

# **Risk factors for oral cancer, a retrospective study among an Indian population.**

**Degree project thesis in Medicine**

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## ABSTRACT

### **Degree project, Programme in Medicine**

*Risk factors for oral cancer, a retrospective study among an Indian population.*

Lars Wennerström, University of Gothenburg, Sweden 2016.

### **Introduction**

Oral cancer or oral squamous cell carcinoma (OSCC) is among the most common types of cancer in India and strongly linked to habits like smoking- and smokeless tobacco, alcohol and Areca nut chewing. In the western world an increasing number of HPV-positive OSCCs are diagnosed and differences in the expression of the immune system indicates that the pathogenesis of HPV-positive and HPV-negative OSCC differs.

### **Aims**

To evaluate risk factors that increase the risk for malignancy in the oral cavity.

### **Methods**

Patients were recruited from Kannur Dental College and asked to answer a question form about risk habits and medical history. The patients were then examined by a dentist and if appropriate, tissue samples were collected for later analysing.

### **Results**

19 patients were included in the study. Data analysing only showed significance for smokeless tobacco in combination with areca nut (P-value=0.021) as a habit that increases the odds (OR= 18.667) for presenting an OSCC. No tissue swabs were analysed during the timeframe for the thesis

## **Conclusions**

The results for smokeless tobacco and Areca nuts highlight the need for a stricter policy (or stricter implementation of the existing policy) to reduce the numbers of users. The negative results for the other habits is most likely due to the small number of patients included in the study.

## **Key words**

*Oral cancer, oral squamous cell carcinoma, OSCC, India*

## INTRODUCTION

Worldwide, 14.1 million new cases of cancer were diagnosed in 2012 and 8.2 million people died because of cancer. The most common cancers are lung (1.82 million), breast (1.67 million) and colorectal (1.36 million). The cancer form causing most deaths are lung (1.6 million deaths), liver (745,000 deaths), and stomach (723,000 deaths) (1).

### Oral cancer

Oral cancers ranks as the 15<sup>th</sup> most common cancer worldwide, with an incidence in 2012 around 300.000 new cases, i.e., 2.1% of the total new cancer cases in the world and with two thirds of cases occurring in men. Each year 145.000 people (1.8% of the total cancer deaths in the world) die from oral cancer (2). Oral cancer is more common in the countries of the Pacific and South Asia than in Europe and North America (3). The majority (95%) of oral cancers that are diagnosed today in the world is oral squamous cell carcinoma (OSCC) (1, 2).

OSCC is often, but not always, preceded by a pre-malignant lesion, this is more often the case with OSCCs that are caused by habits such as alcohol and tobacco.

Leukoplakia, the most common of the pre-malignant lesions, presents itself like a white plaque in the oral epithelium.

Studies have shown that when biopsied, 20% of the leukoplakia contain epithelial dysplasia, and when positioned in the

floor of the mouth or the tongue, the ratio was over 40% (4).



Figure 1: Leukoplakia in the roof of the mouth.

OSCC caused by habits such as tobacco and alcohol use is usually found in the lateral and dorsal sides of the tongue and in the floor of the mouth (4), while human papilloma virus (HPV)-associated OSCC occurs more frequently in the tonsils and in the base of the tongue (5, 6). OSCC is typically presented as a red, white or speckled lesion, often with a central ulceration if the OSCC is of a more advanced stage (4).



*Figure 2: OSCC on the right lateral side of the Tongue.*

In India 70.000 new cases are diagnosed and 48.000 people die of OSCC each year (7-9), being the second most common cancer among men and the fourth among women. It is, however, estimated that even more patients are affected by OSCC since there is no compulsory registration of oral cancer in India and a lot of people have limited access to healthcare (8).

#### Risk factors

Smoking, tobacco chewing and high alcohol consumption are all major risk factors for OSCC (8, 10-13) and the chewing of Areca nut has been strongly linked to OSCC in countries where this habit is common, for example India (8, 12, 14). Viral infections, especially those caused by HPV type 16, also belong to the risk factors (8, 11-13) and HPV-associated OSCC are

associated with oral sex practice and multiple sexual partners (6, 14-16). It is likely that the pathogenesis of HPV-associated OSCC differs from OSCC caused by tobacco and alcohol use. First, HPV-positive OSCCs usually affect a younger population. Secondly, habits such as high alcohol consumption, tobacco and chewing of betel nut are less common among patient with HPV-positive OSCC (13, 16). Thirdly, the prognosis for patients with HPV-positive cancers is better than for non-HPV associated OSCC (16, 17).

Studies in India show that the risk for OSCC increases five times when chewing tobacco among non-smokers and that as much as 50% of all OSCC are caused by smokeless tobacco products (18). In a 24 month study, 35,112 individuals from Sangli in Western Maharashtra (a semi-urban district in India) were screened for OSCC and habits of smoking, chewing tobacco and drinking alcohol. 112 cases of oral cancer were found giving it a prevalence of 0.3% among the study population. Subgroup analyses of individuals with habits of smoking, tobacco chewing and/or drinking alcohol showed a prevalence of oral cancer of 1.12% (19).

Heredity and genetic predisposition are thought to constitute minor risk factors for the development of OSCC. Instead factors such as chronic inflammation, DNA-damage, neo-angiogenesis and alterations in the host immunity are considered as major contributors to the development of OSCC (8).

## Tobacco

There are about 70 different species of the tobacco plant (*Nicotiana*), but few of them are used in tobacco products. Commercial tobacco products usually contain the *Nicotiana tabacum* or *N. rustica* which is common in South America, Africa and Asia, where it is used in smokeless tobacco products. *N. rustica* contains more nicotine and alkaloids than *N. tabacum* (20-22). According to the World Health Organization (WHO) we have approximately 1.1 billion smokers in the world, which gives smoking a prevalence of around 22%. Smoking is more common among men (37%) than women (7%). There are several ways of smoking tobacco, although 90% of smokers use the manufactured cigarette (10). Tobacco has a considerable impact on global health and constitutes the most important preventable risk factor for an early death. It is estimated that 6 million people die because of tobacco use and another 600.000 from second-hand smoking (10).

