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Correlation of Arsenic Levels in Smokeless Tobacco Products and Biological Samples of Oral Cancer Patients and Control Consumers

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Abstract It has been extensively reported that chewing of smokeless tobacco (SLT) can lead to cancers of oral cavity. In present study, the relationship between arsenic (As) exposure via chewing/inhaling different SLT products in oral cancer patients have or/not consumed SLT products was studied. The As in different types of SLT products (gutkha, mainpuri, and snuff) and biological (scalp hair and blood) samples of different types of oral cancer patients and controls were analyzed. Both controls and oral cancer patients have same age group (ranged 30–60 years), socio-economic status, localities, and dietary habits. The concentrations of As in SLT products and biological samples were measured by electrothermal atomic absorption spectrophotometer after microwave-assisted acid digestion. The validity and accuracy of the methodology were checked by certified reference materials. The resulted data of present study indicates that the concentration of As was significantly higher in scalp hair and blood samples of oral cancer patients than those of controls ($p<0.001$). It was also observed that the values of As were two- to threefolds higher in biological samples of controls subjects, consuming SLT products as compared to those have none of these habits ($p>0.01$). The intake of As via consuming different SLT may have synergistic effects, in addition to other risk factors associated with oral cancer.

Keywords Arsenic · Oral cancer · Biological samples · Smokeless tobacco products · Atomic absorption spectrophotometry

Introduction

Oral cancer is a common malignancy among people who have tobacco smoking and chewing habits [1]. The disease is characterized by a high rate of morbidity and mortality [2]. The etiology of oral cancer is multifactorial, major risk factors are tobacco and alcohol consumption [3]. Both tobacco smoking (cigarettes, cigars, and pipes) and chewing SLT products with and without other ingredients have been shown to increase the risk of developing oral cancer [3–5]. Cancers caused by SLT use often begin as leukoplakia or erythroplakia, which has a higher chance to becoming cancerous over the time [6].

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Tobacco plant (*Nicotiana tabacum*) is well-known for its capacity to concentrate toxic elements from its growing environment [7]. Tobacco is known to contain numerous classes of carcinogenic substance such as tobacco-specific nitrosamines, which are often regarded as a major factor in SLT-related carcinogenesis. The combined exposure of nitrosamines and other classes of organic and inorganic substances, including toxic metals enhances the carcinogenetic effects [8].

Chronic exposure to As and heavy metals has long been recognized to enhance the cancer incidence among exposed human populations. In fact, As and heavy metals are considered to be able to act not only as carcinogens but also as co-carcinogens that could activate certain chemical compounds [9, 10]. The As exposure may cause gastrointestinal irritation, decreased production of red and white blood cells, abnormal heart rhythm, damage of blood vessels, pins and needle sensation in hands and feet, as well as damage the internal organs [11–13].

The effect of As exposure on human health was observed in population of south and southeastern Asia, particularly in Bangladesh, Taiwan, India, and Pakistan [14–16]. Numerous studies have demonstrated that high exposure of As caused various cancers, chromosome aberrations, and oxidative stress [17–21]. The As toxicity causes skin cancer, mouth ulcerations, low hemoglobin, leukemia, acute renal failure, and nerve damages [22]. Human exposure to As via different routes such as water and foods can lead to diverse disease processes. However, intake of As from non-food sources are often overlooked although they may be a contributory factor in the development of disease and this requires further investigation [23].

In the last decade, toxic element, As, received much attention as humans may be exposed through occupational and environmental exposure [24]. In a previous study, it was reported that As levels in surface and underground water, vegetables, and tobacco were high and population of southern areas of Pakistan have high exposure to As from food and non-food items [25]. The study reported that chewing SLT products is a risk factor for As-related skin lesions in women [26]. The As in mammals causes lipid per oxidation, glutathione depletion, as well as protein and enzyme oxidation [27–29]. The As carcinogenicity include its ability to alter DNA methylation patterns, induce cell death and proliferation, inhibit DNA repair, and induce genetic damage [30–32].

Several analytical techniques, electrothermal atomic absorption spectrometry [33], and hydride generation atomic absorption spectrometry [34, 35] are used for the determination of trace levels of As with sufficient sensitivity. As the rate of oral cancer is increased in Pakistan, although many risk factors has been well characterized in its pathogenesis, while very common habit of chewing SLT products is also one of the main reasons.

