PERGAMON

Oral Oncology 34 (1998) 558–566

ORAL
ONCOLOGY

The Swedish *Snus* and the Sudanese *Toombak*: are they different?

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Received 11 March 1998

Abstract

In Sweden, snuff (locally known as *snus*), was introduced since the year 1637. Presently, Sweden has the highest per capita consumption and sale figures of snuff in the world, and the habit is becoming increasingly popular. *Snus* is manufactured into a dry form used in the nasal cavity and a moist form used in the oral cavity. *Snus* manufactured for oral use is a moist ground tobacco of Dark Kentucky or Virginia species mixed with an aqueous solution of water and other blending ingredients. This form of snuff is found in two types: (1) loose and (2) portion-bag-packed. These are the most widely used. The loose moist form (1–2 g a quid) is the most popular type consumed by 73% of the males, followed by the portion-bag-packed form (0.5–1 g a quid), consumed by 13% of the males, while 14% of the males are mixed users. The majority of *snus* users place the quid in the vestibular area of the upper lip, and the prevalence among persons 15 years of age or older is 15.9% among males and 0.2% among females. The pH of *snus* has declined from a previous range of 8–9 to a range of 7.8–8.5, moisture content ranges 35–60% and nicotine content is in the order of 5–11 mg/g dry wt tobacco-specific *N*-nitrosamines (TSNAs) in micrograms (*N*′-nitrosonornicotine: NNN 5–9; 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone: NNK 1–2; *N*′-nitrosoanatabine: NAT 2–5). In the Sudan, snuff, locally known as *toombak*, was introduced approximately 400 years ago. It is always processed into a loose moist form, and its use is widespread in the country. Tobacco used for manufacture of *toombak* is of the species *Nicotiana rustica*, and the fermented ground powder is mixed with an aqueous solution of sodium bicarbonate. The resultant product is moist, with a strong aroma, highly addictive and its use is widespread particularly among males. Its pH range is 8–11, moisture content ranges 6–60% and nicotine content is from 8 to 102 mg/g dry wt, and TSNAs contents in micrograms (NNN 420–1 550; NNK 620–7 870; NAT 20–290). *Snus* and *toombak* dippers develop a clinically and histologically characteristic lesion at the site of dipping. Probably due to control of the TSNAs in *snus*, this type of snuff is associated with a lower risk of cancer of the oral cavity (relative risk: RR 5–6-fold), whereas the risk for cancer of the oral cavity among *toombak* users was high (RR 7.3–73.0-fold). In conclusion, the two snuff products significantly differ in many aspects. Most notable differences are tobacco species, fermentation and ageing, nicotine and TSNAs content, pH, expression of the *p53* tumour supressor gene, and keratin types 13, 14, and 19. It was, therefore, the object of the present study to highlight the oral health hazards of *toombak*, and to compare it with *snus* regarding the aforementioned differences.

Keywords: Snus; Toombak; Sudan; Sweden

1. Introduction

Expert committees of the International Agency for Research on Cancer reviewed epidemiological studies from different countries who indulge in smokeless tobacco use and concluded that "the use of snuff can cause cancer in humans, and that the use of chewing tobacco may increase the risk for oral cancer development" [1]. Snuff is a term used to describe a wide variety of products containing finely ground tobacco as a principal constituent and other additives. Snuff is either inhaled to the nasal cavity or dipped in the oral cavity.

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1368-8375/98/$—see front matter
PII: S1368-8375(98)00047-5

Epidemiological and laboratory studies have documented that use of snuff is associated with increased risk for cancer of the oral cavity [1,2]. Snuff contains a number of carcinogens, principally the most abundant ones, the tobacco-specific *N*-nitrosamines (TSNAs), which have been shown to be potent carcinogens in experimental animals [1–3]. In addition, snuff contains other carcinogens including aliphatic and aromatic hydrocarbons, formaldehyde, ketones, alcohols, phenols, amines, amides, alkaloids, metals, radioelements (e.g. polonium-210, uranium-235 and -238) and polyaromatic hydrocarbons (PAHs) [1–3].

The use of snuff is common world-wide, but more common in the southern parts of United States, the Scandinavian countries, southern parts of the Kingdom of Saudi Arabia, southern African countries and the Sudan in northeast Africa. Scandinavian snuff (locally called *snus*), has been used for centuries. In Denmark, the habit of *snus* dipping is widely prevalent and the quid is usually placed between the lower lip and the alveolar process [4]. In Norway, the habit of *snus* dipping is uncommon, but presently is increasing particularly among young individuals, and the quid is placed under the upper lip [5,6]. In Sweden, the habit of *snus* dipping is the oldest in Europe and dates back to the year 1637. In Sweden, *snus* consumption declined for several decades during the period 1920–69 [7], increased by 92% during the period 1970–92, and currently is the only tobacco product with increasing sale figures [7]. *Snus* product for oral use is moist with a pH value in the range 7.8–8.5 [7], and many different brands are commercially available [7,8]. However, the majority of *snus* users prefer only one or two brands [7,8]. The most popular way of practising the habit is by placing the quid in the upper gingivolabial sulcus [7,8]. Sweden has the highest per capita consumption and sale figures of snuff in the world [7,8]. In Africa, consumption of snuff (locally called *toombak*) is particularly high in the Sudan where it has been used for over three centuries [9]. While cancer of the oral cavity is low in Sweden despite widespread use of *snus* [10], an increased risk for cancer of the oral cavity has been documented among *toombak* dippers in the Sudan [9].

