ABSTRACT

Objectives: A variety of smoked and smokeless tobacco products with varying nicotine content are accessible in India. Nicotine quantity in tobacco products has direct bearing on tobacco dependence. Our objective was to estimate nicotine content in various types of smoked and smokeless products. Disclosure for essential health warning was also checked.

Materials and Methods: Liquid-liquid extraction was used for nicotine extraction and high-performance thin layer chromatography technique was applied for quantification of nicotine in seventy-one smoked and smokeless tobacco products.

Results: Significant variation in nicotine content was observed across products. In smoked tobacco, nicotine content varied from 1.01 to 13.0 mg/rod, while in smokeless tobacco products it ranged from 0.8 mg/g to 50.0 mg/g. Moisture content varied from 9% to 21%.

Conclusion: This work lists a range of smoked and smokeless tobacco products available in this region. We report a wide variability in nicotine quantity across smoked and smokeless tobacco products. Such variation in nicotine content may have important implications for tobacco cessation interventions and policies.

KEY WORDS: High-performance thin layer chromatography, nicotine, packaging disclosures, tobacco, tobacco products

Introduction

The use of tobacco is a public health problem worldwide and has a significant impact on socioeconomic status and environment sustainability. Eighty percent of the estimated one billion deaths from tobacco use in the 21st century will occur in the developing countries. In the South-East Asia Region, 29.8%–63.1% of men and 0.4%–15% of women smoke tobacco. This region also has a substantial burden from smokeless tobacco use, with 1.3%–38% of men and 4.6%–27.9% of women using smokeless tobacco. The estimated number of tobacco users in India is 274.9 million, with 163.7 million users of smokeless tobacco, 68.9 million are smokers, and 42.3 million use both smoking and smokeless tobacco.

Smoked tobacco products are cigarettes, bids (hand rolled cigarettes), and hookah (water pipe). Smokeless tobacco products include pan (piper betel leaf filled with sliced areca nut, lime, catechu, and other spices chewed with or without tobacco), pan masala/gutkha (tobacco mixed with areca nut, slaked lime, and catechu), zarda (mixture of tobacco, areca nut, areca catechu, and slaked lime with added fragrance, spices and flavors), khaini (tobacco leaves with slaked lime), mishri (a powder tobacco rubbed on the gums as paste), and kaddipudi (dried tobacco leaves taken orally or with betel leaves). Studies have shown trace metals, moisture, tar, and nitrosamine levels in smoked and smokeless tobacco products. However, only a few have reported the nicotine content of tobacco products.
On the recommendation of the World Health Organization, as of May 2013, 24 states, and 3 union territories have banned gutkha under the Food Safety and Regulation (prohibition) Act 2011. Both for public health and regulatory issues, as well as for appropriate pharmacotherapy for tobacco cessation, there is a need for quantification of toxins present in commonly used tobacco products.

This study was planned for quantification of nicotine in smoked and smokeless tobacco products. Citing consumer safety product disclosures about ingredients and related health-risks packaging were also checked. Subsequently, we plan to quantify other toxins present in locally available tobacco products. Although various chromatographic techniques (high-performance liquid chromatography, liquid chromatography-mass spectrometry, gas chromatography-mass spectrometry) can be applied for nicotine quantification, we found high-performance thin layer chromatography (HPTLC) to be simple, sensitive, cost-effective, and less time-consuming technique suitable in our setting.

Materials and Methods

All smoked and smokeless products were procured from local retailers in Delhi, Uttar Pradesh, Haryana, and Bengaluru, India. Unbranded tobacco products (kaddipudi, zarda, tambaaku) were obtained as unzipped packs while few samples of unconventional tobacco products (mishri and hookah) were obtained from the patients attending the outpatient services. Product packs were checked for pictorial health warning. This is obligatory to as per the Supreme Court of India directive, under the Cigarettes and Other Tobacco Products Act guidelines. All the samples were labeled randomly with unique identifiers and stored in double wrapped plastic bags at 4°C until analysis. Random selection was opted and at a time same type of products (smoked or smokeless) were analyzed. Nicotine quantity was expressed as milligram per gram along with the total weight of unit [Table 1]. Statistical analysis was done using SPSS version 11 (Statistical Package for the Social Sciences by IBM).