The aim of present study was to evaluate and compare the concentration of As in different types of snuff (dry and moist), mainpuri and gutkha, available, and consumed in Pakistan. The As in scalp hair and blood samples of oral cancer patients and controls consumed different SLT products were also analyzed.

Materials and Methods

Reagents and Glassware

Ultra-pure water obtained from ELGA labwater system (Bucks, UK). Concentrated nitric acid (65 %) and hydrogen peroxide (30 %) were obtained from Merck (Darmstadt, Germany). Working standard solutions of As were prepared prior to their use by stepwise dilution of certified standard solutions (1,000 ppm) Fluka Kamica (Buchs, Switzerland), with 0.2 mol/L of HNO_3. Moreover, matrix modifier was employed to analyze As was prepared from $Mg(NO_3)_2$ and 99.999 % Pd (Sigma, St. Louis, MO). All solutions were stored in polyethylene bottles at 4 °C. For the accuracy of methodology, certified reference materials (CRM), human hair BCR 397, Clincheck® control-lyophilized human serum and Virginia tobacco leaves (ICHTJ-cta-VTL-2) were used. Glasswares and polyethylene containers were soaked in 10 % (*v/v*) HNO_3 for 24 h; washed with distilled water, finally with de-ionized water and dried in such a manner to ensure that no any contamination from glasswares occur.

Instrumentation

The determination of As was carried out by means of a double-beam Perkins-Elmer Atomic Absorption Spectrometer Model 700 (Norwalk, CT) equipped with the graphite furnace HGA-400, pyrocoated graphite tubes with integrated platform, an autosampler AS-800, and deuterium lamp as background correction system. A single element hollow cathode lamp used for As was operated at 7.5 mA with a spectral bandwidth of 0.7 nm. Portions of both standards/samples and modifier transferred into autosampler cups, then 20 μL (standard or sample volume 10 μL and modifier 10 μL in each case) was injected into the electrothermal graphite atomizer. The graphite furnace heating program was set for the drying, ashing, atomization, and cleaning steps as temperature ranges (°C)/time (s): 80–120/15, 300–600/15, 2,000–2,100/5, and 2,100–2,400/2, respectively. A horizontal electrical shaker (220/60 Hz, Gallenkamp, England) was used for shaking the samples. The pH was measured by a pH meter (781-pH meter, Metrohm). A PEL domestic microwave oven

(Osaka, Japan), programmable for time and microwave power from 100 to 900 W, was used for digestion of samples.

Study Population

A survey was carried out about the gutkha and mainpuri chewing, while snuff inhaling habits of both genders, age ranged 30–60 years, residing in the different cities of Pakistan. The data of hospital based case–control study population was collected from Nuclear Institute of Medicine and Radiotherapy (NIMRA) Jamshoro and Larkana institute of nuclear medicine and radiotherapy (LINAR), situated in different areas of Sindh, Pakistan, during 2011–2013 years, by collecting files and extracting important information about the oral cancer. During 1-year study period (2011), the information department of both hospitals recorded >5,200 cases of cancers of all types, and mouth cancer comprised of 2.7 % of the total. The oral cancer patients were divided into sub groups according to the different over found locations of oral cancer, Lips, tongue, cheeks, and pharynx (throat). Oral cancer patients and controls were further grouped according to their SLT chewing habits, not consumed any SLT product (NU), gutkha (GU), snuff (SU), and mainpuri users (MPU). Complete demographic information is listed in Table 1.

Physical examinations were performed to measure participant's weight, height, blood pressure, and biochemical data. The biochemical tests of oral cancer patients and controls were performed to estimate hemoglobin, red blood cells, packed cell volume, transferrin iron-binding capacity, mean corpuscular hemoglobin concentration, and volume in the blood.

