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Review Article

Oral Mucosal Membrane Exposure and Adverse Health Risks Secondary to the Use of Smokeless Tobacco

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Abstract

Substantial impact of various smokeless tobacco toxicants, constituents, and chemicals as they pass through the oral mucosal tissues, enter body fluids and systems causing adverse health effects. Greater emphasis should be placed on the early cellular, molecular, physiological, and morphological changes that occur secondary to the harmful chemicals and toxicants in smokeless tobacco, which play a role in the carcinogenesis process. Smokeless tobacco constituents may cause destruction and/or irritation of the oral mucosal tissues resulting in severe tissue damage, carcinogenesis and serious systemic effects.

Keywords: Smokeless Tobacco; Oral Mucosal Cells and Epithelium; Toxicants; Carcinogens

Introduction

There is controversial evidence in the literature as to the level of harm in using smokeless tobacco [1]. Smokeless tobacco products have been promoted in recent years by major tobacco companies as an alternative to smoking [2]; however, these products are not safe and there are known oral cancer risks [3]. In 2020, an estimated 53,000 adults in the United States were diagnosed with oral cavity and oropharyngeal cancer, and an estimated 10,700 people will die of these cancers. The 5-year survival rate for oral and pharyngeal cancer is approximately 50% - 57% [4].

The use of smokeless tobacco is associated with oral cancer [5,6] and is commonly diagnosed in the later stages of the disease [1,7]. Early identification of cellular tissue changes will result in improved smokeless-tobacco-related disease outcomes. Certain cellular and physiological changes in oral mucosal tissues due to smoke-

less tobacco use are considered early markers in the progression of carcinogenesis [1]. This overview will raise awareness about the cellular changes in oral mucosal tissues as smokeless tobacco chemical elements pass through the oral mucosa, which result in an increased risk of oral cancer, other diseases, addiction, and exposure to toxicants. These dysplastic changes are often a result of frequency and duration of long-term smokeless tobacco use [8-10].

There are several types of smokeless tobacco that have been traditionally marketed in the United States: snuff (wet and dry) including snus (wet snuff) and chewing tobacco. Many products such as snus and snuff are in small teabag-like pouches [7,11]. These products do not require spitting, their use can be easily concealed [12]. Most smokeless tobacco use involves placing the product, which contains numerous toxicants and other flavorings between the cheek or lip mucosa and the gum [1,6,11]. Long-term use of smokeless tobacco may lead to pre-dysplastic or malignant foci [12]. Many smokeless tobacco products contain harmful and potentially harmful constituents (HPHCs) [13]. Such constituents include toxicants, carcinogens, addictive components, and chemical compounds, increasing the levels of exposure to the harmful effects of smokeless tobacco use [5, 14].

Smokeless tobacco (ST) products sold in the U.S. vary significantly in yields of nicotine and tobacco-specific nitrosamines (TSNA's) [2,8]. Results showed that independent of the pattern of smokeless tobacco use, and nicotine yields, levels of TSNA's in smokeless tobacco products play a significant role in carcinogen exposure levels [2,8,10].

On the US market, conventional and low tobacco-specific nitrosamines (TSNA's) are the most popular form of smokeless tobacco products [11]. Sales of moist snuff and snus have been increasing over the past decade. In 2018 sales of snus increased to around $1.55 million in US dollars, and sales of moist snuff increased to approximately $3.92 billion in US dollars [15]. Sales of other smokeless tobacco products have also increased. Flavored products tend to contain lower levels of free nicotine and some have a lower pH. Flavored products are often the impetus for many persons to use smokeless tobacco starter products [16]. The resurgence of smokeless tobacco is becoming increasingly popular in the United States and globally. Studies show that the new smokeless tobacco products can be quite dangerous. Additionally, the global use of Swedish snus (oral moist snuff marketed in Sweden), can also be extremely damaging to the oral mucosal tissues and to some organ systems [3].

Oral manifestations and health effects of smokeless tobacco

Oral mucosal lesions that may form as a result of tobacco use include leukoplakia, erythroplakia, cancers of the oral cavity and pharynx, oral diseases of the gingival and periodontal tissues, and after long-term use, snuff-dippers lesions, sores, and snuff submucosal deposits which may lead to dental caries [6,7,17-19]. In addition, there is evidence that smokeless tobacco use can increase the risk of cardiovascular diseases and stroke [7,18,19].

