Variability in addictive and carcinogenic potential of smokeless tobacco products marketed in Mumbai, India: a surveillance study

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Summary

Background India has the highest incidence worldwide of smokeless tobacco (SLT)-associated oral cancer, accounting for nearly 70% of all SLT users globally. Nicotine and tobacco-specific *N*-nitrosamines (TSNA) play critical roles in the addictive and carcinogenic potential, respectively, of SLT products. Our group has previously reported substantial variability in nicotine and TSNA levels across a small SLT product sample in India, calling for systematic surveillance. However, there is no information available on the current levels of these constituents in Indian SLT.

Methods We analysed 321 samples representing 57 brands of eight popular types of manufactured SLT products purchased from five local markets in Mumbai, India between August, and September 2019. The sampling locations were Mumbai Central, Kurla, Thane, Vashi, and Airoli. Product pH, moisture content, total and unprotonated (biologically available) nicotine, and TSNA levels were measured at the Advanced Centre for Treatment, Research, and Education in Cancer (ACTREC) in Mumbai.

Findings Total nicotine content ranged from 0.45 to 35.1 mg/g across products. The unprotonated nicotine fraction contributed 0.1–100% of the total nicotine content. The carcinogenic TSNA levels ranged 0.06–76 ug/g for *N*′-nitrosonornicotine (NNN), 0.02–19.2 ug/g for 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and 0.01–6.51 ug/g for 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL). Consistent with our previous study, we observed substantial variations across different brands of the same product type.

Interpretation This is the most extensive and the first within-country study to report brand-specific nicotine and TSNA levels in SLT products marketed in Mumbai, India. Our results show that levels of these constituents remain extremely variable across Indian SLT and are strikingly high in many products. Enhanced public education and continued efforts to reduce SLT use prevalence in India are critical for reducing the global burden of SLT-associated morbidity and mortality. Regulation of nicotine and TSNA levels in SLT products should be considered.

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Keywords: Smokeless tobacco; Carcinogens; Nicotine; Tobacco-specific N-Nitrosamines; India; Tobacco control

Research in context

Evidence before this study

Smokeless tobacco (SLT) is the predominant form of tobacco in India, with nearly 200 million adults regularly consuming SLT products. India has one of the highest rates of oral cancer worldwide and more than 90% of which can be attributed to tobacco use. The limited published literature suggests that many Indian SLT products contain high levels of alkaloid nicotine and tobacco-specific nitrosamines (TSNA), which play a critical role in the addictive and carcinogenic potential of SLT products. India also has highly diverse SLT products and brands. In our 2017 publication, we reported a 350- to 600-fold variability in nicotine and TSNA content across a small number of products purchased from different markets in Mumbai, drawing attention to the need for systematic product surveillance to inform tobacco control in India. Since then, there have been no publications on the levels of these constituents in Indian SLT.

Added value of this study

This study is intended to provide current, brand-specific data on nicotine and TSNA in SLT sold in Mumbai using the recently established in-country capacity to analyse tobacco products for these constituents. In total, 321 SLT products representing different product types and brands were purchased from five geographical locations in Mumbai. This is the most extensive and the first within-country study to report brand-specific nicotine and TSNA levels in SLT products marketed in India. Our results show that levels of these constituents remain extremely variable across Indian SLT and are strikingly high in many products. The SLT products collected in this study will serve as a repository for the future comprehensive characterisation of other classes of chemical toxicants and carcinogens.

Implications of all the available evidence

Differences in health outcomes associated with SLT use worldwide strongly suggest that products with higher harmful constituent levels pose a higher risk of disease to users. The extremely high nicotine and TSNA levels in many SLT products sold in India are likely responsible for the enormous burden of SLT use and the high incidence of oral cancer and other SLT-associated diseases. Therefore, there is a critical need for enhanced efforts to prevent SLT initiation and facilitate its cessation in India. In addition, product regulation measures, such as setting limits on key harmful constituents, should be considered to reduce the cancer risk among those who continue to use SLT. Given that India is home to the majority of the global SLT user population, such measures are likely to have a major impact on global SLT-associated morbidity and mortality.

Introduction

Smokeless tobacco (SLT) is a broad category of products that do not involve combustion and are used orally and sometimes nasally by 600 million people worldwide. SLT product formulations vary substantially (such as the types of tobacco and other ingredients), as do the associated health risks.[1] For example, cancer risks in users of Swedish SLT products called snus are similar to those of non-users.[2] However, SLT product use in some other countries is associated with a high risk of oral, esophageal, and pancreatic cancers.[3] This is particularly prominent in India, where SLT-induced oral cancer is a leading cause of cancer-related death among men and occurs at rates that are among the highest in the world.[4–6] The extreme diversity of SLT product types and formulations likely contributes to the widespread SLT use in India and confounds tobacco control efforts.[2] Given that India is home to nearly 70% of all SLT users worldwide, understanding and addressing the causes of the high risk of SLT use and its related health outcomes in India are key to reducing the global burden of SLT-associated disease and death.