Table 1 Characteristics of study subjects (30–60) age groups

Controls/oral cancer patients		NU [a]	GU [b]	SU [c]	MPU [d]
Male					
Controls		192	158	135	209
Oral cancer patients	Lips	61	54	47	55
	Tongue	43	51	29	34
	Cheeks	32	37	26	32
	Pharynx (throat)	31	23	30	26
	Total	167	165	132	147
Female					
Controls		136	120	107	98
Oral cancer patients	Lips	43	31	28	24
	Tongue	25	31	23	27
	Cheeks	21	15	22	19
	Pharynx (throat)	24	15	24	26
	Total	110	92	97	96

[a] Non-SLT users

[b] Gutkha users

[c] Snuff users

[d] Mainpuri users

Criteria for the selection of patients was of biopsy proved oral squamous cell carcinoma prior to any treatment and they were not taking any mineral supplements during last 3 months. The criteria for selection of 1,155 referent subjects were same age group, socio-economic status, and dietary habits, being free of any cancer diagnosis and not taking any mineral supplement. The biochemical results are given in Table 2. The histological information is not given in this study. Prior to the biological samples collection, the controls have undergone a standard routine medical examination. This study was approved by ethical committee of Sindh University, working under the auspices of higher education commission of Pakistan.

Sampling of SLT Products

A total of 23 brands of snuff (dry and moist), 11 brands of gutkha, and 12 brands of mainpuri were purchased from local markets of the different cities of Pakistan as per their availability over a 3-year period (January 2011–December 2013). The samples were packed in their original packing and placed in prewashed dried plastic bags separately and stored at 4 °C, until tested. Ten composite samples of each brand of snuff, gutkha, and mainpuri were prepared by homogenizing the mixture after removing the wrappers. Care was taken to avoid any source of contamination, and this preparation was carried out in a clean environment. All samples were dried at 80 °C. The dried samples were ground with agate mortar and pestle, sieved through nylon sieves with mesh sizes of 125 μm, and then stored in the labeled sample bottles.

Biological Samples

Venous blood samples (5 mL) were collected by 7-mm heparinized lithium Vacutainer® tubes (Becton Dickinson). About 2 ml of venous blood samples were stored at −20 °C until elemental analysis. The scalp hair samples were taken from five different parts of the scalp (frontal, cranial, occipital, right, and left lateral). The first 5 cm of hair from the root were used for analysis. Hair samples were put into separate plastic envelopes for each participant, tightly sealed and attached with identification number of the participant and questionnaire. In the laboratory, hair specimens were further cut into pieces, approximately 0.2- to 0.3 cm, and washed four times with a 1:200 *v/v* dilution of Triton X-100, then rinsed three times with ultra-pure water and two times with acetone [36], then dried in an oven at 80–85 °C.

pH Determination of SLT Products

Weighed 1 g sample of each brand of gutkha, mainpuri, moist and dry snuff, added 10 mL of ultrapure water in flask (100-mL capacity), and placed in an electrical shaker at 30 rpm for 30 min, then filtered the solution through

Table 2 Clinical and biochemical characteristics of referents and different types of oral cancer patients

Parameters	Normal range	Controls	Cancer patients			
			Lips	Tongue	Cheeks	Pharynx
Male						
Hb (mg/dL)	13–16	14.6+0.56	9.34±0.51	7.62±0.72	9.38+1.02	7.76±0.69
RBC ($\times 10^{12}$/L)	4–6.6	6.3+0.2	2.35±0.24	1.96±0.31	2.32+0.58	2.25±0.34
PCV (%)	40–54	52.5+1.3	24.9±0.83	18.4±0.59	24.6+1.93	16.8±0.69
MCH (pg)	27–32	30.6+0.9	49.2±1.38	54.8±1.33	48.5+2.52	53.8±2.65
MCHC (g/dL)	32–36	35.4+0.3	18.9±0.91	17.9±0.75	15.5+0.82	14.7±0.55
MCV (fl)	76–94	93.4+0.5	172±7.98	194±9.41	167+8.39	215±9.27
Serum Fe (μg/100 mL)	60–160	149+9.5	106±7.55	90.8±6.58	82.5+8.22	62.8±4.59
TIBC (μg/100 mL)	280–400	365+35.1	164±16.8	185±17.9	192+7.37	153±10.8
Female						
Hb (mg/dL)	11–14.5	12.9±1.4	8.96±0.22	7.63±0.54	7.65+0.92	5.82±0.68
RBC ($\times 10^{12}$/L)	3.5–4.5	4.10±0.32	1.95±0.37	1.83±0.26	2.01+0.18	1.60±0.12
PCV (%)	35–47	44.2±2.6	18.5±1.04	16.2±0.71	23.8+1.06	15.9±0.99
MCH (pg)	27–32	30.3±0.9	46.9±1.62	45.8±1.83	48.9+1.97	54.5±1.71
MCHC (g/dL)	32–36	35.2±0.7	16.9±0.72	17.2±0.58	15.9+0.32	15.1±0.62
MCV (fl)	92.6±2.6	94.2±1.4	165±9.62	185±6.98	169+7.33	210±8.65
Serum Fe (μg/100 mL)	60–160	135±13.2	92.6±5.21	89.6±9.74	79.2+8.55	67.8±5.92
TIBC (μg/100 mL)	280–400	362±35.2	170±12.6	187±9.29	184+10.6	155±7.39

