Smokeless Tobacco, Viruses and Oral Cancer

Lars Sand[1], Mats Wallström[2], Jan-Michaél Hirsch[1]

[1]Department of Surgical Sciences, Oral and Maxillofacial Surgery, Medical Faculty, Uppsala University, Uppsala, Sweden. [2]Department of Oral and Maxillofacial Surgery, Institute of Odontology, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden.

Abstract

Oral Squamous Cell Carcinoma (OSCC) is the most common epithelial malignancy in the oral cavity. OSCCs and their variants constitute over 90% of oral malignancies, and the disease is associated with poor prognosis. OSCC is a complex malignancy where environmental factors, viral infections, and genetic alterations most likely interact, and thus give rise to the malignant condition. The International Agency for Research on Cancer (IARC) in 2007 concluded: "there is sufficient evidence in humans to establish smokeless tobacco as carcinogenic, i.e. smokeless tobacco causes cancer of the oral cavity and pancreas". ST products contain a large array of carcinogens, although the number found is actually smaller than in cigarette smoke. Worldwide, ST products have many different names depending on the region where it is produced. However, there are two main types of ST, chewing tobacco and snuff. It is estimated that approximately 150 million people in the world use ST. Herein, we review available literature regarding smokeless tobacco and oral *Carcinogenesis*. We also discuss the role of viral infections in combination with ST in OSCC development.

Key Words: Smokeless tobacco, Oral cancer, OSCC, Human papilloma virus, HPV

Introduction

Smokeless Tobacco (ST) or unburned tobacco is used worldwide by several hundreds of millions of people [1]. Moist snuff is a mix of finely ground tobacco, flavouring and water and this product is mainly used in the United States and the Scandinavian countries [2]. It is usually placed in the upper or lower vestibulum, and daily exposure as well as the consumed amount of snuff varies [3-6]. The use of moist snuff means continuous exposure to high levels of nicotine, which is highly addictive [7]. It also causes negative effects on visceral and circulatory functions. Further, the snuff dipper is exposed to over 3000 chemicals in moist snuff, including the carcinogenic Tobacco Specific Nitrosamines (TSNA) [8,9]. Moist snuff use cause local and generalized pathological reactions such as oral and pancreatic tumours and also increased risk of cardiovascular disease, and diabetes mellitus [4,10-17]. Similar toxicological reactions have also been observed in animal studies [18-21].

An *in vitro* investigation showed that ST extracts have an immune-stimulating potential [22], while other studies have revealed a number of general negative effects on the immune system [23,24]. It is reasonable to assume that the immune system of the oral mucosa at the site of ST exposure could be affected, so it is also of interest to elucidate some of the most potentially harmful chemicals found in ST [25]. It is still an open question to what extent snuff-induced lesions in the oral mucosa are reversible. In the light of a possible malignant cell transformation later in life, apparent in available reports [17,26], this is an important issue that needs attention. The negative health consequences of moist snuff use and its relatively high prevalence, especially among adolescents and young adults [27], make it necessary to promote snuff cessation. Viral infections, especially Human Papilloma Virus (HPV) is involved in oral *Carcinogenesis* [28]. Studies have been suggested HPV as a possible co-factor together with ST use in the development of OSCC. It is therefore important to explore further opportunities for achieving good results in snuff cessation in a highly nicotine-dependent group of users.

This article provides a narrative review of the possible role of smokeless tobacco and viruses in the etiology of OSCC.

Historical Background

American Indians were probably the first people to use snuff and to chew or smoke tobacco [29-34]. Tobacco was used in those cultures for several reasons including medical treatment, prevention of fatigue and hunger on long distance treks, and various rituals and ceremonial uses. During the 16th century the use of tobacco spread all over Europe. The French ambassador Jean Nicot introduced snuff in 1560 to the French Royal Court to cure Queen Catherine de Medici's severe migraine, recommending her to inhale particulate tobacco nasally [35-39]. The botanical name derived from his surname was established when Carl von Linné named the plant *Nicotiana Tabacum* in his system of plant classification in 1753 [40]. The word "tobacco", from the Spanish "tobaco", derives from an Arawak language word for a roll of tobacco leaves or the tube or pipe in which the plant was smoked, while the name in the Caribbean for the plant itself was "petun". Locally, however, in parts of Mexico, the plant was also referred to as "tabac". In 1828 the active ingredient of tobacco was isolated and called nicotine [40]. The use of snuff also spread throughout Africa, Japan, and China, where it was fashionable among the Ching Dynasty. The Chinese believed that snuff cured toothache, provoked sweating, and alleviated constipation [41-43]. The use of snuff by European royalty during the 16th, 17th, and 18th centuries gave respectability to the habit and increased its popularity. In many Swedish cities, snuff has been manufactured since the beginning of the 18th century.