The levels in SLT products of the major tobacco alkaloid nicotine and tobacco-specific *N*-nitrosamines (TSNA) play key roles in SLT addiction and carcinogenicity, respectively. Nicotine sustains tobacco addiction by binding to nicotinic cholinergic receptors in the brain and facilitating release of dopamine, glutamate, gamma aminobutyric acid (GABA), norepinephrine, serotonin, and other neurotransmitters.[7,8] The addictive potential of SLT products is especially influenced by pH-dependent unprotonated nicotine, a the biologically available nicotine form able to cross cellular membranes.[7,9] TSNA are particularly important because they are specific to tobacco. Extensive toxicological and epidemiological evidence supports the role of two TSNA as key causative agents for tobacco-induced cancers: *N*′-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK).[10–12] The International Agency

for Research on Cancer classified NNN and NNK as human carcinogens.[3] TSNA formation occurs via tobacco alkaloid nitrosation by nitrite during tobacco processing, and additional amounts of TSNA can be formed during finished product storage, facilitated by the bacterial reduction of nitrate to nitrite.[2] More than three decades of publications, including studies from our group, consistently indicate that a wide range of nicotine and TSNA levels occur in Indian SLT products, with some having the highest levels ever reported for tobacco products.[13–17] For example, our most recent study on the chemical composition of several Mumbai-bought product varieties found that unprotonated nicotine levels, when expressed per dry weight of product, varied more than 300-fold and the TSNA content more than 650-fold across the tested products.[17] Alarmingly, an SLT product marketed in India as a safer alternative to other tobacco products actually contained TSNA and unprotonated nicotine at levels that are among the highest found in SLT products worldwide.[18] In that publication, we called for systematic surveillance of Indian SLT products as an important tool for informing the local tobacco control efforts.

Our goal in the current study was to examine the current nicotine and TSNA levels in a significantly expanded number of SLT brands sold in Mumbai, India, using our previously developed procedures. In addition, a key limitation of previous research on Indian SLT constituents was that the product analyses were conducted in laboratories located in the USA.[15–17] A concern with such an approach is the possibility of alteration of SLT chemical composition during product shipment from India to the USA. Furthermore, such an approach is not sufficiently effective in generating timely data for informing tobacco control locally. To address this important limitation, our group collaborated on building laboratory capacity for the analysis of tobacco products and biomarkers at the Advanced Centre for Treatment, Research and Education in Cancer (ACTREC) in Mumbai.[19] Here, we report new data from the ACTREC laboratory related to inter-product and intra-product variability of nicotine and TSNA in SLT products marketed in Mumbai.

Methods

Product samples

Tobacco products were collected between August and September 2019 from five markets representing various geographical locations in the district of Mumbai and its surrounds: Mumbai Central, Kurla, Thane, Vashi, and Airoli. We aimed to purchase three samples of each SLT brand at each site to obtain a representative average for that specific product at the purchase location, and used our previously developed sampling and labelling procedures.[17,20,21] Briefly, at the time of purchase, each sample was placed in an individual plastic bag, the information about the sample was filled out on a pre-printed label attached to each bag, and samples were transferred the same day to the Healis Sekhsaria Institute for Public Health in Mumbai and frozen at –20 °C. The information recorded on the labels included the date and place of purchase, price, and notes on the product that may have been communicated to the vendor (for example, special ingredients). Each sample was assigned a unique identification code. Once collection was completed, all samples were transferred in one batch to ACTREC (approximately 10 miles away), where the products were sealed in plastic sleeves and stored at 4 °C until analysis.

Chemical analyses

Moisture content, pH, total and unprotonated nicotine, and TSNA were analysed at ACTREC using methods previously developed and extensively used at the University of Minnesota.[17,20–22] In addition to tobacco samples, CORESTA reference products CRP1 (Swedish-style snus pouch) and CRP2 (American-style loose, moist snuff) were analysed as quality controls to monitor the performance and analytical accuracy of the assays. Unopened tins of the reference products were provided by the University of Minnesota laboratory from a batch that was used as routine quality control for SLT product analyses. This allowed a cross-laboratory comparison of the data for these products.

Moisture content, pH, and unprotonated nicotine

Moisture was analysed by a gravimetric method, and pH was calculated as a mean of triplicate measurements of an aqueous product extract (at the ratio of 1 mL deionised water per 100 mg product) by an Okaton pH 700 m (Cole Parmer).[17] The pH values were used to calculate the unprotonated nicotine percentage from the Henderson–Hasselbalch equation, using the total nicotine amount in the product (analysed as described below) and the pKa of nicotine, 8.02.[23,24]

Analysis of nicotine and TSNA

These constituents were analysed by liquid chromatography–tandem mass spectrometry (LC-MS/MS) in the positive ion mode using a Shimadzu Nexera X2 ultra-performance liquid chromatography system (Japan) coupled with an AB Sciex QTRAP-4500 (USA). Because many Indian SLT products contain only a small amount of product per sachet, we used a multianalyte extraction approach to extract nicotine and TSNA from the same sample simultaneously.[17] Briefly, tobacco was extracted with 10 mM ammonium acetate, and an extract aliquot was taken for nicotine analysis. The remaining extract was used for TSNA purification and analysis. Stable isotope analogues of nicotine and TSNA, used as internal standards, were purchased from Toronto Research Chemicals (Toronto, Canada).