Hb hemoglobin, *RBC* red blood cells, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *MCV* mean corpuscular volume, *PCV* packed cell volume, *TIBC* transferrin iron-binding capacity

Whatman No. 42 filter paper and extracts was taken to determine the pH.

Microwave-Assisted Acid Digestion

Replicate six samples of each CRM (0.5 mL Clincheck® control-lyophilized human serum, 0.2 g of Virginia tobacco leaves and BCR 397 human hair) and duplicate samples of different types of SLT products (0.2 g), whole blood (0.5 mL) and scalp hair (0.2 g) were taken separately in polytetrafluoroethylene (PTFE) flasks (25 mL in capacity). Added 3 mL of a freshly prepared mixture of concentrated HNO_3 - H_2O_2 (2:1, *v/v*), kept at room temperature for 10 min. Then placed the flasks in covered PTFE container and heated at 80 % of total power (900 W) for 3–4 min. The digested samples were diluted up to 10 mL with 0.1 mol/L concentrated HNO_3. A blank extraction (without sample) was carried out through the complete procedure.

Statistical Analysis

All statistical analyses were performed using computer program Excel X State (Microsoft Corp., Redmond, WA, USA) and Minitab 13.2 (Minitab Inc., State College, PA, USA). The data from triplicate samples of each composite samples were expressed as means ± std. The Student's *t* test was used to assess the significant difference of As in certified and experimentally found values. The one-way ANOVA was used to assess the significance of differences between the concentrations of As observed in the biological samples of oral cancer patients and control subjects. A $P<0.05$ was considered significant difference.

Analytical Figures of Merit

The concentration range of As for calibration curve reached from the quantification limit up to 50 μg/L. The detection and quantification limits, given by $LOD=3\times s/m$ and $LOQ=10\times s/m$ respectively, where *s* is the standard deviation of ten measurements of a reagent blank and *m* is the slope of the calibration graph. The LOD and LOQ, calculated for As were 0.126 and 0.421 μg/L, respectively. The validity and efficiency of the MAD method was checked with certified values of human hair BCR 397, Clincheck® control-lyophilized human serum, Virginia tobacco leaves (ICHTJ-cta-VTL-2), and with those obtained from conventional wet acid digestion method on same CRM (Table 3). The microwave-assisted digestion method was less time-consuming, requiring <10 min to complete the digestion of samples. The mean values for As differed <1–2 % from the certified values. Non-significant

Table 3 Determination of arsenic in certified sample of human hair, serum and Virginia tobacco leaf by conventional (CDM) and microwave digestion method (MAD)

CDM $\bar{x} \pm s$ [a]	% Recovery[b]	MAD $\bar{x} \pm s$	Paired t test[c] $t_{Experimental}$	% Recovery	Certified values
	Certified sample of human hair (μg/g)				
0.305±0.02 (6.55)[d]	98.4	0.307±0.02 (6.51)	0.578	99	0.31±0.02
	Certified sample of serum (μg/l)				
19.4±1.91 (9.84)	98.9	19.5±1.54 (7.89)	0.960	99.5	19.6±4.0
	Virginia tobacco leaf (μg/g)				
0.944±0.04 (4.23)	99	0.946±0.08 (8.45)	0.651	99.3	0.953±0.08

$t_{Critical}$ at 95 % confidence limit=2.57

[a] Average value±confidence interval (P=0.05)

[b] % Recovery=[Experimental value]/[Certified value]×100

[c] Paired t test between certified values vs found values, degree of freedom (n−1)=5

[d] Values in parenthesis RSD

differences (p>0.05) were observed when comparing the values obtained by both methods (paired t test).