1.1. Snus

Snus is manufactured from the Dark Kentucky or Virginia tobacco with relatively a high nicotine content compared to cigarettes [8]. The tobacco is selected according to the content of nicotine which may vary considerably. Both soil and climate where the tobacco is grown determine the quality of the product. The tobacco is dried in air or in barns by heating the air with open fires (dark-fired) [8]. The smoke affects both the aroma of the tobacco and its chemical composition [8]. After cutting and grinding of the leaves, water and salt are added to the dry flour to keep it fresh and thereafter the *snus* is heated at high temperature and humidity. To produce various brands, other ingredients are usually added. The purpose of heating is to eradicate micro-organisms and lower nitrate and subsequent nitrosamine formation [7]. Processing of the Swedish *snus* is a data controlled free-hands tobacco industrial process that involves several modifications through the different stages of processing. However, the exact composition of the blending additives remains a commercial secret [8]. Finally, and after repeated shifts, the *snus* is refrigerated until ready for packing, in waxed cardboard containers with plastic lids, and the product is then stored at $+4^{\circ}C$ [8].

Snus is primarily moist snuff with pH values previously 8−9 but presently 7.8−8.5, and is produced in various commercial brands [7,8]. Sweden has the highest prevalence of use of snuff in the European Union, with 21% of all males (900 000 persons) using moist *snus* [11]. Of the two forms, loose and portion-bag-packed *snus*, 73% of the males consume the loose form and 13% consume the portion-bag-packed form and the remaining 14% are mixed users [7,8]. The habit is practised by taking a pinch of 1–2 g of loose *snus* or sachets of portion-bag-packed of 1-g sachets, and place it in contact with the oral mucosa behind the upper lip [7,8]. The average *snus* dipper keeps the *snus* in the oral cavity for approximately 11–14 h/day, and consumes almost 75 g a week, corresponding to approximately 4 kg of *snus* a year [7,8]. Epidemiological studies have shown that changes of the oral mucosa and gingival margins are less pronounced among users of portion-bag-packed *snus* compared to users of loose *snus* who show more pronounced gingival recessions which seem to be irreversible [7].

1.2. N-*nitrosamines*

Snus leading brands were found to contain levels of TSNAs ranging from 5.5 to 106 ppm [8]. Waxed cardboard *snus* containers were found to contain morpholine. Diffusion of morpholine into *snus* and subsequent *N*-nitrosation may result in formation of *N*-nitrosomorpholines (NMOR), a potent experimental carcinogen [8]. On the other hand, *snus* portion bags wrapped in aluminium foil have been reported to contain significantly fewer quantities of volatile *N*-nitrosamines (VNA) and *N*-nitrosodiethanolamine (NDELA) and TSNAs [8], which demonstrates one possibility to reduce deleterious substances in *snus*. The levels of nicotine and TSNAs in *snus* have been reported during the period 1980–90 (Table 1), and were found in the range of 11.3–18.1 mg/g for nicotine, and 5.5–106 ppm for TSNAs.

Table 1
Levels[a] of nicotine and the tobacco-specific *N*-nitrosamines in *snus* and *toombak*

Sample[b]	Year	Nicotine (mg/g)	NNN (μg/g)	NNK (μg/g)	NAT (μg/g)	NAB (μg/g)	Reference
Snus							
A(5)	1980–90	12.4–15.1	5.67–7.83	1.0–2.08	2.2–5.13[c]		[19]
B(5)	1980–90	12.5–18.1	4.0–7.95	0.61–1.51	1.4–4.43[c]		
C(5)	1980–90	11.3	5.24–8.94	1.40–1.85	2.4–5.50[c]		
Toombak							
A(5)	1990	32.2–102.4	830–3085	630–7870	66–290	40–2370	[15]
B(5)	1990	8.4–26.0	420–1550	1140–2790	30–140	60–210	
C(4)	1990	16.5–22.8	490–960	1170–2270	20–80	20–220	
D(4)	1990	17.9–40.6	850–1800	620–3830	50–130	90–230	
E(2)	1990	26.4–26.6	780–970	1610–1680	20–40	80	
I(5)[d]	1993		241–369	188–362	21–42	14–43	[51]

[a] All values are based on dry weight.
[b] Numbers in parentheses are numbers of samples analysed.
[c] NAT contains 5–10% NAB.
[d] Samples also contain 1.4–20.7 μg iso-NNAL, and up to 22.9 μg NNAL/g tobacco.

1.3. Toombak

In the Sudan, nasal use of snuff is found only in the far southern parts of the country and only oral use which is widespread is described. *Toombak* is a native tobacco plant of the species *Nicotiana rustica* used for manufacture of snuff. *Toombak* is grown in silky or sandy soils which receive heavier rainfalls in the north-west of the Sudan. After the end of the rainy season (September/October), *toombak* is planted during the months November/December and never irrigated. Harvesting starts in the months February/March when the leaves turn yellow and brownish spots start appearing (called the smallpox stage). Harvested leaves are left in the field for uniform drying, tied into bundles, moistened with sprinkling of water and stored for fermentation for a couple of weeks at temperature ranging from 30 to 45°C, during which bundles are separated for uniform drying during the months April/May. Tobacco leaves are ground and stored for a year for ageing [12].