Manufacturing Process, Alkaloids and Nitrosamines

ST is mainly produced from *Nicotiana Tabacum*, although

Corresponding author: Lars Sand, Department of Surgical Sciences, Oral and Maxillofacial Surgery, Medical Faculty, Uppsala University, Uppsala, Sweden; Tel: +46-186116450, Fax: +46-18559129 e-mail: lars.sand@surgsci.uu.se

Nicotiana rustica Linn is used in Turkey for the production of the ST specific to that region [44]. Moist snuff consists of 40% to 45% finely ground air- or fire-dried tobacco mixed with water (45–60%), sodium carbonate (1.5–3.5%), sodium chloride (1.5–3.5%), moisturizer (1.5–3.5%), and flavouring (<1%) [45]. The chemical composition of ST varies due to the type of tobacco used and undergoes substantial changes during curing, processing, and storing [46]. Over the years chemical analyses performed on ST have shown that it contains very large numbers of different chemicals [47,48]; Hoffmann et al. [9] found 23 N- nitrosamines and 28 pesticides, which brought the number of known constituents in tobacco to a total of 3095. All ST products contain nicotine, which is highly addictive, and the speed of absorption is a major determinant of addiction [7,49]. The level of unprotonated nicotine affects the absorption rate and degree of trans-mucosal nicotine absorption, which is facilitated when the tobacco product is more alkaline [50,51]. The pH and the level of unprotonated nicotine vary among the tobacco products and snuff brands, and the ones with the highest content of unprotonated nicotine have the highest market shares. TSNAs are widely considered to be among the most important carcinogens in ST and cigarette smoke [8,52]; about 30 carcinogens have been identified in smokeless tobacco. The high levels of TSNA observed in ST are primarily due to their formation during curing, fermentation, and aging, but they are also produced endogenously during consumption [53] from the precursor alkaloids, nicotine, nornicotine, anatabine, and anabasine where nicotine, nornicotine, and anabasine are the major contributors. Hoffmann et al. provided the most comprehensive insight into the levels of major tobacco carcinogens in the leading brands of most snuff sold in the USA [54]. Since the middle 1980s the concentrations of nitrosamines in some brands on the USA market and in Sweden have declined by up to 85% [55,56], although Richter et al. [3] found a 20-fold difference in the range of sums for total carcinogenic TSNAs. The factors of the Sudanese smokeless tobacco *toombak* believed to have significant adverse health consequences, particularly in terms of addiction and oral cancer development, are its pH and high levels of tobacco-specific nitrosamines (TSNAs) [57]. *Toombak* has a pH range of 8–11, with a moisture content of 6–60%, nicotine content of 8–102 mg/g dry weight, and TSNA contents in micrograms, i.e., nitrosonornicotine (NNN), 420–1550 µg/g; 4-(methyl-nitrosamine)-1- (3-pyridyl)-1-butanone (NNK), 620–7870 µg/g; N-nitrosoanatabine (NAT) 20–290 µg/g (140). TSNAs, particularly NNN and NNK are found in the saliva and body fluids of *toombak* dippers [57-59]. Compared with ST from Sweden and the USA, *toombak* contains 100-fold higher levels of TSNAs [60].

Epidemiology of Smokeless Tobacco Habits

Smokeless tobacco is used in different forms in different parts of the world and approximately 150 million people use it worldwide. There are two main types of ST: chewing tobacco and snuff. Chewing tobacco in the form of loose leaf, cut, or shredded tobacco is universally available. Snuff for oral application, "dipping", or sucking is dry or moist and is commercially available as loose or as portion bag-packed products [61]. Although it is banned by governmental regulation in some countries, ST for oral use is manufactured and consumed on all continents [1,62,63] under various names including betel-quid, chimo, gudhaku, gutkha, gul, iq'milk, khiwam, kahaini, maras, maras powder, mishri, nass, naswar, plug, shamma, toombaak, moist snuff, snus, or some other variant depending upon the locale [1].

