantly elevated in SLT/smoking tobacco (cigar) exposed subjects in group II and III indicating coronary risk.

Keywords: Smokeless tobacco, smoking tobacco, Oxidative stress

Introduction

The usage of tobacco is a risk factor for the sixth leading cause of death in the world (WHO, 2011). These SLT products have been reported as popular tobacco products worldwide. These two countries comprise 80% of the 300 million/30 crores SLT users worldwide (National Cancer Institute, 2014; Mutti Packera et al., 2017). Though SLT products are used globally and across 121 countries, over 80% of SLT users live in the South East Asian Region countries (Sinha et al., 2015; Asthana et al., 2018). SLT Combustible tobacco products (American Cancer Society, 2015; Stanfill et al., 2018). Globally twelve present of all deaths among adults aged 30 years due to consumption of tobacco (WHO, 2012; Kaur and Prasad, 2013). The tobacco industry invests half billion dollars annually to promote SLT products, double as much as earlier years ago (Federal Trade Commission, 2016; Elias et al., 2017). Low socioeconomic status, illiteracy, socially acceptable and easily addictive behavior are the various multiple factors contributing to the consumption of more smokeless tobacco than smoking cigarettes. The smokeless tobacco constituents may also contribute to adverse toxic effects among consumers. Recently, the Food and Drug Administration (FDA) proposed the rule to reduce the amount of tobacco-specific N-nitrosamines (TSNA) in SLT products than the original amount due to their carcinogenic activity (FDA, 2017).

There is evidence which supports that the enactment of smoke-free legislation has been directly proportional to the consumption of smokeless tobacco among adolescent males (Hawkins et al., 2018). The SLT products are used without burning and are applied under the cheek, lip, and gums and have recently emerged as most popular tobacco products during the recent years. About 35-40% of tobacco consumption in India is in SLT forms, most of the species are Nicotiana rustica, while most smoking tobacco is from Nicotiana tabacum species (Gupta and Ray, 2003). People believed that mixture of all tobacco products were harmful, but individual/single products were not harmful (Agaku et al., 2018). The use of multiple tobacco products is not limited to particular demographic groups and could potentially reduce tobacco-related diseases (Unger et al., 2018).

Smokeless tobacco products contain a large array of toxicants and carcinogens, including nicotine, tobacco-specific N-nitrosamines, the most prevalent strong carcinogens in SLT products are the tobacco-specific N-nitrosamines. Hecht and Tricker, (1999) reported that the tobacco-specific N-nitrosamines are synthesized from nicotine, nornicotine, anabasine, and anatabine respectively through the process of nitration (Figure 1). At room temperature, the levels of N-nitrosamines and nitrite of the snuff are increased significantly after storage of tobacco (Djordjevic et al., 1993). The tobacco-specific N-nitrosamines are formed during curing and fermentation of tobacco from nicotine and other alkaloids as such as nornicotine, anatabine, and anabasine (Brunnemann et al., 1996).

Addictive potential to users (Benowitz, 2010). SLT users who dip or chew 8-10 times per day may be exposed to the equal amount of nicotine as individuals who smoke 30–40 cigarettes per day (Department of Health and Human Services, 1986; IARC, 2007). Data (Table 1a) indicates that the smokeless tobacco products are named on the basis of region of the world called as chewing tobacco and snuff in North America, chimo in South America, snus in Sweden, gutkha and dry snuff in United Kingdom, gul, naswar, and zarda in Central Asia, gutkha, panmasala in South East Asia, creamy snuff, gul, gutkha, khaini, mawa, mishri, qiwam, red tooth powder in South Asia, nass, naswar, shammah, zarda in Middle East Asia, toombak in Africa (Smokeless Tobacco Fact Sheet, 2002). The smokeless tobacco products of panmasala, gutkha, and khaini are listed in table 2a and 2b are the commonly used in the retail shops in India.

Cigarette smoking, a serious health problem, is the most preventable cause of death in world (Jhaet al., 2008). It has been estimated that about 1.5 billion people smoke cigarettes worldwide (WHO Report, 2008). Surveys revealed that there are about 120 million smokers in India with 5 million deaths annually (Pasupathi et al., 2009). Scientific literature unanimously demonstrated that smoking is responsible for a broad spectrum of dreadful diseases such as, cancer, coronary artery diseases (CAD), as well metabolic and neurodegenerative diseases. On the other hand, the authentic stimulatory, euphoric, reinforcing and addictive properties of smoking make people not to quit the habit (Kunze et al., 2004; Grunberg, 2007). Further, unlike other drugs like anesthetics and alcohol, cigarette smoke does not impair performance in judgement, cognition and motor behaviour (Grunberg, 2007).

Materials and methods

Study subjects and data collection

Twenty-four human male volunteers were selected and each group consisted of eight volunteers, aged between 25-45 years at the mean age of 28.4±2.15 years residing in Ananthapuramu town, Andhra Pradesh taking local diet. The entire studied population was subdivided into three groups:

1. Non-tobacco users or normal healthy controls individuals do not consume any form of tobacco products
2. Smokeless tobacco (SLT)–Individuals consume only chewing tobacco (Gutkha brands (Khaleja) users or Khaini users–Individuals consume only Khaini brand)
3. Smoking tobacco users (cigarette/cigar, Gold Flake)

The baseline information for the category of SLT/Cigar users were that individuals used SLT/Cigar products habitually, at least > 20 times per week consists of 50-60 g during the last 5 years. The exclusion criteria are the consumed either alcohol or religion of the people are not preferred. In the present study all

volunteers were free from any chronic disease, illness, and teetotallers with no drug habit with free from use of any tranquilizers and anaesthetics.