Products were removed from the wrapping, weighed (average of 10 packs) and pooled for chemical analysis (in duplicate). Smoked and smokeless were subjected to liquid-liquid extraction for nicotine extraction after measuring moisture content. Nicotine quantification was done using HPTLC. Nicotine standard was procured from Sigma-Aldrich (USA).

Results

Seventy-one smoked and smokeless tobacco products were analyzed using HPTLC for their nicotine content. Moisture was 9–21% and pH varied from 5.0 to 7.5 in aqueous solutions. A large variation in nicotine content from 0.2 to 22.5 mg/g across brands was seen and coefficient of variance was 34.29%.

Twenty-two cigarettes of different manufacturers (14 Indian, 8 international) were analyzed [Table 1]. The average amount of nicotine content per rod was 9.74 ± 2.11 mg and 14.55 ± 3.28 mg/g of tobacco. Significant variation in nicotine content per gram tobacco ranging from 8.8 to 21.2 mg across brands was observed. The mandatory pictorial warning of health-risk associated with tobacco smoking was seen on all the cigarette brands from India.

Similarly, 18 local brands of bidi were analyzed [Table 1]. Average nicotine content per rod varied from 4.05 ± 1.82 mg to 20.34 ± 6.21 mg/g of tobacco. Tobacco used in several...
brands had higher nicotine concentration compared to tobacco used in cigarettes. Preliminary scrutiny showed that pictorial health-risk warning was printed on all the brands. In some \((n = 4)\) warning label was positioned on the side of the package and was not completely visible. Two brands of cigars we analyzed carried the essential health warning. Their mean nicotine content was 9.9 ± 4.7 mg/g of tobacco.

Ten different brands of gutka and pan masala had average amounts of 6.26 ± 2.65 and 3.25 ± 0.7 mg/g of tobacco respectively. We report significant variation in nicotine content per gram tobacco ranging from 2.4 to 4.3 mg across brands. Pictorial health warning was seen on nine brands along with some promotional messages. The ingredients (tobacco, betel nut, lime, saffron, spices, catechu, and flavors) were mentioned on four brands only. However, none of them mentioned either the presence or content of nicotine in them.

Six different brands of khaini products [Table 1] had an average of 6.65 ± 2.69 mg nicotine per gram of tobacco. The wide variation in nicotine content per gram tobacco (3.3–10.3 mg) was observed in this orally used smokeless tobacco variety. The presence of ingredients (tobacco, lime, menthol, oil, and spices) was displayed in all packs except one. Pictorial warning of health-risk was seen on all brands. None of them displayed presence and quantity of nicotine content.

The dried tobacco leaves (tambaaku) [Table 1] had an average weight of 9.74 ± 3.24 g/pack and the average tobacco content per pack was 8.36 ± 2.24 g. The average nicotine content per gram of tobacco was 13.77 ± 7.64 mg. Pictorial health warning was displayed on all packs.

Hookah samples collected from urban “hookah bars” from Bengaluru were found to contain nicotine content of 0.8 mg/g, while, in zarda, average nicotine was 20.35 mg/g. Mishri is available as strong and light flavor. We had access to light flavors that was found to contain 5.05 mg/g nicotine. Among kaddipudi, mishri, and zarda samples, the nicotine content was highest in zarda [Table 1]. All these products are sold unbranded in unzipped packs without any health-risk warning.

**Discussion**

A number of tobacco products are available in India. From the vantage of public health interest ingredients of these smoked and smokeless tobacco products needs to be scrutinized and quantified precisely. We carried out a systematic quantification of nicotine in 71 brands of commercially available smoked and smokeless varieties of tobacco products. Our data demonstrates a wide variation in the nicotine content per unit pack and per gram tobacco across different tobacco products. These findings are likely to be relevant to many other countries in the region which also have multiple tobacco products.