Nicotine was analysed after further dilution and addition of [CD_3]nicotine internal standard, using a Hypercarb 3µ, 3 × 150 mm column (Thermo Scientific)

and monitoring m/z 163.2 → 130.0 for nicotine and m/z 166.2 → 130.0 for [CD_3]nicotine. In addition to NNN and NNK, we measured 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), which is a human NNK metabolite but is also a carcinogenic TSNA found in processed tobacco.[10,16,17] Other commonly measured TSNA, such as *N*′-nitrosoanatabine (NAT) and *N*′-nitrosoanabasine (NAB), were not included because of their lack of carcinogenicity.[10] An internal standard mixture containing [$^{13}C_6$]NNN, [D_4]NNK, and [$^{13}C_6$]NNAL was added to tobacco extract, followed by purification on ChemElut cartridges (Agilent Technologies, Bangalore, India). The purified samples were analysed using a Zorbax SB C18, 1.8 µ, 3.0 × 150 mm column (Agilent Technologies), monitoring m/z 178.1 → 148.1 for NNN, m/z 208.0 → 178.0 for NNK, m/z 210.1 → 180 for NNAL, and corresponding transitions for the respective internal standards.

Approach to analysing tobacco sold with pan masala

Tobacco samples sold by vendors as companion sachets with *pan masala* products (intended for mixing prior to consumption) were analysed using as follows: the tobacco sachet content was analysed for nicotine and TSNA, and the *pan masala* (which is usually highly alkaline) was analysed for pH and moisture. The moisture content or pH of tobacco in these products was not analysed because the amount of tobacco consumed per sachet was very limited. The product unprotonated nicotine content was calculated using the tobacco sachet nicotine content and the pH of the *pan masala* mix.

Statistical analyses

We performed a two-way ANOVA in R to test whether the levels of nicotine and TSNA differed by purchase location (Mumbai Central, Kurla, Vashi, Airoli, and Thane) for those product brands that had 3 samples purchased in each market (Gai Chhap tobacco, Om Special Pandharpuri tobacco, Chaini Khaini, and Ekka).

Role of the funding source

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, writing of the report, or decision to submit the paper for publication.

Results

We purchased a total of 321 tobacco samples, representing 57 brands of eight SLT product types commonly available in Mumbai: (i) packaged plain tobacco (shredded tobacco leaves as the only component); (ii) *khaini*; (iii) manufactured *gutkha* (areca nut pieces covered with a tobacco-containing flavored paste) and products with the same formulation but not labelled as such (referred to in this paper as *gutkha*-like products); (iv) *pan masala* with tobacco (sold as companion sachets); (v) *mishri*; (vi) *gul*; (vii) creamy snuff; and (viii) dry snuff (Table 1). Six SLT brands were available from all five markets: packaged plain tobacco brands Gai Chap and Om Special Pandharpuri, *khaini* brands Khaini Chaini and Miraj, *pan masala* with tobacco brand

Product	Description	Brands (number of markets)	No. of samples
Chewing tobacco products, chewed as purchased or mixed with lime before use			
Packaged plain tobacco	Dried or processed tobacco leaves, shredded into small flakes.	Gai Chap (5), Om Special Pandharpuri (5), Kamath Hathi Chhap (3), Aasha Jyoti (2), No 555 (2), Mrugaraj (2), Veer (1), No 777 (1), Pandharpuri Sandeep (1), Kala[a] (2), Barik[a] (2), Jagat[a] (1)	71
Manufactured *khaini*	Moist product made with minced tobacco mixed with lime, with or without spices.	Chaini Khaini (5), Miraj (5), Kuber (1), Dhariwal Special Tobacco (1)	36
Finely ground tobacco used as dentifrice, as an ingredient, or nasally			
Mishri	Roasted tobacco powder, used as dentifrice.	Shaheen (4)	12
Gul	Tobacco powder, used as dentifrice.	Chand Tara (2), Dulhan (1), Musaka (1)	12
Creamy snuff	Dentifrice.	Ipco (2), Tona (2)	10
Dry snuff	Used as an ingredient in mixed products or can be used nasally.	N.B. (2), Swastik (2), Gulab Chhap (1), Ghoda Chhap Joshi (2), SVS (1), Photo Budha Javan (1), Sugandha Sagar (1), Rani Singh (1), Gai Chap (1), Kamath Hati Chhap (1), Panch Photo Brand (1)	42
Products that contain areca nut in addition to tobacco			
Gutkha and *gutkha*-like	Manufactured product containing areca nut, tobacco, and spices. Packages are either labelled as *gutkha* or have no specification (only brand name).	Ekka (5), Shikhar (3), Goa (2), Kolhapuri (2), Sagar (1), 4K (1), RMD (1)	45
Pan masala with tobacco	Sold as companion sachets intended to be mixed before consumption.	Vimal (5), Rajanigandha (4), Raj Niwas (2), Rajashree (2), RMD (2), Musafir (2), Tansen Blue (1), Jafri (1), Shudh Plus (1), Pan Bahar (1), Cash Gold (1), Pukar (1), Kamala Pasand (1), Rokada (1), Hot (1), Banarasi Ashik (1), Vilas[a] (1)	93
Total samples analysed			**321**