Introduction of this tobacco plant to the Sudan was attributed to a Koranic (Islamic) teacher, who came to the Sudan either from Egypt, Timbuktu of Mali or Morocco. It has also been suggested that *toombak* was introduced to the Sudan from Turkey or Arabia. Another popular name for *toombak* is *Sauté*, which means sniffing of the product in the local language, indicating nasal usage when it was first introduced. The commercial names for *toombak* include, *El-sanf* (of high quality), *Wad Amari* (accrediting the person who was believed to have introduced it) and *Sultan El-kaif* (the power to improve one's state of mind) [12].

Processing of *toombak* for sale is usually carried out manually in *toombak* shops by *toombak* vendors [12]. It is performed by preparing four parts of a coarse powder of dried *toombak* leaves in a bowl and in another the concentrate of sodium bicarbonate is added gradually in small amounts to the tobacco [12]. While adding the solution, the product is mixed vigorously by both hands and concurrently tested by sensation of the tips of the fingers until it becomes moist and hardened. The output is then transferred to special air-tight tin-containers which are then covered firmly for about 2 h, thereafter the product becomes ready for sale or use. Before buying, users generally ask for a bit to smell or taste, since the aroma and taste decide the quality rank of the product. Currently, *toombak* is sold in small plastic bags each taking about 100 g. The *toombak* user carries round or box-shaped tin cans in his pocket, named hookah and is similar to plastic bags, though some people use king-size ones. Hookah is still used by some people, and it makes an indentation in the pocket of a user, thus one can easily guess and identify a user. This helps users to communicate with each other for exchange of brand or request of a dip. A regular user consumes an average of 10–20 dips per day, thus requiring a steady supply of *toombak*.

1.4. Natron (sodium bicarbonate)

Natron or atron (sodium bicarbonate, also called sodium hydrogen carbonate), is a mineral rock with the chemical formula $Na_2H(CO_3)_2.2H_2O$. Its colour is grey to yellowish white, and is of alkaline pH. There is no information on either the history or reasons behind use of atron as an additive to *toombak*. It may be used to homogenise the leaves to a fine sticky form as atron is used in the Sudan to homogenise vegetables during cooking.

Atron, opposed to lime in other parts of the world, is probably added to *toombak* for its alkaline effects. It has been shown that at high pH (11.0–11.8) nicotine is

completely protonated and its rate of absorption is increased [13]. Studies of *nass*, a type of snuff used in the former USSR which contains lime and has high pH (11–11.8), have shown that when the product is placed in the mouth, nicotine reaches the central nervous system very quickly [14]. Thus, pH value in tobacco products can influence the absorption and thereby the extent of pharmacological activity of nicotine [14]. Atron probably quickens absorption of nicotine from *toombak* to the central nervous system.

1.5. The habit

Toombak can be bought from innumerable shops in the market, and the product is advertised extensively at points of sale where vendors tend to use commercial names to attract buyers [12]. The habit of *toombak* dipping is practised by taking a small portion from the bag or hookah with the three fore-fingers, usually of the right hand, putting it in the palm of the left hand, and manipulating it by the thumb and middle fingers of the right hand until it forms a ball called saffa which is of about 10 g in weight [12]. The saffa is not chewed but dipped and retained between gum and lip or cheeks or floor of mouth, and sucked slowly for about 10–15 min. Generally, men prefer dipping between the lower lip and gum, while women prefer dipping between cheeks and gum. The dipping continues for a period ranging from a few minutes to several hours, until the saffa becomes bland. Men periodically spit the insoluble debris that is freed from the bullous and the saliva which is secreted during *toombak* use, whereas women retain the saffa, without spiting because of social unacceptability. The mouth is usually rinsed with water after the quid is removed. The *toombak* quid is sometimes retained in the mouth during sleep [12].

1.6. N-*nitrosamines*

Using gas chromatography with thermal energy analysis, the TSNA types; *N*′-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), *N*′-nitrosoanatabine (NAT) and *N*′-nitrosoanabasine (NAB) were quantified in *toombak* and saliva of *toombak* users [15,16]. Exceptionally high levels (mean; range, mg/g *toombak*/dry wt) of NNN (1.13; 0.50–3.08), NNK (2.31; 0.62–7.87; 31), NAT (0.08; 0.02–0.2) and NAB (0.22; 0.02–2.37) were found (Table 1). Previously, the highest levels of NNN and NNK in any oral tobacco found were 0.154 and 0.014 mg/g dry wt, respectively [17]. Two additional TSNAs, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol (iso-NNAL) were found in the saliva of *toombak* users for the first time and were confirmed by gas chromatography mass spectrum [16]. Exposure to TSNAs has also been assessed by measuring the content in the saliva of 21 *toombak* users, where nine out of 10 subjects had detectable levels of TSNAs in their saliva before dipping (total TSNAs: 0.01–1.0 mg/ml). During *toombak* use, TSNAs concentrations have reached g/ml levels (range: number of positive subjects) for NNN (0.6–1.2, 12/12), NAT (0.6–6.7, 2/12), NAB (0.05–1.9, 12/12), NNK (0.06–6.7, 8/12), NNAL (0.05–3.3, 11/12) and iso-NNAL (0.07–0.4, 8/12). These high levels of TSNAs found in *toombak* were partially attributed to the use of tobacco Species, *Nicotiana rustica*, fermentation of *toombak* at elevated temperature, prolonged storage, and contamination during processing [18–20].