The prevalence of smoking has decreased from 41% in 1980 to 31% in 2012 for men and from 11% to 6% for women. The actual number of smokers though, has increased from 721 million to 967 million during the same period due to the increase in the worldwide population (23).

The data available on the consumption of smokeless tobacco makes it difficult to correctly describe its global use to this day (10). In 2010, in a survey that represented about 70% of the world population, it was estimated that over 300 million people were using smokeless tobacco (19), a number being reinforced by WHO, which also states that 90% of the smokeless tobacco users lived in the South-East Asia region (24) and in some of these countries, the use of smokeless tobacco even exceeds that of smoked tobacco (10).



## Tobacco in India

In 2010, 35% of the Indian population used tobacco of some form; smokeless tobacco (60%), smoking tobacco (25%) and both methods (15%). Tobacco consumption is more common in rural (38%) versus urban areas (25%). The habit is more widespread among men where 48% is using tobacco compared to 20% in women. Education is another factor where 44% of the population with no formal education was using tobacco compared to 21% of those with secondary or higher education. The young population aged 15-24 years showed the lowest consumption of tobacco (18%), while people 65 of age and older had the highest percentage smokers (48%). The tobacco industry is very large in India and employs millions of people (25).

For comparison; in 2014 in Sweden, 22% of the Swedish population was using tobacco on a daily basis, 48% used smoking tobacco, 48% smokeless and 2% was using both (26).

In India, smoking tobacco is much more common among men (24%) than women (3%) (25, 27). There are significant differences between states in India with a smoking prevalence ranging from 24-62% among men and 1-25% among women (28). Overall, the most popular smoking product was the Bidi (60%) followed by the manufactured cigarette (40%) and hookah (less than 1%) (25).

In 2010 the numbers of smokeless tobacco users in India were over 220 million, or 26% of the population (9, 20, 25). The most popular product of smokeless tobacco in India is Khaini (46%), followed by Guthka (31%) and the betel quid (23%) (25). In contrast to smoking tobacco, the consumption of smokeless tobacco is more equal among men and women (20% and 17%, respectively). In rural areas, 29% are using smokeless tobacco compared to 18% in urban areas (20, 25). As with smoking tobacco, the percentage of people using smokeless tobacco varies between states in India with prevalence varying between 12-68% among men and 2-64% among women (28).

### Smoking tobacco

The most common way to smoke tobacco in India is the cigarette (22), although the *Bidi* is also very popular. The *Bidi* is a thin, sometimes slightly conical cylinder made out of the leaf of the *temburni* or *tendu* plants. The cylinder is filled with tobacco and is quite similar to a cigarette. It is hand-rolled and secured in one or both ends with a string (22, 29, 30). The water pipe, or *Hookah* in India, is gaining popularity again after a decline, especially among young people. In many Western countries, it is only the cigarette that is more common, and in some Middle Eastern countries the *Hookah* is the primary way to use smoking tobacco (30-32).

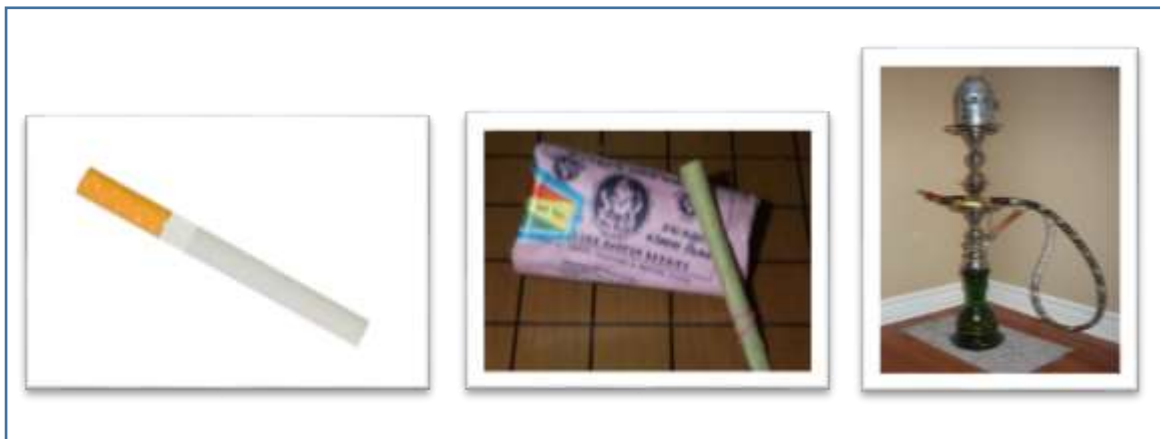


Figure 3: From left to right: Manufactured cigarette, Bidi and Hookah

### Smokeless tobacco

There are a multitude of variations of smokeless tobacco in the world with different countries and areas having their own types. Smokeless tobacco is usually held in the mouth and chewed or sucked upon, but can also be taken nasally. Common variations in India are *Khaini*, *Guthka* and *Betel Quid*. The contents of these are tobacco in some form and slaked lime to raise the pH. Areca nut is usually added as is spices, flavorings and sweeteners and in the case of *Betel Quid*, all the contents are wrapped up in a betel leaf (hence the name) making a small sachet that is then placed in the mouth. Products like *Khaini* and *Guthka* are made both commercially or by a large cottage industry, while the *Betel Quid* is always made on demand

by the provider or user. There are lots of laws in India meant to regulate and even outlawing some of the versions of smokeless tobacco, but these laws are rarely enforced to the point that it makes any difference (20).



Figure 4: Left; Betel Quid or Paan, right; Guthka.

#### Tobacco and cancer

There are about 4000 different chemical components in tobacco that are considered harmful to various degree. For the development of cancer, the tobacco-specific nitrosamines (TSNAs), are considered the most potent due to the high concentration of them in tobacco. International Agency for Research on Cancer (IARC) has classified two of the TSNAs as IARC group 1 carcinogens, i.e., N<sup>2</sup>-nitrosornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) which are believed to be major contributors in the development of oral cancer (20).