[a]For the brands marked with an asterisks, a single sample of the product was obtained from each market.

Table 1: **List of tobacco product brands analysed in this study.**

Product type	Moisture, %	pH	Total nicotine mg/g[a]	Unprotonated nicotine	
				%	mg/g[a]
Chewing tobacco products, chewed as purchased or mixed with lime before use					
Packaged plain tobacco	23.7 ± 3.3	5.45 ± 0.4	21.9 ± 4.5	0.47 ± 0.6	0.08 ± 0.08
Khaini	36.8 ± 6.6	10.17 ± 0.4	5.2 ± 0.8	98.9 ± 1.3	5.2 ± 0.8
Mean ± SD for chewing tobacco	28.3 ± 7.8	7.12 ± 2.3	16.1 ± 8.8	35.2 ± 47.3	1.9 ± 2.5
Finely ground tobacco used as dentifrice, as an ingredient, or nasally					
Mishri	13.1 ± 1.1	6.86 ± 0.1	8.8 ± 0.4	6.6 ± 1.5	0.59 ± 0.2
Gul	10.9 ± 1.4	8.99 ± 1.0	17.7 ± 4.2	78.0 ± 22.6	13.2 ± 3.3
Creamy snuff	34.2 ± 4.1	9.27 ± 0.6	2.3 ± 0.6	90.1 ± 14.2	1.9 ± 0.3
Dry snuff	13.0 ± 6.4	9.20 ± 1.4	8.0 ± 2.3	77.7 ± 26.1	6.4 ± 3.2
Mean ± SD for ground tobacco	15.5 ± 8.9	8.81 ± 1.4	8.9 ± 4.9	68.1 ± 34.8	5.9 ± 4.8
Products that contain areca nut in addition to tobacco					
Gutkha and *gutkha*-like	11.0 ± 1.4	8.89 ± 0.4	1.3 ± 0.7	85.8 ± 9.9	1.1 ± 0.7
Pan masala with tobacco[b]	7.5 ± 2.6	9.87 ± 1.0	18.4 ± 5.2	95.4 ± 17.5	17.1 ± 6.7
Mean ± SD for areca-containing products	8.7 ± 2.8	9.5 ± 1.0	12.8 ± 9.2	92.1 ± 16.2	11.9 ± 9.4

Levels are represented as mean ± SD for all product types. [a]Levels of total and unprotonated nicotine are expressed per gram wet weight. [b]For *pan masala* with tobacco products, pH and moisture content analysis was carried out on *pan masala* sachet and chemical analysis was carried out on the companion tobacco sachets.

Table 2: **Levels of pH, moisture content, and total and unprotonated nicotine in various tobacco product types analysed in this study.**

Vimal, and *gutkha*-like brand Ekka. The remaining products were available only at certain locations.

For data analysis and interpretation, products were grouped into three categories based on their content and intended mode of use: (i) chewing tobacco products that contain mostly shredded tobacco leaves, chewed as purchased or mixed with lime before use (plain tobacco, *khaini*); (ii) finely ground tobacco products (dry or creamy) used in multiple ways, including as dentifrice, as an ingredient in handmade products, or nasally (*gul, mishri,* creamy snuff, dry snuff); and (iii) areca nut-containing products, including *gutkha* and *gutkha*-like products (manufactured, ready-to-use) and *pan masala* with tobacco (two separate sachets intended for mixing before use). Product brands/varieties with at least three available samples were included in the analysis. Complete data on the constituent levels in individual product brands/varieties are provided in Supplemental Table S1 (moisture, pH, and total and unprotonated nicotine) and S2 (TSNA). Tables 2 and 3 summarise the data aggregated by product type, with nicotine and TSNA levels presented per gram wet weight of product (as consumed by users). Dry weight conversion was performed using the data from Supplemental Tables S1 and S2.

Supplemental Table S3 summarises the data for the reference products CRP1 and CRP2, which are consistent with the results obtained at the University of Minnesota laboratory and with available technical reports on these products.

Constituent ranges: overall and by product category

The moisture content, pH, and total and unprotonated nicotine levels by product type are summarised in Table 2. The moisture content in individual product samples ranged from 1.5% (*pan masala* Cash Gold) to 51.7% (*khaini* Kuber). It was generally lower in dry snuff, *gutkha* and *gutkha*-like products, and *pan masala* than in other product types. The pH across individual product samples ranged from 4.95 (*pan masala* Banarasi Ashik) to 12.82 (dry snuff Kamath Hathi Chhap), with most products having alkaline pH except for the plain tobacco, which is intended for mixing with slaked lime before use.