Users of smokeless tobacco and users of cigarettes have comparable levels of nicotine in the blood. Nicotine which is absorbed through the oral cavity tissues directly continues into the blood, where it goes to the brain [14,20] Even after the tobacco is removed from the mouth, nicotine continues to be absorbed into the bloodstream [21]. Orally absorbed nicotine stays in the blood longer for users of smokeless tobacco, than for cigarette smokers and is easily absorbed through the oral mucosal lining [3]. Approximately twice as much as nicotine is absorbed per dose of smokeless tobacco than cigarettes (4 mg vs. 2 mg) [7,18,19,22]. It is estimated that one pouch of smokeless tobacco currently on the market contains on average about twenty times lower amounts of analyzed agents than an average portion of traditional products.

Smokeless tobacco products deliver substantial doses of nicotine along with powerful cancer-causing chemicals. Local irritation from salt may increase the absorption of smokeless tobacco carcinogens in the oral cavity, and may lead to chronic inflammation [11,23]. Absorption of nicotine across biological membranes is highly pH dependent. Free nicotine (unionized and pH above 6.5) is readily absorbed into biological tissues, and well absorbed through the mouth and buccal membranes [24].

The tobacco cut, size, and the product's pH (a measure of its acidity or basicity) are all a part of the absorption process as constituents cross oral mucosal membranes, enter the bloodstream and subsequently the brain where is exerts the pharmacological effects that may lead to addiction. When the pH was reduced in one study by Andersson and Warfvinge [25], it was noted that fewer pronounced clinical and histological changes were noted at the sites of placement [25]. Increasing the pH of nicotine transforms nicotine to a unionized form and is more readily absorbed than ionized forms such as salt [21,24,26,27]. The results of two studies by Hatsukami., *et al*. confirm that the frequency, duration, the amount of product used, residence, time and flux within the mouth cavity affect nicotine exposure from smokeless tobacco, and addic-

tion. These behavioral aspects of smokeless tobacco use result in high variability in nicotine exposure and addiction [28].

Nitroso compounds and chemicals

Thousands of chemical substances have been found in tobacco [14] and according to the National Cancer Institute (NCI), more than 28 cancer causing chemicals, have been identified in smokeless tobacco [18,22]. Tobacco-specific N-nitroso compounds have been detected at high concentrations in both snuff and chewing tobacco [29]. Tobacco and (quid chewing) may cause oxidative stress to tissues through the release of reactive oxygen species, which initiate free radical reactions, causing damage of proteins, lipids, carbohydrates and DNA. Smokeless tobacco products offer a more direct link between harmful and potentially harmful constituents (HPHC) levels in the products and corresponding exposures in users [13,14]. Several priority HPHC's include nicotine, the carcinogenic TSNA, N'-nitrosonornicotine (NNN) and 4-(methylnitrosaminor)-1-(3-pyridyl)-1butanone (NNK) and other polycyclic aromatic hydrocarbons (PAH), [30,31]. These chemicals are of particular concern, as they are believed to be responsible for toxic and harmful effects associated with the use of smokeless tobacco [14]. A number of studies revealed that two TSNA's (tobacco-specific nitrosamines, NNN (N-nitrosonornicotine) and NNK 4-(methyl-N-nitrosamino)-1 -(3-pyridyl)-1-butanone are carcinogenic by oral or parenteral routes in rats and mice [30,31]. In rats, NNN produces tumors in the nasal cavity and esophagus (oral cancer) while NNK produces tumors primarily in the lung, liver [32,33] (Figure 1). Another study based on the Shanghai Cohort Study explored the dose-response relationship between biomarkers of exposure to NNN and risk for esophageal cancer in smokers. Urine samples were used from 77 esophageal cancer cases and 223 cancer-free controls (all smokers) were analyzed for urinary total NNN. The results showed that odds ratio of esophageal cancer risk for the second and third tertiles of urinary total NNN were 4.0 and 17.0 compared to the lowest quartile [32]. NNN alone is strongly associated with esophageal and oral cavity cancer [7,14,34]. Epidemiologic study data indicate the TSNA's are associated with increased risk for cancer in smokers and likely, smokeless tobacco users [14,31]; NNN and NNK are likely causative agents for cancers of the oral cavity, esophagus, and pancreas in smokeless tobacco users, and are classified as human carcinogens by the International Agency on Cancer [7,14].