Levels of nicotine and TSNAs in *snus* and *toombak* were previously compared [21]. Tables 1 and 2 show quantitative data on nicotine and TSNAs in various *snus* brands and saliva of *snus* dippers and compare that with concentrations of nicotine and TSNAs in various samples of *toombak* and saliva of *toombak* dippers. These data clearly document that *toombak* contains concentrations of TSNAs 100-fold higher than those found in *snus* (Table 1). The level of NNN and NNK in the saliva of *toombak* dippers is also significantly higher than those found in the saliva of *snus* dippers (Table 2). It has been estimated that more than 80% of the carcinogenic TSNAs are extracted from the *toombak* by saliva and negative pressure during sucking of dipped *toombak* quid [20]. The concentrations of TSNAs in the saliva of *toombak* dippers exceeded their concentration in a solution that produced mouth tumours in rats swabbed with this solution twice daily [22]. Whereas epidemiological studies suggest low incidence of oral cancer in Sweden [10], both high relative frequencies of oral cancer as well as increased risk of oral cancer development among *toombak* dippers in the Sudan have been reported [9,21]. These data strongly support the widely held idea that the TSNAs NNN and NNK play an important role in carcinogenicity of *toombak*.

1.7. Prevalence

Large-scale surveys from Sweden in comparison with similar surveys from the Sudan have shown that *snus* is increasingly used by young individuals whereas *toombak* is increasingly used as persons get older. Use of *snus* by young individuals in Sweden suggests an alternative to smoking of cigarettes. Table 3 shows a large-scale survey in Sweden and its comparison with similar surveys from the Sudan. An informative report on prevalence of *toombak* use and cigarettes smoking in a random population sample of 4535 (21 433 household members; 22.6% males, and 1.7% females), in the population of age 4 years or older, in the Nile Province north of Khartoum, Sudan, has been described [9]. Among individuals of age 18 years or older, the average prevalence rate for use of *toombak* was found to be 34% in males

Table 2
Levels of the tobacco-specific *N*-nitrosamines in the saliva of *snus* and *toombak* dippers

Product	Year	No. of samples	TSNA (ng/ml saliva)				
			NNN	NNK	NAT[a]	NAB	Reference
Snus	1988	4	3–140	nd–16	4–85		[52]
Toombak	1991	12[b]	582–21 000	63–6690	nd–471	46–1944	[16]
	1993	6	14.8–105.7	20–135	2.3–20.4	2.6–14.2	[53]

nd, not detected.
[a] NAT contains 5–10% NAB.
[b] Saliva sample [12] contains 52–3272 ng NNAL/ml saliva.

Table 3
Prevalence of use of *snus* in Sweden and *toombak* in the Sudan

Survey	Year	Age (years)	No.	%	Reference
Snus					
University of Malmö	1970–73	15	20 333	15.9	[39]
Toombak					
University of Kartoum	1992	⩾20	2868	41.6	[21]
Toombak					
Toombak Research Centre and Oral Cancer Campaign, Khartoum	1998	4–17	3795	1.7	(Dr A Idris, University of Khartoum, Sudan, personal communication)
		18+	7237	34.1	
		⩾4	11 068	23.0	

compared to 2.5% in females [9]. Use of *toombak* has ranged between 40 and 47% among males of 40 years of age or older, while among adults in rural areas, 40% of males were found to use *toombak* compared to 24% found in urban areas [9]. Rural populations were found to use home processed *toombak* whereas urban ones use more vendor-processed *toombak*. However, both groups follow a similar processing procedure [9].

Almost all *snus*-dippers in Sweden in the 15–65-year age group are males, implying about 17% of the population [8]. When stratified, prevalence of the habit accounted for 19% for the age group 15–30 years, and 10% for the age group 65 years and above. Only 1% of Swedish women used *snus* [8,23]. Among school children aged 13–16 years, 11–15% of the boys, but none of the girls used *snus* regularly [24,25]. There are regional differences in the habitual of use of *snus* in Sweden. Accordingly, the habit is widely spread in the northern part of the country where almost 25% of the male population over 15 years use *snus* daily [23]. The habit is also more common in rural areas (20%) and in small towns (13%) than in larger towns (7%) [26]. It has been reported that people with a high consumption of *snus* smoked less (12 cigarettes/daily) than people with limited *snus* habits. Of the male *snus* dippers, 43% did not smoke at all, while 22% smoked occasionally and 35% were regular smokers as well as *snus* dippers [8]. Recently, it has been reported that about 21% of all males in Sweden use moist *snus* regularly, implying 900 000 persons, of which > 500 000 use only smokeless tobacco and about 335 000 use both cigarettes and smokeless tobacco, and only 8% of the users are women [11].

1.8. Oral cancer

The first study from Scandinavia on *snus*-associated soft tissue changes was published in 1937 [27]. In that report, among 496 men with cancer of the oral cavity, pharynx, larynx and or oesophagus registered between 1930–36, 91% were daily *snus* users, and 70% of 68 cases of outer oral cavity cancers used *snus*. In another case control study, it has been concluded that chewing of tobacco is common among men, with the cases being too small to suggest that it could be a risk factor. It has been stated that, *snus* dipping in Sweden apparently increases the risk of oral cancer at the site where snuff is placed five- to six-fold [28]. In addition, the role of the Swedish *snus* alone or in combination with live HSV-1 as an aetiological factor in development of oral cancer has been studied [29]. More recently, quite a number of studies have been carried out in Sweden to elucidate a possible risk of *snus* use and oral cancer [7,8,29–32].