Total nicotine content in all tobacco-containing samples (i.e., excluding *pan masala* sachets) ranged from 0.45 mg/g (*gutkha* Kolhapuri) to 35.1 mg/g (companion tobacco to *pan masala* RMD), averaging 16.1 ± 8.8 in chewing tobacco, 8.9 ± 4.9 in ground tobacco products, and 12.8 ± 9.2 in areca-containing products (Table 2). Among products that contained primarily tobacco (i.e., chewing and ground tobacco products), the highest total nicotine levels were found in plain tobacco and *gul* products (21.9 ± 4.5 and 17.7 ± 4.2 mg/g product, respectively), whereas creamy snuff had the lowest levels (2.3 ± 0.6 mg/g). Unprotonated nicotine content in all products varied from 0.1% (companion tobacco to *pan masala* Banarasi Ashik) to 100% (dry snuff Kamath Hathi Chhap) of total nicotine, averaging 35.2 ± 47.3% in chewing tobacco, 68.1 ± 34.8% in ground tobacco products, and 92.1 ± 16.2 in areca-containing products. Among products that contain primarily tobacco, unprotonated nicotine was highest in *gul*, followed by dry snuff and *khaini*: 13.2 ± 3.3, 6.4 ± 3.2 and 5.2 ± 0.8 mg/g product, respectively.

The TSNA levels by product type are summarised in Table 3. Across all product samples, TSNA levels ranged from 0.06 µg/g (*gutkha* brands Shikhar and Kolhapuri) to 76 µg/g (dry snuff [*naswar*] Panch Photo Brand) for

Product type	TSNA (µg/g)[b]			
	NNN	NNK	NNAL	TSNA sum[c]
Chewing tobacco products, chewed as purchased or mixed with lime before use				
Packaged plain tobacco	4.8 ± 4.9	1.7 ± 0.8	0.37 ± 0.6	6.8 ± 6.0
Khaini	8.1 ± 7.9	1.4 ± 1.6	0.95 ± 0.9	10.4 ± 10.3
Mean ± SD for chewing tobacco	5.9 ± 6.3	1.6 ± 1.1	0.57 ± 0.8	8.1 ± 7.9
Finely ground tobacco used as dentifrice, as an ingredient, or nasally				
Mishri	3.86 ± 0.26	1.5 ± 0.1	0.20 ± 0.03	5.5 ± 0.2
Gul	3.53 ± 2.74	0.64 ± 0.5	0.39 ± 0.3	4.6 ± 3.5
Creamy snuff	4.17 ± 0.79	1.4 ± 0.2	0.49 ± 0.2	6.0 ± 1.0
Dry snuff	16.9 ± 19.8	6.3 ± 5.6	1.1 ± 1.6	24.3 ± 25.8
Mean ± SD for ground tobacco	11.0 ± 16.0	3.9 ± 4.7	0.77 ± 1.3	15.8 ± 21.4
Products that contain areca nut in addition to tobacco				
Guthka and *gutkha*-like	0.27 ± 0.28	0.12 ± 0.1	0.03 ± 0.02	0.42 ± 0.4
Pan masala with tobacco[d]	1.67 ± 0.51	0.93 ± 0.5	0.21 ± 0.1	2.8 ± 0.9
Mean ± SD for areca nut-containing products	1.21 ± 0.80	0.67 ± 0.5	0.15 ± 0.1	2.0 ± 1.4

[a]Abbreviations: TSNA, tobacco-specific *N*-nitrosamines; NNN, *N*′-nitrosonornicotine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol. [b]Levels are represented as mean ± SD for all product types; the results are presented per gram wet weight. [c]Sum of the three carcinogenic TSNA: NNN, NNK, and NNAL. [d]For *pan masala* with tobacco products, TSNA analysis was carried out on the companion tobacco sachets.

Table 3: **Levels of tobacco-specific *N*-nitrosamines NNN, NNK, and NNAL in various tobacco product types analysed in this study.[a]**

NNN; 0.02 µg/g (*gutkha* Kolhapuri) to 19.2 µg/g (dry snuff [*naswar*] Panch Photo Brand) for NNK; and 0.01 µg/g (multiple brands of *gutkha*) to 6.5 µg/g (dry snuff [*naswar*] Panch Photo Brand) for NNAL. The sum of NNN, NNK, and NNAL (referred to in this study as total carcinogenic TSNA) ranged from 0.09 µg/g (*gutkha* Kolhapuri) to 102 µg/g (dry snuff [*naswar*] Panch Photo Brand) and averaged 8.1 ± 7.9 µg/g in chewing tobacco, 15.8 ± 21.4 µg/g in ground tobacco products, and 2.0 ± 1.4 µg/g in areca-containing products (Table 3).