During growth, the tobacco plant will synthesize alkaloids, for example nicotine, from compounds absorbed by the soil. The alkaloids are the most important precursors of TSNAs. Nitrate (NO<sub>3</sub><sup>-</sup>) in the tobacco and microorganisms, bacteria and fungi, on the leaves will facilitate the conversion of alkaloids to TSNAs. During the manufacturing process the microorganisms will proliferate and convert the nitrate to nitrite (NO<sub>2</sub><sup>-</sup>) which in turn will

react with the alkaloids in the tobacco, generating TSNAs. The amount of microorganisms on the tobacco leaves during the manufacturing process is the most important factor for the final content of TSNAs. This has been proved by using different manufacturing methods, such as pasteurization, which reduce the number of bacteria and fungi on the tobacco. This will in turn reduce the transformation of nitrate to nitrite, resulting in lower concentrations of TSNAs in the end product (20).

Besides inducing addiction to tobacco, nicotine is an alkaloid and a precursor to TSNAs, and as such, one of the major contributors to the levels of NNN and NNK in the finished product. Nicotine exists in bound (ionized and mono-protonated or di-protonated) and free (un-ionized or un-protonated) form in tobacco. The total nicotine is normally declared on the tobacco product, i.e., both the bound and free nicotine are declared. It is the free, uncharged, form that is the most active since it can pass through cell membranes more easily. The amount of free nicotine in a tobacco product is determined by the pH, i.e., the higher the pH, the higher the amount of free nicotine (20).

## Alcohol

On average, every adult in the world consumed 6.2 liters of pure alcohol in 2010. The highest consumption is found in middle- to high-income countries in Europe and North America. It should also be taken into consideration that 48% of the global population has never consumed alcohol, so the consumption will vary considerable between regions and countries.

Worldwide, 3.3 million deaths, or 5.9% of all deaths, were related to the alcohol consumption in 2012 (10, 33).

According to a WHO global report on alcohol the average consumption for an Indian adult was 4.2 liters of pure alcohol per year during the years of 2008-2010. But in India 74% of the population is lifetime abstainers, (59% of men and 90% of women) and 13% (16% of men and 5% of women) are former drinkers (people that have not consumed alcohol the last 12

months). Taking this into the equation the actual alcohol consumption among people that drink is much higher, i.e., 28.7 liters of pure alcohol per year and (drinking) person. In comparison, a Swedish adult consumes 13.3 liters of pure alcohol per year when adjusted for abstainers. The type of alcohol consumed in India is dominated by spirits (93%), followed by beer (7%) and wine (<1%) (33), again, as comparison when translated to pure alcohol, in Sweden wine stands for the bulk of the consumption (42%), followed by beer with an alcohol content over 3.5% (30%), liquor (22%), beer with an alcohol content up to 3.5% (5%) and cider and premade alcoholic drinks (1%) (34).

### Areca nut

Areca nuts come from the Areca palm (*Areca catechu*) which grows in South-east Asia and Eastern Africa. It is used as an ingredient in smokeless tobacco products as well as on its own. The nut can be used as it is, without any preparation or dried, roasted or baked. Before consumption the nut will usually be cut into small slices or cubes or crushed (20, 35, 36).

Worldwide, Areca nut is one of the four most commonly used psychoactive substances with 600+ million users (3, 36-38), the other three are caffeine, nicotine and alcohol (36, 38, 39). Most of the users come from East Asia, South-East Asia and Africa. Immigrants from these regions have also brought the habit to their new countries. For example, Great Britain is the largest importer of *Gutkha* outside Asia and in the USA, both *Betel Quid* and *Gutkha* is legal and the consumption there is increasing (36). The top consumer in the world is India (36), and the prevalence there ranges from 3% to 29% depending on which state you are looking at (28).

### Areca nut and cancer

The Areca nut is an IARC group 1 carcinogen. It contains the compounds guvacoline and arecoline which when reacting with nitrate or during chewing form areca-specific nitrosamine compounds (ASNAs) such as N-nitrosoguvacoline (NGL) and 3-(N-nitrosomethylamino)

propionaldehyde. The latter of these are proved highly genotoxic as well as cytotoxic to buccal mucosal epithelial cells in humans, partly explaining why Areca nut users are overrepresented among OSCC patients in countries where this habit is common (20, 37, 40). When combined with other ingredients the carcinogenic properties can be increased further. For example, slaked lime will, when combined with areca nut, form reactive oxygen species (ROS) which will lead to oxidative damage to DNA (37).

### Human papillomavirus

There are more than 150 different sequenced phenotypes of the Human papillomavirus (HPV) (41).

#### Structure and genome

The HPV is a 52-55nm non-enveloped virus. Its genome contains double-stranded DNA with approximately 8000 base pairs. The genome encodes for about 8 open reading frames divided in three parts, early, middle and late expression genes. Proteins needed for replication, viral transcription and cellular transformation are expressed early, and hence the name “E #” for these proteins. Proteins needed later in the virus cycle (denoted L#) are those for assembly of the virus, e.g., the capsid proteins L1 and L2 (13). The cell cycle progression is stimulated by the proteins E6 and E7 and differences in the expression of these proteins decide if a HPV is a high- or low risk oncogenic phenotype of HPV. High risk HPV includes HPV-16, 18, 31, 33, 35, 45, 51, 52, 56, 58 and 59 (13, 41).

#### Transmission

The favored sites for the HPV to infect are the squamous epithelial cells and usually the basal cells of the tonsils, oropharynx, cervix, vagina, vulva, penis, perianal area and anal canal (13, 42). In the case of oral transmission there are multiple ways of infection such as autoinfection, open mouth kissing and sexual transmission. Rare cases of perinatal transmission of HPV have been observed as well (13).

### Reproduction and malignancy

After gaining entry to the cell, to maximize viral reproduction the HPV must increase the proliferation rate of the infected cell. Initially only a few copies are made of each virus and these viruses are forming the base for the latent part of the infection. In the productive phase of the infection viral proteins are produced at a much higher rate, regulated by the E1 and E2 proteins. In the case of high risk (HR)-HPV infection, E6 and E7 are also expressed with E6 preventing apoptosis and E7 making the cell DNA replication cycle available for viral DNA production by enabling the cell to re-enter the S-phase of the cell cycle. This means that the epithelium will start to proliferate thereby creating an increasing number of HPV infected cells that may result in an intraepithelial or invasive neoplasm. By the help of the E1 and E2 proteins, the virus eventually leaves the infected cells and can infect new ones (13).