However, this has been supported by some studies [8,29] and questioned by others [7,30–32]. According to disagreement between the results of these studies and others, the Swedish Board of Health and Welfare symposium, held in September 1996, questioned the law which orders the manufactures to label the *snus* product with the text "Causes cancer", adopted after the IARC statement [1]. Nevertheless, the IARC statement was based on intense epidemiological as well as laboratory findings identified from different populations. Thus, it is clear that there is a need for further research into the field before any further questioning of the IARC statement.

Although a high relative frequency of oral cancer in the Sudan has been observed since the years 1959 and 1963 [33,34], the earliest observation on the association between oral cancer and use of *toombak* was reported in 1980 [35]. Much later, it was found that 81% (50/62) of patients with oral squamous cell carcinoma (SCC) from the Sudan used *toombak* [36]. Recently, abundant information on *toombak* use and development of oral cancer has been reported, and the cancer was frequently found at the site where the *toombak* quid is placed [9,12,15,16,21]. Studies of a similar nature from Saudi Arabia have suggested the aetiological role of Shamma for development of oral cancer [37,38]. So far, only one case control study from the Sudan has quantified the risk associated with use of *toombak* [12]. In that study, the history of the use of *toombak* was compared in four case groups including: (1) 375 with SCC of the lip, buccal cavity and floor of mouth; (2) 271 with SCC of the tongue, palate and maxillary sinus; (3) 204 with benign neoplasms in the oral cavity and/or other sites of the body; and (4) 2820 with no malignancy selected from the general population [12]. The adjusted odds ratio (OR) associated with *toombak* dipping for the first case group (cancer of the lip, buccal and floor of mouth) in comparison with the other hospital and general population control groups was 7.3 and 3.9 (95% confidence limits, 4.3–12.4 and 2.9–5.3), respectively, and among long term users of *toombak*, the OR was 73.0 for the hospital controls (95% confidence limits, 9.8–542.2) [12].

The clinical and histological features of *snus*-induced lesions in Scandinavia have been the subject of several studies [4,7,8,39]. The clinical characteristics of *snus*-induced lesion at the site of quid placement have been described as white or yellowish to brownish colour changes, a wrinkled or folded surface and a clear demarcation to diffuse merging into surrounding normal mucosa [4,7,8,39]. A four-point scale of clinical grading of these lesions has been introduced [39]. The severity of the grade is correlated with increased consumption (h/day/year). Two major histological patterns of changes in the surface epithelium were recognised [7,39]. The first pattern is increased epithelial thickness with vacuolated cells. The second was frequent chevron type changes and variable thickness with evidence of keratinization. High daily use was associated with relatively more pronounced epithelial changes [7]. It has been concluded that tissue changes clinically as well as histologically including presence of epithelial dysplasia are reversible following cessation of the *snus* habit [7].

The clinical and histological features of *toombak* dippers' lesions have been described [40]. The characteristics of the clinical and histological features of *toombak* dippers' lesions were found to be similar to those of *snus* dippers' lesions. This similarity is probably due to high alkalinity of both products, although the previous high alkalinity pH (8–9) of *snus* has recently reduced to 7.8–8.5 [7]. A positive correlation between severity of the lesion and longer life-time duration (>10 years) but not daily frequency, of the habit of *toombak* use was found [40]. However, increased severity of the lesion in *snus* users, was found associated with both daily and longer life-time duration of use [7]. Similar to *snus* dipper's lesion, increased epithelial thickness, Parakeratosis, pale surface staining and chevron type keratinization were observed in *toombak* dipper's lesions, but epithelial dysplasia was found to be low [40].

1.9. p53*, keratins (Ks) and human papillomavirus (HPV) infection*

The *p53* tumour suppressor gene encodes a nuclear phospho-protein that plays an important role in cell proliferation and diffrentiation [41,42]. The most common genetic alterations identified in human cancers are mutations in the *p53* tumour suppressor gene [42]. A high frequency of *p53* mutations has been observed in tobacco-related cancers [43], including head and neck [44]. Expression of p53 in premalignant oral lesions and oral squamous cell carcinomas from Swedish and Sudanese snuff dippers, as well as in premalignant and malignant oral lesions from non-snuff dippers from the Sudan, Sweden and Norway, have been investigated by immunohistochemistry [45]. Only 2/15 fibro-epithelial hyperplasia from *snus* dippers and 3/14 carcinomas from *toombak* dippers expressed p53, whereas expression of p53 in carcinomas from non-dippers from the Sudan, Sweden and Norway was found in 9/14, 39/60 and 28/41, respectively [45]. Although results of immunohsitochemistry are not always conclusive, these findings suggested that the low expression of p53 found in *toombak* and *snus*-associated oral lesions may be due to formation of the p53 protein to cytoplasmic complexes with other proteins, mainly Ks and HPV proteins. In another study, *toombak* dippers' lesions (49 including 40 dysplastic), and *snus* dippers' lesions (15 fibro-epithelial hyperplasias) were examined by immunohistochemistry for expression of K types 13, 14, and 19 and for presence of HPV DNA by in situ hybridization [46].