Constituent variations by brand within product type

Fig. 1A and B illustrate the variability in unprotonated nicotine content and total carcinogenic TSNA across individual brands within the seven product types for which multiple brands were available. The highest variation in unprotonated nicotine was found across brands of *pan masala* with tobacco, followed by dry snuff and packaged plain tobacco brands at 2000-fold, 11-fold, and 7-fold, respectively (Fig. 1A). The highest variation in TSNA was found across dry snuff brands, followed by *khaini* and *gutkha* (including *gutkha*-like) brands: 32-fold, 28-fold, and 11-fold, respectively (Fig. 1B).

Constituent variations by market and vendor for the same brand

Fig. 2 illustrates the variation in unprotonated nicotine and total carcinogenic TSNA across samples of the same brand purchased from the five markets. Some differences across markets were statistically significant (Fig. 2). TSNA content in Chaini Khaini was most variable, with NNN varying from 10.4 ± 1.1 (Mumbai Central) to 15.2 ± 0.9 (Kurla) µg/g, NNK from 1.3 ± 0.2 (Mumbai Central) to 3.4 ± 0.2 (Thane) µg/g, and NNAL from 1.6 ± 0.07 (Mumbai Central) to 2.2 ± 0.3 (Kurla) µg/g.

Discussion

Our study demonstrated that the nicotine and TSNA levels in Indian SLT continue to be extremely variable and are exceptionally high in some products. These findings are consistent with previous reports on these constituent levels in Indian SLT. In addition to providing a snapshot of current nicotine and TSNA levels in SLT sold in Mumbai, this is the first study to report extensive brand-specific data across various SLT product types in India. This is also the first report in which the data was generated in an Indian laboratory using analytical methods routinely used in the USA to analyse multinational tobacco products. Our findings emphasise the critical need for effective educational and policy measures in India to prevent SLT use and encourage its cessation. Another important policy measure would be to establish product standards that limit harmful constituent levels in Indian SLT. Given that India is home to the majority of the global SLT user population, such measures are likely to have a major impact on global SLT-associated morbidity and mortality.

The data presented in this report are expressed per gram of "wet" product weight to illustrate the constituent level variability in products as they are sold to consumers. Across all product samples analysed (that is, individual SLT samples purchased from a specific market/vendor), the per-gram product nicotine levels varied 78-fold. Furthermore, because many Indian SLT