When causing a malignancy, the viral genome breaks during integration at the site for the E1 and E2 proteins, resulting in a loss of control of the E6 and E7 proteins, which in turn will be directly involved in the cell cycle by removing the influence of p53 and pRb (10, 43). The E6 protein binds to p53, inhibiting the cells ability to go into apoptosis. E7 interacts with pRb, removing its ability to prevent the cell cycle moving from the G1 phase to the S phase, promoting cell proliferation. In conclusion, by working together, the E6 and E7 proteins can induce uncontrolled cell growth, while preventing normal apoptosis of cells (2, 10, 42, 51). As a result of this, mutations of the p53 gene are rare among HPV positive tumors when compared to the HPV negative ones, indicating that it is the direct influence of the HPV that causes HPV positive malignancies (10).

HPV infections are responsible for about 5% of all cancers worldwide, which translates to over 600.000 new cancer cases each year. HPV has more impact on less developed countries, where it is responsible for 7% of the new cancer cases compared to 2% for the developed countries. The majority of these HPV-related cases is cervical cancer (85%) but the incidence of other cancers such as OSCC is increasing worldwide (43).

### HPV and oral cancer

In contrast to cervical cancer that may be caused by multiple HPVs, HPV-16 dominates in HPV-associated OSCC (6, 13, 14, 43). As mentioned earlier, HPV-associated OSCC occurs most frequently in the tonsils and in the base of the tongue (5, 6, 14, 44). However, dormant infections of HPV in the tonsils are uncommon in patients undergoing tonsillectomy for benign causes (5, 44). It is at present not understood why HPV-positive tonsillar cancers are common but dormant infections of the tonsils are uncommon. One reason could be that dormant infections are difficult to detect in the tonsils or that tonsils are rarely infected by HPV but when HPV infection occurs, cancer is easily developed (43).

### The immune system and oral cancer

Toll-like receptors (TLRs) and nod-like receptors (NLRs) belong to pattern recognition receptors (PRRs) and play crucial roles for the innate immune response. PRRs are expressed on immune cells and different tissues including the oral mucosa and are important for recognition of antigens of pathogens and molecules of damaged cells. TLRs are known to be activated by “damage-associated molecular patterns” (DAMPs) that are released from stressed, dying or dead cells. Many clinical trials are now focusing on how to boost the immunological response in order to eradicate tumors by targeting TLRs (45). NLRs recognize molecular patterns of pathogens after e.g. tissue damage (46).

The innate immune system seems to play an important role for the development of OSCC. TLRs are expressed in OSCC (47, 48), and, studies show that higher expression of TLR5 in oral tongue squamous cell carcinoma is associated with increased recurrence rate (49). TLR5 is activated by bacterial flagellin and TLR5 has therefore been suggested to be the link between bacterial infection and the development of carcinogenesis (50). Animal models suggest that HPV vaccines targeting TLRs besides HPV may be beneficial in the therapy of already established HPV-associated cancers (51).



## Hypothesis of the Master thesis

Smoking, chewing tobacco and/or Areca nut and overuse of alcohol increase the risk of developing OSCC.

## AIMS

- To evaluate risk factors that increase the risk for malignancy in the oral cavity.

## ETHICS

- The Helsinki declaration regarding ethical principles for medical research involving human subjects was taken in consideration when conducting this study.
- The study was approved by the Kannur medical college hospital ethical committee.

## METHODS

The original design of the study were to include two groups of patients:

1. A study group recruited from the patients receiving care at the Malabar Cancer Centre in Kannur, a hospital treating oncology patients exclusively. The patients from this hospital had a present use or history of habits that are considered risk factors for OSCC, i.e. alcohol and/or tobacco and/or areca nut consumption and one or more clinical and/or histopathological verified malignant lesion/lesions in the oral cavity.
2. A control group recruited from patients that were seeking care at the dental clinic at the Medical and Dental Hospital of Kannur, the dental clinic treated patients with a wide variety of diagnoses. As for the study group, the inclusion criteria were that the recruited patient to the study had a present use or history of habits that are considered risk factors for OSCC, but in the case of the control group, there was no demand for a verified malignant lesion in the oral cavity.

The data collected from the study- and control group would then be matched against each other, using IBM SPSS Statistics version 22.

Unfortunately, due to unforeseen circumstances (a missing memorandum of understanding), the project plan could not be forwarded to the ethical committee at the Malabar Cancer Centre and we were therefore unable to include patients from that hospital in the study.

Therefore, the patients recruited from the dental clinic at the Medical and Dental Hospital of Kannur was instead divided into two groups, one with one or more premalignant or malignant lesions in the oral cavity, and one with non-malignant or no lesions in the oral cavity. Once included, there were no exclusion criteria unless the patient decided to end his or hers participation in the study. The data collected would then be matched against each other, using IBM SPSS Statistics version 22.