The average nicotine content of cigarette was 14.55 ± 3.28 mg/g of tobacco. This was consistent with previous reports\(^{10,12}\) Bidi is the most popular form of smoked tobacco used in India.\(^{17}\) The dependence potential of bidis is evident in India where bidi smoking accounts for 40% of tobacco consumption.\(^{18}\) We found the nicotine content of bidi to be 20.3 mg/g and 4.1 mg nicotine being delivered per unit. Malson\(^{18}\) reported a nicotine content of 21.2 mg/g and 4.7 mg nicotine per unit of bidi while Reddy and Shaik\(^{19}\) found an average nicotine content of 26.9 mg/g and 5.0 mg/unit.

Smokeless tobacco products have lower nicotine content, both per pack and per gram of tobacco consistent with earlier reports.\(^{15}\) However, it was observed that users often abuse it more frequently than smoked tobacco products. Oral use of tobacco has an increased risk of cancers and squamous cell and verrucous carcinomas of oral cavity and pharynx.\(^{18,19}\)\(^{10}\)

Apart from gutkha, dried tobacco leaves, and khaini other readily available smokeless products include kaddipudi, mishri, and zarda. Kaddipudi samples contained an average of 5.3 mg/g of tobacco. Nicotine content in hookah samples obtained from local hookah bars was low (0.85 mg/g). However, hookah samples from rural areas contain higher nicotine content.\(^{18}\) Zarda is added to betel leaves as per the customer’s taste as tez (strong) or light. Zarda was found to contain 20.35 mg/g nicotine. The quality of the product, additives, and nicotine content can all influence the patterns and consequences of use of this diverse range of tobacco products.\(^{13,14}\)

From the public health perspective, this work can be further expanded to quantification of nitrosoamines and polycyclic aromatic hydrocarbons and disease markers as well as gaseous toxins of each product (carbon monoxide, tar, and other oxides). To ensure the consumer’s right to be informed about the health-risk from a product, there is a need to monitor and regulate the emission and ingredients among all smoked and smokeless tobacco products used in the country. In addition, awareness should be created in the general public.

**Table 1:**

| Tobacco Product | Average weight/unit (g) | Average nicotine content mg/g tobacco |
|-----------------|--------------------------|-------------------------------------|
| **Khaini**      |                          |                                     |
| Mean=5.9±0.89   | Mean=6.6±2.64            |                                     |
| 5.243           | 3.3                      |                                     |
| 5.900           | 5.0                      |                                     |
| 4.900           | 5.0                      |                                     |
| 6.179           | 7.2                      |                                     |
| 6.431           | 9.1                      |                                     |
| 7.400           | 10.3                     |                                     |
| **Kaddipudi**   | Mean=49.0±1.4            | Mean=5.3±0.565                      |
| 50.000          | 5.7                      |                                     |
| 48.000          | 4.9                      |                                     |
| **Tambaaku**    | Mean=5.7±3.9             | Mean=13.7±7.6                       |
| 6.950           | 8.3                      |                                     |
| 8.986           | 10.5                     |                                     |
| 13.30           | 22.5                     |                                     |
| **Gutkha**      | Mean=2.0±0.92            | Mean=3.2±0.70                      |
| 0.408           | 4.1                      |                                     |
| 1.749           | 2.4                      |                                     |
| 2.093           | 2.4                      |                                     |
| 2.055           | 2.5                      |                                     |
| 1.866           | 3.4                      |                                     |
| 1.761           | 3.8                      |                                     |
| 2.064           | 3.3                      |                                     |
| 2.106           | 3.5                      |                                     |
| 1.838           | 4.3                      |                                     |
| 4.230           | 2.8                      |                                     |
about the harmful effects of these products. This study also has implications for effective pharmacological management for tobacco cessation, which have been shown to improve treatment rates in India and elsewhere.

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Conflicts of interest

There are no conflicts of interest.

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