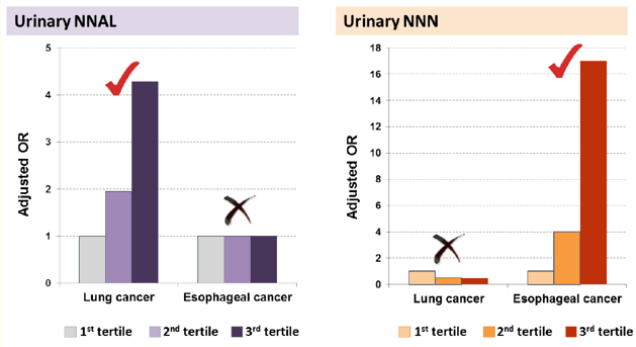

Figure 1: S J.-M. Yuan., *et al*. Carcinogenesis 32(9): 1366-1371 (2011); I. Stepanov., *et al*. Int J Cancer 134:2278-2283 (2014).

NNK exposure linked to lung cancer and NNN exposure linked to oral cancer

NNN and NNK are likely causative agents for cancers of the oral cavity, esophagus and pancreas in smokeless tobacco users, and are classified as human carcinogens by the International Agency on Cancer (IARC). NNN is strongly associated with esophageal and oral cavity cancer.

A mixture of naturally occurring amounts of NNN and NNK produced a few benign tumors at the site of application was applied to the artificial lip canal in rats [30]. Several older and smaller investigations have found at least 16% of biopsied cases show mildly transformed dysplastic cells, white patches and chemically-induced structural changes were found, but no tumor formation noted [35,36]. Extensive bioassays have demonstrated that NNN and NNK are strong carcinogens in snuff. NNN given to F344 rats in the drinking water or in a liquid diet induces a high incidence of esophageal and nasal cavity tumors [30,32,33].

Patterns of cellular alterations

The histopathology of oral leukoplakia or snuff induced lesions were reported by Greer [12,37] and Daniels. Common epithelial changes noted were hyperorthorthokeratosis, hyperparakeratosis, chevron pattern keratinization, pale surface staining koilotosis-like changes with vacuolated cells and basal-cell hyperplasia [38,39]. Keratinization has been found to be one of the early indicators and poor prognosis of oral squamous cell carcinoma and may be involved in the etiology of oral cancers [39,40]. The placement of

smokeless tobacco, regardless of type, in direct contact with the oral mucosa produces a thickened layer of keratin of the oral epithelial surface. This thickening, or hyperkeratotic-like lesion usually occurs directly at the anatomic site where the smokeless tobacco was placed [1,12,20]. Smokeless tobacco keratoses may occur in approximately 60% of smokeless tobacco users, within six months to three years of product usage. Kaugars., *et al*. recorded the highest prevalence of epithelial dysplasia mild dysplasia, and severe dysplasia, and noted that the majority of these biopsies were taken from the site of tobacco quid placement [41]. Malhotra., *et al*. and other researchers also reported that oral lesions were observed to be at the anatomical site of placement and occurred in 50 - 60% of smokeless tobacco users [42]. Constituents of smokeless tobacco can cause local irritation and are absorbed systemically through the oral or nasal mucosa by swallowing saliva that contains tobacco particulates [43].

Additionally, in a study by Arimilli., *et al*. smokeless tobacco use is also reported to be associated with immune dysfunction, including the dysregulation of immune cells and their components [44]. Mohammed., *et al*. and others reported that studies from India showed the presence of binucleated buccal mucosa cells in smokeless tobacco users [39,45]. In another study, squamous epithelial cells sampled from the oral mucosa of smokeless tobacco users demonstrated significant cytologic alterations associated with tobacco exposure. In this study, there were exposure-dependent nuclear alterations, including micronuclei, in the oral epithelium associated with the use of smokeless tobacco. Results suggest that use of smokeless tobacco, may cause genetic damage to cells in the oral epithelium.