Result

Arsenic Concentration in Different Smokeless Tobacco Products

Multiple samples of different brands of each SLT products were analyzed and the mean concentrations along with the standard deviation for ten composite samples of each brand are provided in Table 4. While the range of As levels in brown and green moist snuff was found to be 0.574–1.46 and 0.995–1.53 μg/g, respectively. In dry brown and black snuff products, the As levels were observed in the range of 0.733–1.04 and 0.642–1.07 μg/g, respectively. The contents of As in different brands of mainpuri (n=12) was found in the range of 0.419–0.874 μg/g, respectively. The contents of As in different brands of gutkha (n=11) was found in the range of 0.246–0.622 μg/g.

Demographic Characteristic of Study Population

The controls and patients have informed that they consumed mainpuri, snuff, and gutkha for ≥8.0±2.5 years. Clinical characteristics including basic medical data were obtained from medical records with the help of paramedical staff. The occupational history included (jobs held for more than 1 year over the lifetime), the study subjects (patients and controls) were mostly drivers, working in workshops (automobile, battery recycling) and labors in construction buildings. The females are mostly house wife or working as maid and in garment factories. The exclusion criteria for patients and controls were smoking or drinking alcohol.

Arsenic Concentration in Biological Samples of Controls and Oral Cancer Patients

The mean values of As in biological samples of oral cancer patients and control subjects are presented in Table 5. The resulted data indicated that the contents of As was significantly higher in scalp hair and blood samples among cancer patients (lips, tongue, cheeks, and pharynx) than those of controls (P<0.001). The ranges of As in the scalp hair samples of male control subjects (NU, GU, SU, and MPU) were found at 95 % confidence intervals (CI) 0.92–0.98, 1.31–1.43, 1.20–1.38, and 1.47–1.56 μg/g, respectively, were significantly lower as compared to resulted data obtained from oral cancer patients (p<0.001). The same trend was observed in females. The As concentrations in blood samples of male and female control subjects (NU, GU, SU, and MPU) at 95 % CI 1.62–2.07, 2.54–2.70, 2.33–2.67, 2.70–3.02 μg/L and 1.58–1.97, 2.40–2.59, 2.23–2.40, 2.49–2.82 μg/L, respectively, were found to be significantly lower than patients who consumed different SLT products (p<0.001) (Table 5).

The unpaired student t test between cancerous patients and controls at different degrees of freedom, were calculated for different probabilities. Our calculated t_{value} exceeds that of $t_{critical\ value}$ at 95 % confidence intervals, which indicated that the difference between mean values of As in controls and cancerous patients (both gender) showed significant differences (p<0.001).

Discussion

The pH of all SLT products was highly basic, found in the range of 8.1–8.7, which favors the formation of tobacco-specific amines thus making the product potentially toxic. The production of nitrosamines is major contributors to the

Table 4 Arsenic concentrations in gutkha, mainpuri, dry and moist snuff samples (μg/g)