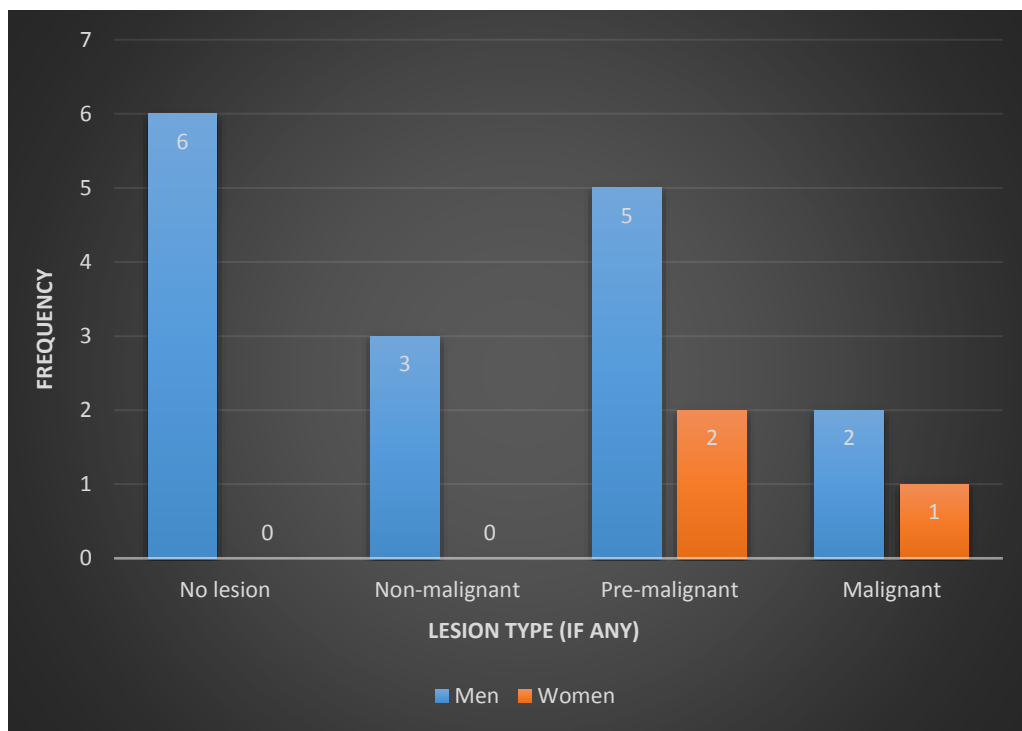
Patients included in the study were given information about the study before signing an informed consent form (appendix A) and filling out a questionnaire (appendix B) for obtaining anthropometric data about tobacco-, alcohol-, areca nut habits and a short medical history. A witness was present to translate the content of the informed consent and health information to Malayalam. After the questionnaire the patients underwent an oral examination by a dentist at the department of oral medicine. Any findings of lesions were registered in the health information form.

Tissue swabs were taken for later HPV analysis. If a malignant lesion was present, the samples were collected from both the tumor (HPV+ and HPV-) and from the healthy contralateral side in the mouth. Patients without malignant lesions underwent swabbing of the tonsillar area, the basal part of the tongue, the buccal mucosa, the hard palate, the side of the tongue and the lip. The equipment used to take the samples were “Isohelix™ SK1 DNA Buccal Swabs (Cell Projects Ltd. Harrietshan, Kent, UK). The samples were then marked with a unique identification code and stored in a freezer. The samples will later be analyzed with RT-PCR for a panel of inflammatory markers within the innate immune response.

## RESULTS

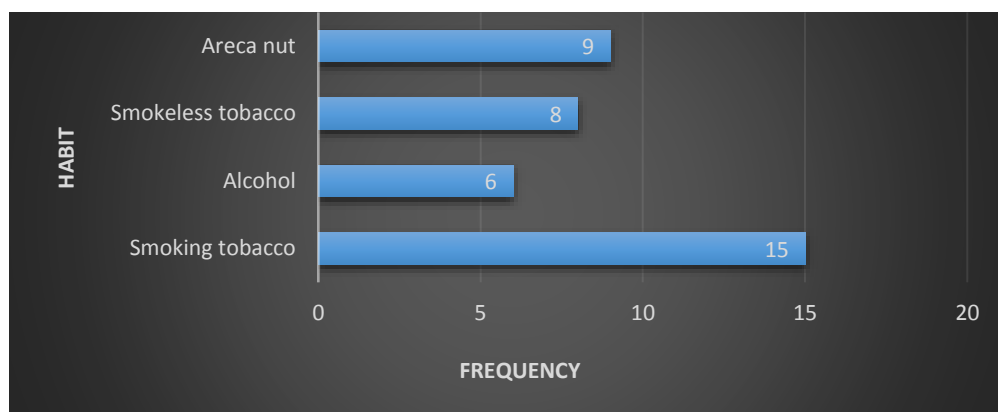
In the period 2016-02-25 to 2016-04-05 19 patients were identified as viable for the study, i.e., presented one or more of the risk factors for OSCC, and asked to participate. 18 of the patients were seeking dental care at the dental clinic at Kannur dental college and 1 patient was recruited during a free dental camp in the town of Kannur. The age of the patients ranged from 19 to 87 years with a mean value of 53.2 years. Of the 19 patients, 16 were men and 3 women. 7 (36.8%) of these patients presented no lesions at all (having sought dental help for other reasons, for example, adjusting dentures and damaged teeth from working accidents), 3 (15.8%) presented non-malignant lesions, 6 (31.6%) pre-malignant lesions and 3 (15.8%) presented histopathological verified malignant lesions. The men showed an equal distribution among the no-/non-malignant and the pre-malignant/malignant lesions while all of the women had pre-malignant or malignant lesions.

*Chart 1: Different lesions in men and women*



Of the 19 patients, 15 (78.9%) were using smoking tobacco, 6 (31.6%) were drinking alcohol, 8 (42.1%) were using smokeless tobacco and 9 (47.4%) were using Areca nut.

*Chart 2: Habits shown by test subjects.*



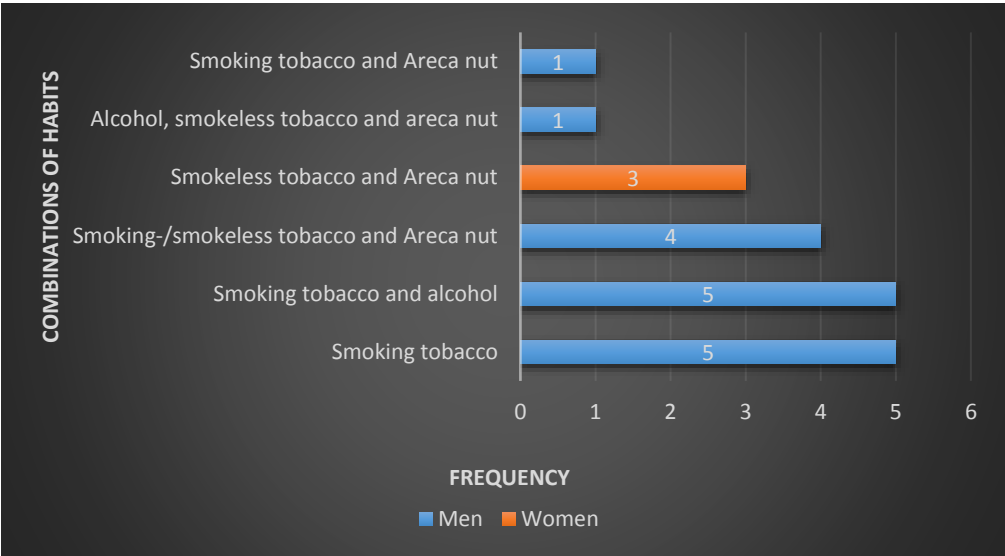
When looking for trends according to age the patients was divided in two groups; under and over mean age (53.2years). Except for the finding that drinking alcohol is twice as common in the older group with 4 patients versus the 2 patients in the younger group, no specific trends could be found this way as shown in table 1.