Additional studies showed snuff insertion in hamster cheek pouch became hyperplastic and hyperkeratotic with a prominent granular layer. By transmission electron microscopy an increased number of mitochondria and rough endoplasmic reticulum were also observed. By scanning electron microscopy, the changes in the epidermis consisted of the development of an irregular arrangement of the micro ridges and a disappearance of the normal honeycomb pattern [36,46,47]. The previous studies were not long enough to determine an outcome of long-term changes.

Mechanisms of oral tissue changes, pre-cancerous and cancerous processes

Two specific tobacco- related lesions of the oral mucosa, nicotine stomatitis and tobacco pouch keratosis, have often been included under the broad umbrella of leukoplakia. Such lesions usually occur in the buccal or labial vestibule where the tobacco is held, but they can also extend onto the adjacent gingiva and buccal mucosa [1,3].

In one analysis, the type of smokeless tobacco used (snuff vs. chew), increased amount used (cans or pouches per day), increased length of use (months), number of days since last use of brand or snuff used were significantly associated with the risk of developing leukoplakic lesions among smokeless tobacco users. Smokeless tobacco (ST) placed in the muco-buccal folds causes direct damage to the periodontium (gingivitis, periodontal recession) and oral soft tissue [3]. Smokeless tobacco (chew) has also been noted to cause tooth decay along the root surfaces of teeth.

Previous studies have shown that soft tissue changes of the oral mucosa and gingival margin are less pronounced, both clinically and histologically, among users of portion-bag packed snuff [48]. Gingival blood flow was measured in 22 healthy snuff consumers from Norway. Application of commercial snuff (500mg that contained approximately 1% nicotine) caused a markedly rapid increase in gingival blood flow on the exposed side as well as on the contralateral side [49]. The significance of the transfer of tobacco related constituents and oral mucosal contact is the cause of occurrence of oral manifestations and other health consequences. Tomar., *et al*. and others reported in a different study that adolescents who used snuff daily had oral lesions, and over a period of many years, was considered an early indicator in the development of oral cancer [19] and found that dysplasia may occur in Snuff Dippers' lesions. Kaugars., *et al*. 1989 noted that women were more likely to have dysplasias than men, which may have occurred because their lesions were detected a decade or so later, or because the women were older [41]. The risk of developing oral cancer due to the use of smokeless tobacco increases with age, can result in significant health risks, and the majority of cases occur after the fifth decade of life [50].

Lesions were strongly associated with duration, monthly frequency, and daily minutes of use of snuff and chewing tobacco [7]. In North Carolina women working in the textile industry had a high prevalence of snuff dipping and elevated death rates from oral cancer. The risk of having oropharyngeal cancer was four times greater in white women and, lower for black women. Women who used snuff for 50 years had exceptionally high oral cancer mortality rates [17]. Preventing high risk behaviors such as the use of

smokeless tobacco, are critical in preventing general cancer and oral cancers. Early detection and cessation are the keys to increasing the survival rate for these cancers. Future studies are needed to moderate the carcinogenic and toxic constituents in smokeless tobacco products.

Conclusion

Continued efforts are needed to assess the public health impact and dangers associated with the long-term use of smokeless tobacco products. There is a continued need to evaluate cytological and morphologic alterations of oral mucosal tissues. There are numerous epithelial layer cells and oral mucosal membranes that are altered by chemicals and constituents in smokeless tobacco, additionally there are significant health risks to major organ systems within the human body. The breakdown of many cellular systems may concurrently result in a complex process of smokeless tobacco-induced carcinogenesis. The oral mucosa may undergo significant destruction in the later phases of carcinogenesis and loose its function as a protective barrier. The body of evidence would be strengthened by future studies that focus on tissue, cellular and molecular components of smokeless tobacco-induced lesions and the association of carcinogen and constituent exposure levels. The abnormal and altered pattern of eosinophils, neutrophils, binucleated buccal cells, lymphocytes, plasma cells, mitochondria and endoplasmic reticulum, and many other structures in the presence of numerous chemically induced, inflammatory, and cellular changes at the anatomic site of product placement should be further investigated. Longitudinal studies are also needed to validate the significance of frequent and long-term use as well as exposure level of the new smokeless tobacco products on the market. Morphologic and cytologic oral mucosal alterations and smokeless tobacco-related diseases which occur in response to chemicals such as N-nitrosonornicotine (NNN), can be minimized through future studies that better define early changes in the process of carcinogenesis.

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