Scandinavia

Moist snuff is the most popular form of orally used ST in North America and parts of Europe, particularly the Scandinavian countries [64]. Earlier data on the prevalence of daily snuff use in Sweden varies from 7% of men over 45 years of age in the southern part of the country [65], to 24% of the male population and 5% of the female population in central Sweden, and 30% of men and 6% of women in the north [66]. Gradually the number of users has increased in the southern and central regions of Sweden, regional variations have diminished, and the prevalence of daily snuff use throughout Sweden was recently reported as 19% of men and 4% of women [67]. Since 1971 an annual drug habit survey has been conducted among schoolchildren in Sweden, and it is clear that the use of snuff has varied over time. In lower secondary school children from 1983 to 2000 the prevalence of daily or occasional snuff use rose among 15-year-old boys from 16% to 25% and among 15-year-old girls from 2% to 8%; by 2009, however, it had declined to 15% and 4% respectively [68]. Data on snuff use in Norway has been collected by Statistics Norway since 1985. From 1988 to 2009 the prevalence of daily snuff use increased among men aged 16 to 74 years from 3% to 11%, and 2% of women were using snuff daily by 2009. The highest prevalence was registered in the age group 16 to 24, where 21% of men and 7% of women were daily users [69].

North America

In the USA in 1970, ST use was most prevalent among adults over 65 and the dominant form was chewing tobacco. Among younger males 16 to 24 years of age, 2.2% used ST. By 1987 this had changed and 6.1% of men over 65 used ST compared with 8.9% of men aged 16 to 24. In 1995, 19.7% of males in higher education reported use of ST and 80% of those used moist snuff [64]. Eaton et al. [27] conducted a nationwide US survey in 2010 and found that 8.9% of all students reported current ST use. The overall prevalence in males was 15%, while only 2.2% of females reported current use. The highest prevalence was documented among white males (20.1%), followed by Hispanic (7.5%) and black males (5.2%).

India and South East Asia

The use of betel quid is an old habit and is commonly practised in Southeast Asia, on the Indian subcontinent and in the Asian Pacific region. It is common among migrant communities in Africa, Europe, and North America. Because of its ancient history, its use is socially acceptable throughout society, including women and, quite often, children. Areca nut (usually incorporated into betel quid) is the fourth most common psychoactive substance in the world, after caffeine, alcohol, and nicotine, being used by several hundred million people [70]. The betel quid is composed by a combination of areca nut, betel leaf and slaked lime. When an industrially manufactured mixture of areca nut, lime, a catechin-containing substance, sandalwood fragrance, and tobacco was

introduced in small aluminium foil sachets, a major change in betel use was seen in India. This product was termed *gutka*, while the same product without tobacco was termed *pan masala*. It is now well accepted that the use of areca nut causes oral submucous fibrosis (OSMF) [70]. According to IARC, there is evidence of the carcinogenic risk of chewing betel quid [1]. The use of betel causes cholinergic effects as well as mild psychoactive effects. The saliva is stained red by the product and the teeth may be stained red/brown after years of betel chewing. In various studies, betel use has been associated with OSMF, leukoplakia and OSCC (for review see IARC Monograph, 2004; p.231) [71].

Sudan

In Sudan, ST is usually used in a form of oral dipping tobacco, locally called *toombak*, and was introduced over 400 years ago [72]. *Toombak* is not chewed but dipped and retained between the gums and the lips, cheeks, or floor of the mouth, and sucked slowly for approximately 10–15 min [58]. The tobacco used for manufacturing *toombak* is *Nicotiana rustica*, and the fermented ground powder is mixed with an aqueous sodium bicarbonate solution. The resulting product is processed into a loose moist form with a strong aroma, and its use is widespread in the country; popular brands of *toombak* are *Saute'*, *El-sanf, Wad Amari*, and *Sultan El-kaiz* [57]. Sudanese snuff or *toombak* differs from the types of ST used in Scandinavia and the USA in terms of tobacco species, fermentation, aging, manufacturing methods, pH, moisture, and nitrosamine content [57]. *Toombak* dippers develop a clinically and histologically characteristic lesion at the site of dipping. Researchers have demonstrated that the use of *toombak* plays a significant role in the etiology of OSCCs, the TSNAs present in *toombak* possibly acting as principal carcinogens [72-74]. In addition to playing a major role in the aetiology of oral cancer, *toombak* is suspected to be associated with neoplasm of salivary glands [75-77].