Moderate to intense expression of K13 and K14 was seen in all *toombak* dippers' lesions compared to moderate to weak expression of K13 in 12/15 (80%) and K14 10/15 (67%) in the *snus* dippers' lesions [46]. K19 was expressed in the basal layer in 16/49 (33%) of the *toombak* dippers' lesions compared to no expression in the *snus* dippers' lesions [46]. All tissues were found negative for HPV except two dysplastic *toombak* dippers' lesions. The low expression of these markers in *snus* dippers' lesions suggests differences in the biological effects of these products on oral tissues as well as a lower rate of malignant transformation of *snus* dippers' lesions [46]. Later, carcinomas from Sudanese (*toombak* dippers/non-dippers), Swedish and Norwegian (non-snuff dippers) were examined for expression of K13, K14 and K19 and HPV infection. For the oral carcinomas from *toombak* dippers, moderate to intense expression of K13 (71%; 10/14), K14 (86%; 12/14) and K19 (93%; 13/14) was found [47]. For the oral carcinomas from non-snuff dippers, weak to moderate expression of K13 (64%; 47/74), K14 (43%; 32/74) and K19 (45%; 33/74) was found [47]. HPV DNA was not detected in any of the carcinomas from the three countries [47]. The study suggested that high levels of expression of the Ks investigated in oral carcinomas from *toombak* dippers, compared to those from non-*toombak* dippers is perhaps due to dysregulation of keratinocyte proliferation and maturation caused by damaging effects of *toombak* [47]. Otherwise, it may explain the low level of p53 expression previously found in *toombak*-associated oral carcinomas which is currently under investigation at the gene level [45].

1.10. Other health effects

The increased popularity of *toombak* use in recent years seems to be due to it satisfying some psychosocial, pharmacological, economical and social demands. Regarding the psychosocial demands, *toombak* helps to alter mood, and ambiguously helps both concentration, and relaxation and distraction that is provided by both the intervals of preparation of the saffa and the dipping. The pharmacological effects are mainly attributed to nicotine which is a powerful pharmacological agent that changes the cardiovascular, neural, endocrine, and muscle function and induces effects in the gastrointestinal tract [48]. The cardiovascular changes include increased heart rate, blood pressure and decrease in skin temperature due to vasoconstriction in the extremities [48]. The neural effects in the brain and the peripheral nervous system are associated with changes in electrical cortical activity, i.e. induction of both stimulation and relaxation [48]. In the gastrointestinal tract, nicotine stimulates the parasympathetic autonomic ganglia and brain stem, causing the release of pharmacologically active substances which may produce nausea, vomiting and occasionally diarrhoea [48]. Therefore, it is now accepted that tobacco causes physical dependence, addiction and habituation [48]. A case control study from Sweden showed an odd ratio for myocardial infarction of 0.89 (95% confidence interval 0.62–1.29) in non-smokers who used *snus* daily [49]. On the other hand, smokers showed a clear increase in risk (odds ratio 1.87, 95% confidence interval 1.40–2.48), blood pressure and serum cholesterol levels were found similar in *snus* users and smokers among the controls. Another study (cohort) revealed a relative risk for death from cardiovascular disease of 1.4 (95% confidence interval 1.2–1.6) in *snus* users who had never smoked [50]. In non-*snus* using smokers of >15 cigarettes/day, the corresponding relative risk was found to be 1.9 (1.7–2.2). The results were unchanged when adjusted for cardiovascular risk factors, such as body mass index and blood pressure. An excess risk was indicated in those dying before 55 years of age in both *snus* users and smokers [50]. Recently, it has been concluded that *snus* use in Sweden involves as high exposure to nicotine as smoking causes release of sympathoadrenergic stimuli which may signify an elevated risk of cardiovascular stress including higher heart rate and blood pressure levels, thus influencing the risk of fatal cardiovascular events [11]. In the Sudan, nicotine content estimated from a *toombak* sample was found to be 32.2–102.4 mg/g dry wt compared to 17.1–23.4 mg/g dry wt and 12.4–15.1 mg/g dry wt of snuff used in USA and Sweden, respectively [21]. These data clearly document that *toombak* contains a range of two- to five-fold higher concentration of nicotine than does the commercial snuff manufactured in the United States and in Sweden. Therefore, during dipping nicotine is extracted from *toombak* and immediately deprotonated in the alkaline media provided by the natron; thus its absorption to the central nervous system is accelerated. These data support the conception that non-smoked tobacco product is a highly addictive product and its self-administration cannot be resisted.

In conclusion, despite *snus* and *toombak* both being snuff forms, the two products differ in many respects, and on their biological impact. They differ in type of tobacco species, ageing, fermentation, methods of processing and manipulation, packaging, pH value, moisture, nicotine, and nitrosamines content and the way the habit is practised and age of predominant use. The major differences which are expected to have significant biological consequences, particularly addiction and carcinogenesis, are pH and levels of the TSNAs.

Clearly, quantitative risk estimates on occurrence of cancer among *snus* and *toombak* dippers in non-smokers are needed. This research program will document the role of snuff, particularly *snus* and *toombak*, as an inducer of cancer in humans and it will affirm the importance of nicotine, *nor*-nicotine, anatabine and

anabasine defined *N*-nitrosamines in snuff carcinogenesis. Such an intervention program will be useful for populations at high risk in the Sudan, Sweden, the rest of Europe and the USA which could also specifically contribute to a reduction of *toombak* and *snus* use, modification of the product or use of an alternative product.

Acknowledgements

We would like to acknowledge the logistic support we received from the *toombak* Research Centre and Oral Cancer Campaign, Khartoum, Sudan.

References

[1] IARC, International Agency for Research on Cancer. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 37. Tobacco Habits other than Smoking; Betel Quid and Areca-nut Chewing; and some Related Nitrosamines, 1985, France.