Methodology

Collection of blood and sample analysis

Blood samples from overnight fasted subjects were used for the study. Blood samples, drawn from human male volunteers by vein puncture between 7 and 10 AM into heparinized test tubes, were used immediately for plasma analysis.

Results and Discussion

Data presented in Tables 2 and 3 reveal the impact of the use of SLT users and Cigar users on plasma biochemical profile especially the levels of glucose, lipids and lipoprotein patterns, proteins, free amino acids and iron (Tables 2 and 3). The data presented in Table 2 indicate that SLT/smoking tobacco (cigar)-exposure caused permissive but significant effect role by elevating the plasma glucose levels by 12% and 15% in group II and III human exposed subjects respectively when compared to control group I subjects who do not use any SLT/smoking tobacco (cigar) products, there is a net drop in plasma iron, glycolipids with a significant increase in free amino acids. Data presented in the Table 3 reveal that plasma cholesterol levels decreased in SLT users and Cigar users exposed subjects (group II and III) with 12%, 10% respectively when compared to controls. There was no change in HDL-C levels among the three groups. Triglycerides and VLDL-C were significantly elevated in SLT/smoking tobacco (cigar) exposed subjects in group II and III indicating coronary risk.

Table 2. Alterations of SLT and cigar products on plasma constituents.

Parameter	Groups		
	Controls	SLT Users	Cigar Users
Blood Glucose (mg/dl)	86.65 ± 2.58^a	96.45 ± 1.30^b	99.52 ± 2.34^b
Glycolipids (mg/dl)	330.12 ± 4.17^a	293.54 ± 4.48^b	285.40 ± 1.35^b
Total Proteins (gm/dl)	6.92 ± 0.17^a	6.30 ± 0.03^a	6.42 ± 0.04^a
Albumin (gm/dl)	4.71 ± 0.19^a	4.37 ± 0.17^a	4.64 ± 0.40^a
A/G ratio	1.84 ± 0.03^a	1.95 ± 0.04^a	1.95 ± 0.01^a

Parameter	Controls	SLT Users	Cigar Users
Plasma amino acids (mg/dl)	5.48 ± 0.10^a	6.11 ± 0.04^b	6.10 ± 0.03^b
Plasma iron (µg/dl)	116.12 ± 6.65^a	81.81 ± 6.78^b	87.31 ± 8.12^b

Values are expressed as Mean±SEM, in each column, followed by the same letter are not significantly different (P≤0.05) from each other according to Duncan's Multiple Range (DMR) test. n=8.

Table 3. Effect of SLT and cigar products on plasma lipids and lipoprotein.

Parameter	Groups		
	Controls	SLT Users	Cigar Users
Cholesterol (mg/dl)	210.00 ± 5.06^a	182.94 ± 6.44^b	187.70 ± 4.25^b
HDL-Cholesterol (mg/dl)	44.85 ± 4.38^a	48.73 ± 2.73^a	47.31 ± 7.35^a
LDL-Cholesterol (mg/dl)	71.65 ± 6.65^a	55.41 ± 5.95^a	54.22 ± 6.18^a
VLDL-Cholesterol (mg/dl)	10.52 ± 1.80^a	35.34 ± 2.42^b	35.71 ± 2.68^b
Triglycerides (mg/dl)	151.04 ± 9.12^a	180.94 ± 15.12^b	173.83 ± 12.54^b

Values are expressed as Mean±SEM, in each column, followed by the same letter are not significantly different (P≤0.05) from each other according to Duncan's Multiple Range (DMR) test. n=8.

The prevalence of smokeless tobacco consumption was increased across states and state-wide tobacco control and use prevention efforts that should be very effective towards the diverse tobacco products used among adults (Hu et al., 2019). We found that nicotine was able to induce the production of nitric oxide and peroxynitrites at the highest concentration in SLT and cigar users. The levels of nitric oxide and peroxynitrites have been reported to be directly proportional to nitrosative stress and peroxidation of bio molecules. Our results are consistent with those from (Bagchi et al., 1995) demonstrating that tobacco snuff could be enhanced the synthesis of reactive oxygen species, which results in lipid peroxidation, protein oxidation, and formation of DNA adducts. Overproduction of nitric oxide is correlated with the upregulation of iNOS expression. Reactive nitrogen species include nitric oxide and peroxynitrite, while reactive oxygen species include superoxide (O_2^-) and hydrogen peroxide (H_2O_2). The exposure of SLT and cigar results in the production of reactive oxygen species and the level of iNOS/NO was significantly higher of smokeless tobacco users than that of non-tobacco users. In the presence of nicotine and tobacco-specific nitrosamines, the red blood cell membrane undergoes functional and structural alterations resulting in increased NO bioavailability. Peroxynitrites could be reacting with proteins leads to formation of nitrotyrosine adducts, may indirectly induce the risk of atherosclerosis (Beckman et al., 1994). The direct or indirect effect of nicotine and TSNA might contribute to increased ROS results in the increased expression of inducible nitric oxide synthase. The nitric oxide and peroxynitrites serve as potential biomarkers that helpful in the assessment of toxicity of SLT and cigar products.

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