Smokeless Tobacco and Oral Cancer

In 1985, the International Agency for Research on Cancer (IARC) [78], in their monograph. *Tobacco habits other than smoking; betel-quid and areca-nut chewing; and some related nitrosamines*, concluded that "there is sufficient evidence that the use of smokeless tobacco can cause oral cancer in humans and that chewing tobacco may increase the risk for oral cancer development". The issue was reviewed again in 2007 in another IARC monograph, and the conclusion was that several studies in a number of countries have identified the use of smokeless tobacco as a cause of oral cancer. The working group now stated that "there is sufficient evidence in humans to establish smokeless tobacco as carcinogenic, i.e. smokeless tobacco causes cancer of the oral cavity and pancreas" [1]. Tobacco contains high levels of TSNAs, which are the major carcinogens in the several forms of ST used around the world. Studies in India, Pakistan, and Sudan have reported large increases in the risk of oral cancer related to the use of various ST products. Benzo[a]pyrene and other polycyclic aromatic carcinogens (PAHs) are the most important carcinogenic agents in cigarette smoke; in unburnt tobacco, however, nitrosamines are the strongest carcinogens [60]. The metabolites of nitrosamines, particularly nitrosonornicotine (NNN) and 4-(methylnitrosamine)- 1-(3-pyridyl)-1-butane (NNK), are found locally in the saliva of ST users and in their body fluids. These agents are known to cause toxic effects, particularly cancer [60] and other cellular and DNA changes, either at the local placement sites or indirectly and systemically. Even ST products that are claimed to be low in nitrosamines likely raise the risk of oral cancer among users by up to 30% [79].

Among the high-income countries, Sweden has the highest per capita consumption of ST, predominantly in the form of oral moist snuff. In Sweden the amount of TSNA in snuff has been reduced compared to many brands available in North America and some low-income countries [56,80]. This is due to an improvement in its production, including a shift to anaerobic fermentation among other things. The carcinogenic effect of Swedish snuff is controversial and since a high proportion of the male population in Sweden (20%) use snuff regularly, studies regarding its cancer risks are needed. Animal studies have shown that Swedish snuff, as well as snuff from USA, can cause cancer in the oral cavity in rats [81-83]. An association between Swedish snuff use and oral cancer was found in a population-based survey of 9976 men, and the authors concluded that snuff-related risks for oral cancer should not be dismissed lightly [17]. In a case report, Zatterstrom et al. [84] described a case of well-differentiated oral squamous cell carcinoma in a 90-year-old Swedish man who had been a habitual snuff- dipper for 70 years. Further, Hirsch et al. reported 16 oral cancer cases among Swedish snuff dippers where the cancers developed at exactly the location where the snuff was placed, all pathologically confirmed as SCCs [85]. A few clinical studies have not been able to confirm a carcinogenic effect in active snuff dippers though [26,86,87]. Three Swedish-based case controlled studies on oral snuff found no significant association between snuff use and the risk for head and neck cancers [26,87,88]. In one of the articles however [26], a nearly fivefold elevated risk for head and neck cancer was reported in the subgroup of men with snuff use and no history of smoking, and in the IARC analysis [1], a borderline statistically significant increase was found for the risk for oral cancer among former snuff users.

Winn et al. in a case-control study investigated 255 women and 502 controls in the USA regarding risk factors for OSCC development, and they showed an almost 50-fold increased risk for oral cancer development among snuff users [89,90]. Oral cancer in India correlates strongly with the use of ST, with up to 80% of oral cancers occurring in ST users. Ghosh et al. found that among 71 Indian tobacco chewing patients with OSCC, a statistically significant increase was found in patients using the quid overnight [91]. Studies have revealed that the high prevalence of oral cancer in the Sudan has a strong association with the use of *toombak*, and Idris et al. described that among 62 Sudanese patients with OSCC, 50 were *toombak* users [75,76,92]. In a study of the interactive effect of Swedish snuff and Sudanese *toombak* on human oral cells, Costea el al. demonstrated that *toombak* has greater potential to induce abnormal development of normal mucosa than does Swedish snuff [93].

Viruses, Smokeless Tobacco and Oral Cancer

Long-term daily repeated exposure to snuff in rats has been shown to be carcinogenic for the lip and oral cavity [83]. In both animal and human studies, the association between Herpes simplex virus 1 (HSV-1), smokeless tobacco or smoking, and malignant tumours has been investigated, and possible interactions proposed [19,94]. From animal studies in rats it was clear that repeated infection with HSV-1 virus together with daily repeated exposure to snuff resulted in increased number of tumours [18]. Hirsch et al. [19] studied the effect of snuff extracts on HSV-1, and they showed that extracts of snuff have inhibitory effects on the production of cytolytic HSV-1 infections. They suggested that an interaction between tobacco ingredients and HSV-1 might be involved in development of dysplastic lesions. Larsson et al. [95] showed that snuff products inhibit the replicative cycle early. This resulted in increased alpha-protein production in the HSV-infected cells and hence, prolonged maintenance of cellular functions. They suggested a possible HSV-1 induced malignant cell transformation.