[2] Department of Health and Human Services. The Health Consequences of Using Smokeless Tobacco. DHH Publication no. 86-2874, Bethesda, MD: National Cancer Institute, 1986.

[3] Brunnemann KD, Hoffmann D. Chemical composition of smokeless tobacco products. In: Smokeless Tobacco or Health, Vol. 2. National Cancer Institute Monographs, 1992, p. 96–108.

[4] Pindborg JJ, Poulsen HE. Studies in oral leukoplakias. 1. The influence of snuff upon the connective tissue of the oral mucosa. Preliminary report. Acta Pathologica Microbiologica et Scandinavica 1962;55:412–4.

[5] Schei E, Fonnebo V, Aaro LE. Use of smokeless tobacco among conscripts: a cross-sectional study of Norwegian army conscripts. Preventive Medicine 1990;19:667–74.

[6] Ström E. *Snus*—en oversikt. Den Norske Tannlegeforenings Tidende 1998;108:4–8.

[7] Andersson G. Snuff-induced changes associated with the use of loose and portion-bag-packed Swedish moist snuff (Ph.D. thesis, University of Malmö, Malmö). Swedish Dental Journal 1991; Suppl. 75.

[8] Hirsch J-M. Snuff-induced lesions: a clinical and experimental study. Ph.D. thesis, University of Göteborg, Sweden, 1983.

[9] Idris AM, Ahmed HM, Mukhtar BI, Gadir AF, El-besheir EN. Descriptive epidemiology of oral neoplasms in the Sudan, 1970–1985 and the role of *toombak*. International Journal of Cancer 1995;60:1–4.

[10] Östman J, Anneroth G, Gustafsson H, Tavelin B. Malignant oral tumours in Sweden 1960–1989—an epidemiological study. Oral Oncology, European Journal of Cancer 1995;31B:106–12.

[11] Bolinder G. Long-term use of smokeless tobacco: cardiovascular mortality and risk factors. Ph.D. thesis, University of Stockholm, Stockholm, 1997.

[12] Idris AM. The etiological association between use of *toombak* and oral squamous cell carcinoma. Ph.D. thesis, University of Khartoum, Khartoum, 1992.

[13] Brunnemann KD, Hoffmann D. The pH of tobacco smoke. Food Cosmetic and Toxicology 1974;12:115–24.

[14] Brunnemann KD, Genoble I, Hoffmann D. N-nitrosamines in chewing tobacco: an international comparison. Journal of Agricultural and Food Chemistry 1985;33:1178–81.

[15] Idris AM, Nair J, Oshima H, Friesen M, Bronet I, Faustman EM, Bartsch H. Unusually high levels of carcinogenic tobacco-specific nitrosamines in Sudan snuff (*toombak*). Carcinogenesis 1991;12:1115–8.

[16] Idris AM, Nair J, Friesen M, Oshima H, Bronet I, Faustman EM, Bartsch H. Carcinogenic tobacco-specific nitrosamines are present at unusually high levels in the saliva of oral snuff users in Sudan. Carcinogenesis 1992;13:1001–5.

[17] Tso TC. Physiology and Biochemistry of Tobacco Plants. Stroudsburg, PA: Dowden, Hutchinson and Ross, 1982.

[18] Anderson RA, Burton HR, Fleming PD, Hamilton-Kamp TR. Effects of storage conditions on nitrosated, acylated, and oxidizedpyridine alkaloid derivatives in smokeless tobacco products. Cancer Research 1989;49:5895–900.

[19] Djordjevic MV, Fan J, Bush LP, Brunnemann KD, Hoffmann D. Effects of storage conditions on levels of tobacco-specific N-nitrosamines, N-nitrosamines and N-nitrosaminoacids in U.S. moist snuff. Journal of Agricultural and Food Chemistry 1993; 11:1790–4.

[20] Prokopczyk B, Wu M, Cox JE, Hoffmann D. Bioavailabilty of tobacco-specific nitrosamines to snuff dippers. Carcinogensis 1992;13:863–6.

[21] Idris AM, Prokopczyk B, Hoffmann D. *Toombak*: a major risk factor for cancer of the oral cavity in sudan. Preventive Medicine 1994;32:832–9.

[22] Hecht SS, Rivenson A, Braley J, DiBello J, Adams JD, Hoffmann D. Induction of oral cavity tumours in F344 rats by tobacco specific nitrosamines and snuff. Cancer Research 1986;46:4166.

[23] Axell T. Snusning och dess skadeverkningar. In: Tobaksrökning eller hälsa. Tobakens medicinska skadeverkningar. Socialstyrelsen och Liber Förlag, 1979, p. 72–80.

[24] Ramström I, Barroll A. Tobaksvanor i sverige, 1976–77. National Föreningen för Upplysning om Tobakens Skadeverkningar, Stockholm, 1979.

[25] Modeer T, Lavstedt S, Åhlund C. Relation between tobacco consumption and oral health in Swedish school children. Acta Odontologica Scandinavica 1980;38:223–7.

[26] Hibell B., Jonsson, E. Undersökning av skolungdomens alkohol, narkotika, tobaks och sniffningsvanor Våren, 1981. Skolöverstyrelsens Rapport, 1982.

[27] Ahlbom HE. Prä disponierende faktoren für plattenepitelcarzinom in mund, hals and speiseröhre. Acta Radiologica 1937;18: 163–185.