Gutkha			Snuff			Mainpuri		
(G)	$\bar{x} \pm s$ [a]	μg/10 g[b]	Moist snuff	$\bar{x} \pm s$	μg/10 g	MP	$\bar{x} \pm s$	μg/10 g
G1	0.398±0.03	3.68–4.34[c]	BM1[d]	0.611±0.03	5.74–6.35	MP1	0.753±0.05	6.83–7.95
G2	0.520±0.04	4.89–5.78	BM2	1.05±0.05	9.95–11.0	MP2	0.466±0.04	4.19–5.14
G3	0.564±0.05	5.23–6.12	BM3	1.33±0.09	12.4–14.6	MP3	0.480±0.05	4.19–5.33
G4	0.592±0.03	5.56–6.22	BM4	0.712±0.07	6.41–7.83	MP4	0.760±0.04	7.22–7.98
G5	0.337±0.03	3.12–3.68	BM5	1.23±0.06	11.7–13.1	MP5	0.471±0.03	4.38–5.14
G6	0.440±0.02	4.23–4.67	BM6	1.02±0.06	9.60–11.0	MP6	0.661±0.06	5.89–7.22
G7	0.271±0.03	2.46–3.01	BM7	1.30±0.07	12.1–13.9	MP7	0.784±0.07	7.03–8.55
G8	0.473±0.04	4.34–5.12	BM8	1.00±0.03	9.60–10.3	MP8	0.831±0.04	7.79–8.74
G9	0.404±0.01	3.90–4.23	GM9[e]	1.16±0.05	11.0–12.1	MP9	0.675±0.07	5.89–7.41
G10	0.348±0.03	3.12–3.68	GM10	1.46±0.06	13.9–15.3	MP10	0.594±0.03	5.52–6.27
G11	0.487±0.04	4.45–5.34	GM11	1.18±0.03	11.4–12.1	MP11	0.504±0.02	4.76–5.33
			GM12	1.08±0.09	9.95–12.1	MP12	0.637±0.05	5.52–6.84
			GM13	1.19±0.05	11.4–12.4			
			GM14	1.23±0.07	11.4–13.1			
			DB1[f]	0.892±0.06	8.22–9.62			
			DB2	0.817±0.05	7.64–8.86			
			DB3	0.992±0.04	9.46–10.4			
			DB4	0.809±0.06	7.33–8.86			
			DBK5[g]	0.711±0.07	6.42–7.94			
			DBK6	1.02±0.05	9.77–10.7			
			DBK7	0.840±0.04	7.94–8.86			
			DBK8	0.924±0.08	8.55–10.4			
			DBK9	0.855±0.06	7.94–9.16			

[a] Average value±confidence interval (P=0.05)

[b] Intake of As from all SLT products were based on μg/10 g

[c] The intake of As via different types of SLT product are presented in range (minimum–maximum)

[d] Brown moist

[e] Green moist

[f] Dry brown

[g] Dry black

increased risk of chewing SLT products for cancer of upper digestive tract [8].

This case–control study was conducted to evaluate the possible association between As exposure via consumption of different types of SLT products and its altered levels in blood and scalp hair samples of oral cancer patients with related to controls of both gender. The As concentrations in mainpuri, snuff, and gutkha samples consumed by cancerous patients and controls were determined. The high level of As was observed in both types of snuff, while it was observed that the levels of As varies in biological samples of controls and patients, according to the types of SLT products consumed, but difference was not significant (p>0.05).

In all SLT products, significant variation in elemental contents would be expected [37]. Though SLT is described as a group 1 carcinogen by the International Agency for Research on Cancer, little is known regarding bioavailability, absorption, and toxicological effects of toxic and carcinogenic inorganic substances from them. The resulted values of As in biological samples of oral cancer patients of both gender, confirms that chewing SLT products could be major risk factors for the oral disease. The resulted data indicated that in controls of both gender, who not consumed any SLT have two- to threefold lower levels of As in their biological samples as compared to those results obtained from controls consumed SLT products. The significant high levels of As was observed

Table 5 The As concentrations in scalp hair and blood samples of controls and different types of oral cancer patients

Biological samples	Types of SLT products consumed	Controls	Different types of oral cancer patients			
			Lips	Tongue	Cheeks	Pharynx (throat)
Male						
Scalp hair(μg/g)	NU[a]	0.95±0.05	1.72±0.15	1.98±0.21	2.19±0.17	2.34±0.35
	GU[b]	1.37±0.13	2.55±0.31	2.79±0.26	2.92±0.29	3.45±0.38
	SU[c]	1.29±0.18	2.41±0.36	2.68±0.43	2.85±0.32	3.32±0.45
	MPU[d]	1.52±0.09	2.69±0.33	2.95±0.27	3.24±0.55	3.69±0.52
Blood(μg/L)	NU	1.85±0.24	3.25±0.18	3.51±0.47	3.75±0.35	3.94±0.29
	GU	2.62±0.15	4.16±0.24	4.35±0.35	4.56±0.48	4.73±0.64
	SU	2.50±0.37	3.89±0.35	3.96±0.54	4.18±0.31	4.18±0.48
	MPU	2.84±0.30	4.35±0.51	4.67±0.70	4.85±0.58	4.98±0.56
Female						
Scalp hair(μg/g)	NU	0.90±0.08	1.65±0.19	1.95±0.24	2.10±0.15	2.27±0.25
	GU	1.35±0.12	2.48±0.27	2.75±0.23	2.84±0.28	3.42±0.32
	SU	1.24±0.20	2.34±0.15	2.64±0.19	2.73±0.37	3.25±0.21
	MPU	1.46±0.15	2.63±0.22	2.92±0.40	3.18±0.51	3.62±0.30
Blood(μg/L)	NU	1.76±0.32	3.18±0.25	3.43±0.29	3.68±0.17	3.82±0.35
	GU	2.49±0.19	4.05±0.14	4.29±0.25	4.38±0.32	4.60±0.51
	SU	2.36±0.28	3.74±0.43	3.79±0.45	4.06±0.27	4.13±0.51
	MPU	2.65±0.35	4.21±0.25	4.51±0.39	4.69±0.48	4.85±0.37