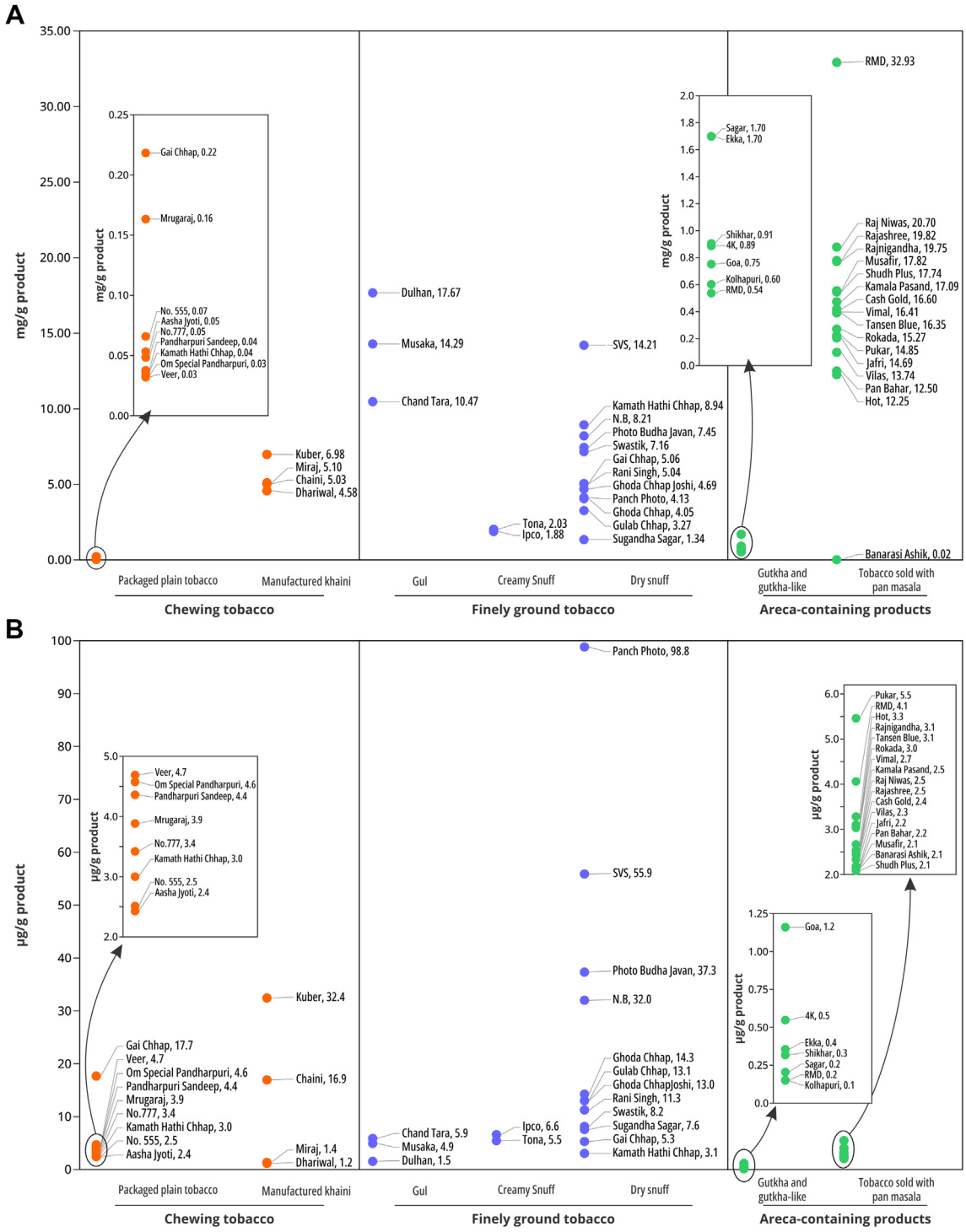

Fig. 1: **Constituent variability by brand within the same tobacco product types.** **A**: Unprotonated nicotine; **B**: The sum of measured carcinogenic TSNA (*N*′-nitrosonornicotine [NNN], 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [NNK], and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol [NNAL]).

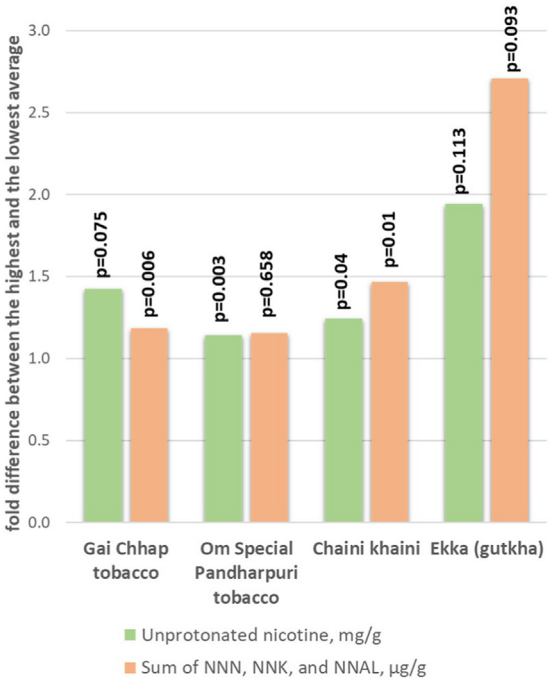

Fig. 2: **Examples of variation of constituent levels within the same product brand, by place of purchase.** The figure includes only those products that had samples purchased at all five markets, 3 samples per market. Bars represent, for each product, the ratio (fold difference) between the highest and the lowest average levels of unprotonated nicotine and tobacco-specific *N*-nitrosamines (TSNA) across the five markets. Numbers above bars are p-values for differences across markets (one way ANOVA, see Methods section).

products contain slaked lime and other alkaline agents, pH values ranged widely across the products (Table 2). The combined effects of the total nicotine and pH variability resulted in a more than 2500-fold range of unprotonated nicotine levels across the 321 individual product samples, whether expressed per wet weight or dry weight of the product (i.e., after accounting for the moisture content). Such extreme variations in unprotonated nicotine levels have major implications for the addictive potential of these products and cessation interventions. For example, individuals using products with very high unprotonated nicotine levels may be more addicted to SLT than those who use low-nicotine products and may therefore require tailored interventions. The total carcinogenic TSNA levels (i.e., the sum of NNN, NNK, and NNAL) in all products analysed here also varied drastically: more than 1100-fold when expressed per wet product weight and more than 1700-fold when calculated on a dry weight basis. Biomarker-based studies have shown a dose-dependent relationship between TSNA levels in SLT products and exposures in users[25] and a prospective association between exposure to TSNA and the subsequent risk for developing cancer.[26] Therefore, the extreme TSNA variability in Indian SLT products is likely to have direct implications for cancer risk in users of these products. The variability in unprotonated nicotine and TSNA content across various brands of the same product type is another important study finding (Fig. 1) and is consistent with our previous report.[17] Such variability emphasises the importance of collecting SLT brand information as part of the tobacco use questionnaires used in tobacco research studies in India. Finally, we also observed constituent variability within the same product brand by purchase location for some analysed products (Fig. 2), which was likely due to lack of manufacturing standards and/or the duration and conditions of product storage.[2]