Clear evidence is lacking that there are similar effects of tobacco chemicals on HPV replication. However there are *in vitro* experiments that have shown that malignant transformation of oral keratinocytes can be caused by a sequential, combined effect of "high-risk" (HR) HPV and tobacco-related carcinogens [96]. The HPV induced cancers are biologically different from those related to alcohol and tobacco and most studies conclude that HR-HPV-related oro-pharyngeal SCC have a better prognosis and the therapy could be different, less aggressive as HPV positive tumours appear to be more susceptible to radiation [97-101]. In India, the prevalence of OSCC and OSMF is among the highest in the world, which is mainly attributed to the use of betel quid containing areca nut and tobacco. An intriguing finding was reported by Jalouli et al. who found 91% HPV 16 and 18 in OSMF compared to only 24%, from patients with OSCC [102]. Using PCR/DNA sequencing, Jalouli and co-workers in a subsequent study investigated the prevalence of HPV in a Sudanese population. In brush tissue samples from *toombak* users, HPV was detected in 40%, while the corresponding figures for non-users were 68%. In OSCC samples HPV was detected in only 27% in *toombak* users, and in non-users 21% [103]. From these data it is not clear that cancer risk is increased with a combined effect of virus and tobacco. These findings are in line with an earlier report by Sand et al. who found no statistical difference between the use of tobacco and alcohol and HPV prevalence in 24 OSCC, 6 lichen planus, 7 leukoplakias and 12 control subjects [104].

In India, HPV DNA was detected less frequently in tumour specimens from tobacco chewers than in those from non-chewers [28]. Further, Gillison et al. [105] reported that HPV DNA was detected statistically significantly less often among tobacco smokers and/or chewers than among non-smokers and/or non-chewers in head and neck cancers. One explanation could be that DNA damage response genes and pathways controlling the stability of HPV episomal DNA, and tobacco extracts might play a role. In contrast, Mehrotra and co-workers in a study to assess the correlation of chewed as well as smoked tobacco and alcohol and HPV infection in subjects diagnosed with OSMF found 31.4% of the patients to be positive for HR-HPV. No significant correlation between the infection and habits such as smoking, chewing of tobacco with areca nut or alcohol consumption was reported. Even if Mehrotra et al. found less than half the percentage of HR-HPV it is still quite an extensive figure [106]. Normally the glandular tissue of the Waldeyer's lymphatic ring and base of tongue are the predisposed areas for HPV associated cancer. In patients with OSMF, one can only speculate that the non-smoked tobacco affected tissue is more permissive to HPV and the infection might be a part of the malignant cell transformation. The OSMF finding is surprising and the implication of the data warrants further studies.

It has been argued that tumour HPV status is a strong and independent prognostic factor for survival among patients with oropharyngeal cancer. Herrero et al. investigated the combined effect of tobacco use and HPV infection by using antibodies against HPV16 and antibodies against HPV16 E6 and E7. For the latter, the risks appeared to be additive, indicating the absence of synergism between tobacco use and HPV [28]. Schwartz et al. [107] reported a multiplicative effect of smoking and HPV, as measured by antibodies against HPV16 which was not found in the above study by Herrero et al. The issue of a presence of additive rather than multiplicative risks between HPV and smoking/chewing tobacco use is not clear. An additive risk suggests that these factors operate, in part, at the same step of multistage *Carcinogenesis* in the oral cavity and oropharynx (e.g., p53 inactivation). Still, HPV infection appears to contribute to an increased risk for cancer of the oral cavity and oropharynx also among tobacco smokers and chewers.

Conclusion

There is sufficient evidence for a causal association between ST use and oral cancer in the USA, Asia and Africa. In the Scandinavian studies the situation is not as clear. There are contradicting results in various studies. Even though the risk seems to be lower for OSCC development in Scandinavia due to ST use, it cannot be regarded as a safe habit. The lower risk could be attributed to differences in tobacco species or in the practice of ST habits, i.e. amounts used, years of usage, differences in TSNA content. Further, oral hygiene status, immune status, genetic susceptibility and nutritional status may be factors to consider in the risk assessment of OSCC. Case report and case series of OSCC development in ST users in Sweden emphasize that large prospective studies are needed to clarify the risks of Scandinavian ST habits.

HPV infection is a common event in the oro-pharyngeal area, but is of no significance unless a chronic infection is established i.e. HPV is upregulated, which is usually seen after an extensive time period. It can be further concluded that there are sparse data to support a multiplicative effect of exposure to tobacco and HPV in development of oral cancer but if any, rather the opposite. It seems that HPV DNA is statistically significantly less often found among tobacco chewers than among non-chewers with oral cancer, and more studies are needed to elucidate any synergistic effects of HPV and ST use in oral *Carcinogenesis*.

Conflicts of Interest

The authors declare no conflict of interest.

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