[28] Axell T, Mörnstad H, Sundström B. Snuff and the cancer of oral cavity—a retrospective study (in Swedish). Lakartidningen 1978;75:2224–6.

[29] Hirsch J-M, Svennerholm B, Vahlne A. Inhibition of herpes simplex virus replication by tobacco extracts. Journal of Cancer Research 1984;44:1991–7.

[30] Andersson G, Axell T, Larsson Å. Impact of consumption factors on soft tissue changes in Swedish moist snuff users. Journal of Oral Pathology and Medicine 1990;19:453–8.

[31] Hirsch J-M, Heyden G, Thilander H. A clinical, histomorphological and histochemical study on snuff-induced lesions of varying severity. Journal of Oral Pathology and Medicine 1982; 11:387–98.

[32] Andersson G, Axell T, Larsson Å. Histologic changes associated with use of loose and portion-bag packed Swedish moist snuff—a comparative study. Journal of Oral Pathology and Medicine 1989;28:491–7.

[33] Hickey BB. Malignant epithelial tumours in the Sudanese. Annual Reports of the College of Surgeons England 1959;24:303–22.

[34] Lynch JB, El Hassan AM, Omer A. Cancer in Sudan. Sudan Medican Journal 1963;2:29.

[35] Idris AA. Elsaffa wassratan elshifa esufla. Elmagala al—Urdunia 1980;1:1–12.

[36] El-besheir EI, Abeen HA, Idris AM, Abbas K. Snuff dipping and COAM in Sudan: retrospective study. British Journal of Oral and Maxillofacial Surgery 1989;27:243–8.
[37] Salem G, Juhl R. Oral malignant and pre-malignant changes in Shamma users from the Gizan region, Saudi Arabia. Acta Odontologica Scandinavica 1984;42:41–5.
[38] Stirling G, Zahran F, Jamtoom A, Eed D. Cancer of the mouth in Western of Saudi Arabia: a histopathologic and experimental study. King Abdulaziz Medical Journal 1981;1:10.
[39] Axell T, Mörnstad H, Sundström B. The relation of the clinical picture to the histopathology of snuff dipper s lesion in a Swedish population. Journal of Oral Pathology 1976;5:229–36.
[40] Idris AM, Warnakulasuriya KAAS, Ibrahim YE, Nilsen R, Copper D, Johnson NW. Toombak-associated oral mucosal lesions in Sudanese show a low prevalence of epithelial dysplasia. Journal of Oral Pathology and Medicine 1996;25:239–44.
[41] Harris AL. Mutant p53: the commonest genetic abnormality in human cancer? Journal of Pathology 1990;162:5.
[42] Nigro JM, Baker SJ, Preisinger AC, et al. Mutations in the p53 gene occur in diverse human tumour types. Nature 1989;342:705–8.
[43] Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in p53 tumour suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Research 1994;54:4855–78.
[44] Field JK, Spandidos DA, Malliri A, Yiagnisis M, Gosney JR, Stell PM. Elevated p53 expression correlates with a history of heavy smoking in squamous cell carcinoma of the head and neck. British Journal of Cancer 1991;64:573–7.
[45] Ibrahim SO, Johannessen AC, Idris AM, Hirsch J-M, Vasstrand EN, Magnusson B, Nilsen R. Immunohistochemical detection of p53 in non-malignant and malignant oral lesions associated with snuff dipping in the Sudan and Sweden. International Journal of Cancer 1996;68:749–53.
[46] Ibrahim SO, Warnakulasuriya KAAS, Idris AM, Hirsch J-M, Johnson NW, Johannessen AC. Expression of keratin 13, 14 and 19 in oral hyperplastic and dysplastic lesions from Sudanese and Swedish snuff-dippers: association with human papillomavirus infection. Anticancer Research 1998;18:635–46.
[47] Ibrahim SO, Bertelsen B, Kalvenes M-Y, Idris AM, Vasstrand EN, Nilsen R, Johannessen AC. Expression of keratin 13, 14 and 19 in oral squamous cell carcinomas from Sudanese snuff-dippers: lack of association with human papillomavirus infection. Acta Pathologica, Microbiologica et Immunologica Scandinavica (in press).
[48] The Health Consequences of Using Smokeless Tobacco. A report of the advisory committee to the Surgeon General. Bethesda, MD: US Department of Health and Human Service, 1996.
[49] Huhtasaari F, Asplund K, Lundberg V, Stegmayr B, Wester PO. Tobacco and myocardial infarction: is snuff less dangerous than cigarettes? British Medical Journal 1992;305:1252–6.
[50] Bolinder G, Alfredsson L, Englund A., de Faire U. Smokeless tobacco use and increased cardiovascular mortality. American Journal of Public Health 1994;84:399–404.
[51] Prokopczyk B, Wu M, Cox JE, et al. Improved methodology for the quantitative assessment of tobacco-specific N-nitrosamines in tobacco by supercritical fluid. Journal of Agricultural and Food and Chemistry 1995;43:916–22.
[52] Ôsterdahl B-G, Slorach S. Tobacco-specific N-nitrosamines in the saliva of habitual male snuff dippers. Food Addiction and Contamination 1988;5:581–6.
[53] Prokopczyk B, Hoffmann D, Cox JE, Djordjevic MV, Brunnemann KD. Supercritical fluid extraction in determination of tobacco-specific N-nitrosamines in smokeless tobacco. Chemical Research Toxicology 1992;5:336–40.