*Table 1: Habits within age groups.*

Habit	Under mean age		Over mean age	
	Yes	No	Yes	No
Smokeless (8)	4	5	4	6
Smoking (15)	8	1	7	3
Alcohol (6)	2	7	4	6
Areca nut (9)	5	4	4	6

Analysing combinations of habits showed that 5 patients (26.3%, all men) were using smoking tobacco only, 5 (26.3%, all men) was using both smoking tobacco and alcohol, 4 (21.1%, all men) were using both smoking and smokeless tobacco and chewing areca nut, 3 (15.8%, all women) were using smokeless tobacco and chewing areca nut, 1 (5.3%, all men) was using alcohol,smokeless tobacco and chewing Areca nut and 1 (5.3%, all men) was using smoking tobacco and chewing Areca nut.

Chart 3: Combination of habits shown by patients (total).



The most common combination of habits was to use smokeless tobacco together with Areca nuts and vice versa (8 out of 8). Alcohol on the other hand was rarely used in combination with smokeless tobacco or Areca nut (1 out of 6).

Table 2: Common and uncommon combinations of habits shown by patients (individual).

Habit	Smokeless (8)		Smoking (15)		Alcohol (6)		Areca Nut (9)	
	Yes	No	Yes	No	Yes	No	Yes	No
Smokeless (8)	X	X	5 (62.5%)	3 (37.5%)	1 (12.5%)	7 (87.5%)	8 (100.0%)	0 (0.00%)
Smoking (15)	5 (33.3%)	10 (66.7%)	X	X	5 (33.3%)	10 (66.7%)	6 (40.0%)	9 (60.0%)
Alcohol (6)	1 (16.7%)	5 (83.3%)	5 (83.3%)	1 (16.7%)	X	X	1 (16.7%)	5 (83.3%)
Areca Nut (9)	8 (88.9%)	1 (11.1%)	6 (66.7%)	3 (33.3%)	1 (11.1%)	8 (88.9%)	X	X

The variables tested for statistical significance to increase the odds for the patient to present a pre- or malignant (OSCC) lesion was; age, gender, smoking tobacco, alcohol consumption, chewing smokeless tobacco and chewing areca nut. When tested alone, chewing smokeless tobacco and Areca nuts showed statistical significance while age, gender, smoking tobacco and alcohol consumption did not. But, as stated earlier, *all* of the patients chewing smokeless tobacco also chewed Areca nuts, which means the results for smokeless tobacco and Areca nut cannot be interpreted on their own.

*Table 3: Statistical analysis of habits.*

Habit	P-value	Odds ratio
Age	0.080	1.051
Gender	0.999	0.000
Smoking tobacco	0.999	0.000
Alcohol	0.262	0.313
Smokeless tobacco	<b>0.021</b>	18.667
Areca nut	<b>0.047</b>	8.167
Smokeless tobacco and Areca nut (in combination)	<b>0.021</b>	18.667

## DISCUSSION

The aim of the study was to evaluate the risk factors for developing an oral malignancy in an Indian population.

It should be noted from the start that it is difficult to interpret the results for significance and odds ratios because of the small number of test subjects. Despite this, it was decided to include them since at least some of them showed similarities to prior research, and for those who did not, it was interesting to speculate why.

Our findings show that the consumption of smokeless tobacco and Areca nuts increase the odds for presenting a pre-malignant or malignant lesion in the oral cavity. This is consistent with, and reinforce, the results from multiple prior studies (8, 10-13). But since all the smokeless tobacco users also used areca nuts it was not possible to say anything about the individual results for these factors and they had to be analyzed together. The reason for this is that the most popular smokeless tobacco products in India also contain areca nut and it is rare to chew areca nut on its own, and therefore smokeless tobacco and areca nut are usually used together. The odds ratios presented was very high, especially for the combination of smokeless tobacco and areca nut (or smokeless tobacco on its own) but this phenomena be attributed to the low amount of patients included in the study. It may also be because of an additive or synergistic effect of using smokeless tobacco in combination with Areca nut as suggested by Garg et. al. (37),but larger studies are needed to confirm this.

Age seemed to be a rather low risk factor in this study. This is most likely dependent on the small number of patients included in the study when a single younger patient presenting a pre-malignant or malignant lesions will heavily affect the odds ratio of age. Previous data from other studies, among others by Gupta et. al. (8), show that the typical patient with OSCC in India is of higher age since the malignancy is usually caused by habits and is not HPV-associated as in western countries where OSCC is affecting a younger population .With a



larger study sample, age would most likely show significance and a higher odds ratio. Since there was little difference in specific habits when different age groups was put against each other we can draw no conclusions that age would have any impact on the probability for an individual to show certain habits, indicating that the use smokeless and smoking tobacco, alcohol and Areca nut are evenly spread in our study population.

Despite alcohol consumption as a variable not being significant, its protective ability shown in this study should be addressed. This is likely due to that alcohol consumers are less prone to chew smokeless tobacco and Areca nuts, the two factors that were over-represented among the patients with pre-malignant and malignant lesions and as prior Indian studies (8, 10-13) has shown, greatly increase the risk for OSCC. That smoking and gender did not show significance or any odds ratio at all is most likely again attributed to the small study sample size.

The fact that all the women all presented premalignant or malignant lesions is most likely explained by all of them also combining chewing tobacco and Areca nut, the main risk factors in India for not only oral cancer, but also for pre-malignant lesions like Leukoplakia as explained by Kademani (4).