[a] Non-SLT users

[b] Gutkha users (GU)

[c] Snuff users (SU)

[d] Mainpuri users (MPU)

in biological samples of tongue, cheeks, and pharynx cancer patients as compared to controls consumed SLT products ($p<0.001$), as shown in Table 5.

It was reported in previous studies that certain toxic elements were found in SLT products [38] and thus their intake via inhaling (snuff) or ingestion (gutkha and mainpuri) can cause serious diseases, including oral cancer [39]. It was reported that exposure to As via different routes and smoking synergistically increases the risk of lung cancer, bladder cancer, and induction of skin lesions [21, 40]. An experimental study showed that As and cigarette smoke act synergistically to cause DNA damage [41]. At present, millions of people worldwide suffer from chronic As poisoning [42, 43] mainly due to consumption of As-contaminated water and food. Lindberg et al. [26] reported that the As content in tobacco and other gradients (betel nut and betel quid) can further increase the risk of As induced skin lesions among people of As endemic areas.

Several studies have been reported that As is present in measurable quantity in tobacco products, although concentrations are relatively smaller than those of other metals, such as cadmium and lead [44, 45]. The As has been detected in cured or processed tobacco leaves at concentrations of approximately 400 ng/g, at dried basis [46], while in certain SLT products, its concentrations ranging between 130 and 360 ng/g of dry tobacco [44, 45, 47]. The As has been shown to induce carcinogenesis via a wide range of cellular changes including alterations in cell differentiation and proliferation [48, 49]. It was reported by Hayes that As has been found to induce chromosomal aberrations and sister chromatids exchange [48]. Studies have been reported that cells exposed to As have also been shown to increase cellular tyrosine phosphorylation, which is related to the aberrant cell signaling and uncontrolled cell growth associated with cancer development [50–52].

Epidemiologic studies have documented that long-term exposure to As is associated with an increased risk of cancer of the lung, skin, and probably other anatomic sites. The As is also one of major risk factors for black foot disease, a unique peripheral vascular disease identified in endemic areas of arsenicosis in Taiwan, where residents had used high As tainted artesian well water for more than 50 years. Exposure to As causes different types of cancers (head & neck, bladder, lung, skin, kidney, prostate, and liver) as well as cardiovascular disease, diabetes, developmental and reproductive effects [53–56]. It was suggested by Marano et al. and Cox that due to potential mechanisms of As carcinogenicity, its removal from cigarette tobacco might reduce human health risks [57, 58]. Epidemiology studies were reviewed, As biomarker

concentrations in a population representative of the US were evaluated, and a probabilistic risk assessment was undertaken [59, 60].

The International Agency for Research on Cancer (IARC) now regards the betel nut which is part of mainpuri and gutkha, itself known as a carcinogen [61]. It is demonstrating that reactive oxygen species, such as hydroxyl radical, are formed in the human oral cavity during SLT products chewing, and that the activity might cause oxidative DNA damage, which transformed into oral cancer [62].

Conclusion

The results of this study revealed that the significant differences were observed in As concentration in biological samples of oral cancer patients as compared to noncancerous controls consumed or/not any type of SLT products. The imbalance in As level in oral cancer patients could be due to change of cellular metabolism in the cancer process. It was also observed that the socioeconomic factors may also play a role in higher mortality rates for oral cancer patients, such as poor nutrition, irregular screening, late diagnosis, and unequal access to health care due to poverty because the cost of cancer treatment is very high. Since the role of As in the mechanism of oral cancer development is still unclear, further detailed and comprehensive investigations are necessary.

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