It is important to emphasise the strikingly high TSNA levels in the products analysed in this study (Supplemental Table S2). For example, if the moisture content is taken into account, the highest calculated level of NNN—a potent oral and esophageal carcinogen[3]—in this product set reaches 112 µg/g dry weight. This is more than 100-fold higher than the U.S. FDA-proposed limit for NNN in SLT products (1 µg/g dry weight).[27] The relatively high NNK and NNAL levels in many products are also of concern: although these TSNA are primarily recognised for their lung carcinogenicity, they can also cause SLT-associated cancers of the nasal cavity and pancreas.[10] The TSNA levels found in some product types in this study are consistent not only with the previous reports for products sold in India but also with these constituent levels in similar products sold in other countries. For example, in the study by Stanfill and colleagues[16] that reported a wide range of NNN levels (0.045–368 µg/g tobacco) across SLT products from 10 countries, the lowest level of this carcinogen (0.045 µg/g) was found in a *gutkha* product from Pakistan. The same study reported 8 µg/g NNN in a *gul* product and 28.6 µg/g in a *zarda* product from Bangladesh. A more recent publication reported a somewhat higher range of NNN levels in similar products from Bangladesh: 13–25 µg/g NNN across four *gul* brands and 2.8–59 µg/g NNN across 22 *zarda* brands, although NNN levels were also relatively high in a reference product CRP1.2 in that study.[28] The highest TSNA levels ever reported in SLT products have been found in the product *toombak* from Sudan, with NNN ranging from 115 to 3080 µg/g and NNK ranging from 147 to 7870 µg/g across studies.[1,16]

This study underscores the urgent need for enhanced efforts aimed at preventing SLT initiation and facilitating cessation in India and other countries with a similarly high prevalence of SLT use and high carcinogen levels in SLT products. Effective and innovative approaches to educate consumers and healthcare providers about the addictive and carcinogenic potential of SLT products could be powerful tools in such efforts. Furthermore, previous publications have suggested that the high variability and extremely high levels of TSNA in

some Indian SLT products date back to at least 1988[13] (Supplemental Fig. S1). Therefore, product regulation measures, such as setting limits on key harmful constituents, should be considered to reduce the cancer risk among those who continue to use SLT. In fact, even prior to the NNN product standard proposed by the U.S. FDA, setting limits on NNN and NNK in SLT products at 2 μg/g dry weight of tobacco has been proposed by the WHO Workgroup on Tobacco Product Regulation (although no action has been taken to date to follow this recommendation).[29] Reduction of NNN and NNK to such levels in SLT, including in Indian products, is achievable by adjusting the tobacco processing and product manufacturing approaches.[2] Of course, reduction of a select few harmful constituents in Indian SLT will not fully eliminate the global harm caused by these products. However, the potential public health benefits of such reductions are supported by epidemiological data comparisons from countries with different carcinogen levels in SLT products. For example, the risks of oral, esophageal, and pharyngeal cancers in Sweden and the USA are lower than those in India (Supplemental Fig. S2), consistent with the differences in the relative abundance of NNN and NNK in SLT products in these countries.[2] Other countries in South and Southeast Asia, where SLT products similar to those in India are being marketed, also experience high risk for oral cancer.[30] High risk for oral cancer has also been reported in Sudan, where the local SLT product *toombak* contains extremely high levels of NNN and NNK.[2,3,5]

This study is the result of a long-term collaborative effort by the authors to develop laboratory capacity for tobacco products and biomarker analyses in India. The study had some limitations. We focused on a limited set of SLT products and a specific geographic area; not all brands had samples available from all five markets, and a limited range of constituents were analysed. While comprehensive surveillance of Indian SLT products was beyond the scope of this report, our team will address such limitations in our ongoing study, in which we collect additional product samples from other parts of India, and through future research. However, further research is likely to reveal even wider ranges of harmful constituents and greater brand variability within product types. It is time to leverage existing evidence to strengthen SLT use interventions, including consumer education, and take firm steps towards regulating harmful constituents in Indian SLT.

Contributors

I.S. and S.S.K. conceptualised the study. I.S., V.G., S.S.K., P.C., D.K.H. and P.C.G. designed the study. P.C.G., N.P., and A.S. were involved in product collection and maintenance of the product repository. I.S. and V.G. planned and supervised the analysis. P.W.V. provided the overall guidance on the analytical methods. S.S.N completed the analysis and I.S. and V.G. verified the generated data. I.S., V.G. and S.S.N wrote the first draft of the manuscript. All authors, including J.S.A. and S.B. contributed to data interpretation, revised subsequent drafts, read and approved the submitted version.

Data sharing statement

Study results reported in this article will be made available upon request. Requests should be directed to Irina Stepanov (stepa011@umn.edu) and Vikram Gota (vgota@actrec.gov.in).

Declaration of interests

S.K. provided an expert testimony, with the payment received institutionally by the University of Minnesota. J.S.A. received travel compensation for the 2021 and 2022 Annual Global Tobacco and Nicotine Forum and is a consultant and has equity in Qnovia, a start-up company developing a nicotine replacement product for U.S. Food and Drug Administration prescription product approval. We declare no other competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lansea.2024.100457.

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