Although, one can speculate that since smoking is a known risk factor for, and men are twice as likely to be affected by, OSCC, in this study where only patients with habits were included, smoking, gender and alcohol (that has indicated a protective ability in this study) are the “lighter” habits while chewing smokeless tobacco and Areca nuts and maybe age are the “severe” habits and more prone to result in a patient presenting a pre-malignant or malignant lesion. But to prove this, a study with a significantly higher number of test subjects and with a control group without habits of risk factors must be conducted.

## Conclusion and implications

India as a country stand before a challenge to reduce the usage of products that are known to cause OSCC and the laws already in place needs to be enforced more firmly.

Part of the problem though, is that the tobacco and Areca nut industry is very large and employs a lot of people, usually with a lower socioeconomic status. New jobs need to be created for these people, making it a political “hot potato” that few wants to handle. That some of the smokeless tobacco/Areca nut products are deeply rooted in Indian culture, like the Betel Quid, does not make it any easier.

## Weaknesses

The obvious weakness that has already been addressed is the small size of the study population since this means that single patients will have an unproportioned effect on the statistical outcome. The reason for this was partly the fact that we could not recruit any patients from the Malabar Cancer Center as planned in the original study design. Hopefully, following projects will be able to include patients from this hospital since this design will supply a study with a large study group when researching patients with malignancies. The other part is most likely, that when it comes to studies about lifestyle factors that are associated with stigma of some sort, such as tobacco, alcohol, and for most people in India today, Areca nut chewing there will be a risk of underreporting or not admitting use at all. This is especially true if the patient in question are seeking help for some problem that are obviously not related to habits, like in this study, where some of the patients was seeking help for work-related injuries to the teeth or needed adjustment of their dentures. Because of this, it is likely that the actual number of patients, with one or more of the risk habits, seeking care at the dental clinic was higher than the ones actually recruited. How to address this problem is hard to say since it has to do with social stigma and the fear from the patients that their level of care will be affected if admitting to these habits.

Since the question form was in English it needed to be translated to Malayalam, this was not done by a professional interpreter which means that all information in the form may not always be presented to the patient. It should be noted that the translation was always done by a professional dentist with good English and native Malayalam language skills.

If repeated, the original study design would probably give a good view of the respective danger of the risk factors for developing oral cancer, if given access to the Malabar Cancer Centre patient pool this would most likely provide a large enough study group. Another way to recruit a large enough control group with the difficulties encountered in this study might be to try to recruit them from patients that are seeking help to quit their habits as classes are being held for this reason, but unfortunately we were not able to try this strategy during the time this study was conducted.

## Populärvetenskaplig sammanfattning

**Munhålecancer**, en studie om riskfaktorer i Indien.

Munhålecancer är en mycket vanlig form av cancer i Indien, den nästa vanligaste för män och den fjärde vanligaste för kvinnor, som jämförelse så kommer den på 15:e plats bland alla cancerformer. I Indien är munhålecancer starkt förknippad med livsstilsvanor som rökning, hög alkoholkonsumtion, att tugga tobak och Areca nötter. Att tugga tobak och Areca nötter är vanligt i Indien och resten av Sydostasien och är bevisat cancerframkallande. Många olika former av tuggtobak och Areca nötter är förbjudna i Indien men dessa förbud har liten effekt då de vanligtvis inte beivras. Över 200 miljoner människor i Indien använder tuggtobak dagligen och majoriteten av dessa tuggar också Areca nötter.

Den här studien gjordes för att undersöka och bekräfta sambandet mellan livsstilsvanor och munhålecancer bland Indier och samla in information om detta. Studiedeltagarna rekryterades på plats vid Kannur Medical College i delstaten Kerala i Indien. De besvarade frågor om livsstilsvanor och sjukhistoria samt undersöktes av en tandläkare. Om man hittade något i munhålan som kunde misstänkas vara cancer eller ett förstadium till cancer så togs det vävnadsprover av dessa för senare analys.

19 deltagare inkluderades i studien. När informationen från frågorna analyserades så fann man en starkt ökad chans att ha en munhålecancer eller förstadium till detta om man tuggade tobak tillsammans med Areca nötter. Resultatet stämmer bra överens med tidigare forskning i ämnet och belyser nödvändigheten av en strikta regler för tobak och Areca nötter, eller att de lagar som redan finns beivras, för att minska antalet användare.

Den här studien kunde inte bevisa ett samband mellan alkoholkonsumtion eller rökning och munhålecancer, detta var antagligen på grund av att antalet deltagare var så lågt. Studier med större antal deltagare behövs för att mer exakt bevisa riskerna.

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## APENDIX

### A – Informed consent

#### Informed consent

##### Project Information

Title of Project: *Risk factors leading to oral squamous cell carcinoma, a retrospective study among an Indian population.*

Principal Investigators: *Dr. Prathima Sreenivasan, Department of Oral Medicine, Kannur Dental College, Anjarakandy, Kerala*

Co-investigators: *Lars Wennerström, dr Daniel Giglio Department of Pharmacology, dr. Bengt Hasséus and dr. Göran Kjeller Institute of Odontology, the Sahlgrenska Academy, Gothenburg University, Sweden. Dr Rajeev Ayilyath Kutyeri, postgraduate student, Department of Oral Medicine, Kannur Dental College, Anjarakandy, Kerala*

Participant's Printed Name/Id code:

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##### Introduction

We invite you to take part in a research study at *Kannur Medical and Dental College* which seeks to investigate risk factors leading to oral squamous cell carcinoma (OSCC).

This is a master thesis project performed by a medical student from the Gothenburg University, Sweden. Taking part in this study is entirely voluntary. If you decide to participate, you must sign this form to show that you want to take part.

##### Research financing

This study is financed by SIDA\* and the Sahlgrenska Academy at Gothenburg University, Sweden.

\*SIDA (Swedish International Development Cooperation Agency) is a government agency of the Swedish Ministry of Foreign Affairs.

##### Purpose of the research

The purpose of this research study is to obtain information on risk factors leading to OSCC.

### Procedures

The participant will be asked to answer a questionnaire about general health, use of smoking/smokeless tobacco, areca nut and alcohol. A clinical examination, including inspection of oral mucosa and palpation of lymph nodes, will then be performed by a professional dentist. If needed, a non-invasive tissue sample will be taken from the buccal mucosa to collect DNA, further investigation will be carried out by professional medical staff at location.

### Discomforts and Risks

There are no risks or side-effects. There might be a slight discomfort when palpating the throat for lymph nodes and collecting the tissue sample.

### Potential Benefits

Participation will not be beneficial for the individual. Medical science may gain further understanding of risk factors leading to OSCC and how to prevent the risk of development of said OSCC.

### Statement of confidentiality

The samples collected for research purposes will be labeled with an anonymous code number that will follow the sample throughout the study. No other health information except the one given in the questionnaire by the participant will be collected or used in this study.

### Voluntary Participation

Taking part in this research study is voluntary. Participation is *not* mandatory. If you choose to take part, you have the right to stop at any time. If you decide not to participate or if you decide to stop taking part in the research at a later date, there will be no consequences.

### Questions

You have the right to ask any questions you may have about this research. Direct your questions to the principal investigators. The principal investigator will also present the information to you orally at the time of examination.

**Participant:** By signing this consent form, you indicate that you are voluntarily choosing to take part in this research.

Date: \_\_\_\_\_

Time: \_\_\_\_\_

Signature of Participant: \_\_\_\_\_

Printed Name: \_\_\_\_\_

Date: \_\_\_\_\_

Time: \_\_\_\_\_

Signature of Investigator: \_\_\_\_\_

Printed Name: \_\_\_\_\_

I confirm that the information of this form was translated to Malayalam and conveyed to the participant:

Date: \_\_\_\_\_

Time: \_\_\_\_\_

Signature of Witness: \_\_\_\_\_

Printed Name: \_\_\_\_\_

## B – Question form

### Health information

Gender: Female  Male  Year of birth: \_\_\_\_\_ Id code: \_\_\_\_\_

#### General health

Do You have or have had any of the following:

- Heart disease
- High blood pressure
- Blood infection: \_\_\_\_\_
- Diabetes
- Sexually transmitted disease/Venereal disease: \_\_\_\_\_
- Tumor disease: \_\_\_\_\_
- Lung disease: \_\_\_\_\_
- Radiotherapy in head & neck region
- Other diseases: \_\_\_\_\_

#### Drinking habits

Do You drink alcohol? Yes  No

How often do You drink alcohol?

- Every day
- 3-6 times a week
- 1-2 times a week
- Twice a month or more
- Once a month or less

When you drink beer, how much, on the average, do you usually drink at any one time?

- 6 or more bottles or tavern glasses
- 4 or 5 bottles of beer or tavern glasses
- 2 or 3 bottles of beer or tavern glasses
- 1 or less bottle of beer or tavern glass

When you drink  wine or  toddy, how much, on the average, do you usually drink at any one time?

- 6 or more glasses
- 4 or 5 glasses
- 2 or 3 glasses
- 1 or less glass

When you drink liquor, how much, on the average, do you usually drink at any one time?

- 6 or more drinks
- 4 or 5 drinks
- 2 or 3 drinks
- 1 or less drink

**Tobacco use**

Do You use or have previously used any type of tobacco? Yes  No

If Yes, which type/types of tobacco do/did You use?

Smoking:

Cigarettes

Bidi

Hookah

Other: \_\_\_\_\_

Smokeless tobacco

Betel quid (pan/paan)

Khaini

Gutkha

Mishri

Gul

Gudakhu

Other: \_\_\_\_\_

**Areca nut use**

Do You use or have used any product containing areca nut?

Yes  No

If you use or have used tobacco or areca nut, please answer the following:

How often do/did You use (times/number per day or week)

Tobacco: \_\_\_\_\_

Areca nut: \_\_\_\_\_

When did You start using (age)

Tobacco: \_\_\_\_\_

Areca nut: \_\_\_\_\_

Have You in the past year considered quit using tobacco or areca nut?

Tobacco: Yes  No

Areca nut: Yes  No

If You *stopped* using tobacco more than 6 months ago

– how long since You stopped? \_\_\_\_\_

If You *stopped* using areca nut more than 6 months ago

– how long since You stopped? \_\_\_\_\_

**Mucosal lesion – status**

Oral mucosal lesion site:

Lesion site	Lesion size in mm	Reaction pattern	Occlusal trauma? Yes / No

Comment:

**Diagnosis**

Provisional clinical diagnosis?

Histopathological diagnosis?

Definitive clinical diagnosis?


Secondary diagnosis?

Comment



## C - Poster

# Risk factors for oral cancer, a retrospective study among an Indian population.




UNIVERSITY OF  
GOTHENBURG


Master thesis, Programme in Medicine, University of Gothenburg: Lars Wennerström  
Supervisors: Daniel Giglio, MD, PhD and Dr.Prathima Sreenivasan, Professor.

### Background

In India, Oral Squamous Cell Carcinoma (OSCC) is the second most common cancer among men and the fourth among women with 70,000 new cases diagnosed and 48,000 deaths each year. Smokeless tobacco and areca nut chewing, smoking tobacco and alcohol consumption are the main risk factors for OSCC in India.



Betel Quid or Paan: a combination of chewing tobacco (bar right) and Areca nut (top left) wrapped in Betel leaves (bottom left).



OSCC at the right lateral side of the tongue.

### Aim

To evaluate risk factors for OSCC in an Indian population.

### Method

19 patients, 3 women and 16 men, were recruited from Kannur Dental College and the city of Kannur in India. The patients included answered a question form regarding risk factors as well as general health information. The data obtained was analyzed in SPSS statistics version 22.

### Results

The factors that showed statistical significance to increase the odds of presenting an OSCC or a pre-malignant lesion among the patients were chewing *smokeless tobacco* and chewing *Areca nut*. *Smoking tobacco* or *alcohol* consumption did not show significance to increase the odds for OSCC or a pre-malignant lesion. Statistical results are presented in the table below.

Habit	P-value	Odds ratio
Smokeless tobacco	0.021	18.667
Areca nut	0.047	8.167
Smoking tobacco	0.999	0.000
Alcohol	0.262	0.313

### Discussion

The result for smokeless tobacco and Areca nut was anticipated and consistent with prior studies. The lack of results for smoking tobacco and alcohol consumption is most likely caused by the small study sample which is the main weakness of this study.

### Take home message!

To avoid oral cancer, do not chew tobacco!  
...or Areca nuts, do not chew on Areca nuts either!

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