

# Analysis of Sub-Mucous Fibrosis and Micronucleus in Tobacco and *Supari* Users: A Review

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**Abstract:** *Introduction:* Oral cancer is one of the most prevalent cancers worldwide including India. The most common cause of oral cancer has been established as tobacco chewing. Tobacco and *Supari* are chewed by people all over the world. But the long-time consumption of tobacco and *Supari* can lead to many diseases related to mouth like Trismus (reduced opening of the jaw), oral leukoplakia, erythroplakia that can eventually develop into oral cancer. OSF (oral sub-mucous fibrosis) is a precancerous condition that can develop into squamous cell carcinoma. Chewing of areca nut can also damage the exfoliated cell of buccal mucosa in which the frequency of micronucleus, an indicator of genetic damage increase as well as increased cytogenetic damage can be seen in the peripheral blood lymphocytes and exfoliated buccal mucosal cells of pan masala chewers. The current review work explores the role of tobacco and *supari* chewing habits in causing precancerous lesions that have the potential to ultimately develop into oral cancer. *Methods:* We conducted a thorough electronic search by Cochrane, EMBASE and PubMed to identify relevant studies. Studies published on the role of tobacco and *supari* and such products on the development of oral sub-mucous fibrosis and eventually oral cancer were included. Studies on micronucleus assay in reference to oral cancer development were also included. After assessing and selecting articles based on eligibility criteria, studies were analyzed and elaborated according to the objective. *Results:* The various studies successfully established the huge role that tobacco and *supari* products play in the carcinogenesis process and early prelesions are significant in these users that can be utilized for early diagnosis and treatment.

**Keywords:** Areca nut, buccal mucosal cells, fibrosis; micronucleus (MN); oral disease tobacco; squamous cell carcinoma.

## 1. Introduction

In India, tobacco is used mainly in form of cigarettes and water pipe<sup>1,2</sup> (Sedigheh-Sadat *et al.*, 2009, Hays *et al.*, 2008) and oral cancer is one of the most prevalent cancer. Over half of all tobacco consumed in India is smoked as *bidi* (Indian specific non-filtered cigarette) and about one-fourth of tobacco consumption is in smokeless form, such as chewing tobacco and *mishiri* (tooth cleaner applied tobacco)<sup>3</sup> (Panchamukhi *et al.*, 2008). In India, smokeless tobacco like pan masala, Gutkha, tobacco mixture with betel nut and quenched lime, mainpuri tobacco, chewable tobacco, suns, sugar candy, Gul, Buzzer, clap, creamy tobacco powder, tobacco water and tobacco are used as smoking *bidi*, cigarette, cigar, charette, chutta, pinch drink, dusty, pipe, hookley, chilam, and hookah are used by men and women and these tobacco contains different types of toxic and carcinogenic substances responsible for different types of cancers and shown the strong risk of OSMF (oral submucous fibrosis) and oral cancer<sup>4</sup> (Babu *et al.*, 1996).

Further areca nut is also commonly used in India and other South Asian countries. Areca nut (naturally crud areca nut) form is used traditionally with betel quid or paan wrapped in leaves of piper betel, with lime, saffron and some additives like catechu, cinnamon, cloves. Since 1970's and 80's, the industries of areca nut (used in Indian technology traditionally) along with the industry of tobacco discovered a new product similar to betel quid ready to be used immediately. The product without tobacco was called "pan masala" and with tobacco was called "Gutkha". The indigenous preparation of areca nut is the *Supari* (a naturally crude areca nut) without adding other ingredients<sup>5</sup> (Rooban *et al.*, 2005). Areca nut chewing also increases the chance of oral cancer because it contains carcinogenic substances, most of South African Indians with cheek cancer are

chewers of areca nut and most of these cases evidently show OSMF<sup>6</sup> (Van *et al.*, 1993).

### Major Components of Tobacco and Areca Nut

Tobacco included following chemical components nicotine, carbon monoxide, Tar, Arsenic, Ammonia, Acetone, Toluene, and Methylamine.

According to IARC study of 2004 on the evaluation of carcinogenic risks to humans, Areca nut compounds are mostly water or ethanol soluble. The fraction of alkaloid contains arecoline, arecaidine, guvacoline, arecolinide and others. The most chief poly-phenols are catechin, flavonoids, and flavan 3:4-diols, leucocyanidines, hydroxyl-flavan and tannin. Minor phenols include epicatechin, gallic acid, D-catechol, phicbatannin, and others. Moreover, nitrosamines have been identified in areca nuts along with trace elements like copper, bromide, vanadium, manganese, chlorine and calcium<sup>7</sup>.

In a study done by Al- Rmali *et al.*, (2011), heavy metal toxicity caused by betel chewing was explored and they found out that the potentially hazardous compounds like arsenic, cadmium, and lead concentrations are pretty high in betel quid chewer, which can have devastating effect on their health<sup>8</sup>.

### OSMF (Oral sub-mucous fibrosis)

Oral sub-mucous fibrosis (OSMF) is a painful and disabling potentially pre-cancerous condition of the oral mucosa, which has been associated with areca nut use. In this condition, elasticity of the mucosa is reduced and fibrous band develops. Uses of Gutkha, a mixture of areca nut, lime, catechu, unspecified flavoring agent etc. and tobacco have been found to be associated with this painful condition while chewing of areca nut is considered a risk factor for oral sub-mucous fibrosis. In South Asian population, the

habit is prevalent but now-a-days it has been recognized in Europe and North America also. Significant morbidity causes OSF. It is also responsible for mortality after transformation into squamous cell carcinoma (SCC). The frequency of OSF increases sharply with combination of areca nut and tobacco. Inflammation is the initial presentation of OSF followed by fibrosis and hypovascularity visible as blanching of oral mucosa with a marble like appearance. Blanching may be localized, reticular or diffuse small vesicles may develop in some cases that rupture and form erosion<sup>9,10</sup> (Wollina *et al.*, 2015).

**Pandya *et al.*, 2009** and **Patel *et al.*, 2014** studied OSF in detail. Their study explained that in advanced stage of OSF, mouth opening is less or restricted by fibrous band (Trismus), which can cause a series of problems like problem with speech, in keeping oral hygiene, mastication, as well as thickening and rubbery appearance of lips. The measuring of inter-incisor or opening and mouth opening can be graded. The severity of Trismus (restricted opening of mouth) are divided into Stage 1 (>3 cm), Stage 2 (2-3 cm), Stage 3 (<2 cm). Cheeks become thick and rigid due to fibrosis<sup>11,12</sup>.

#### Micronucleus as biomarker for genetic damage

Oral cancer is the sixth most common cancer in the world, but in India it is the second most prevalent cancer. The good news is it can be detected and controlled very early on as the pre-lesions are very much visible in the form of patches what are termed as premalignant disorder of mouth because significant number of these lesions transforms into oral cancer<sup>13,14</sup>. (Gupta *et al.*, 2007; Dakubo *et al.*, 2007).

An important biomarker of genetic damage and consequently cancer is the presence of micronucleus (MN) in peripheral blood or other types of cells. It can be said that the stimulation of micronuclei serves as a signal of genetic damage that has taken place. It is a multi-stage process of accumulated genetic damage, which also leads to cell de-granulation along with cell de-granulation along with cell, which is an important risk factor for carcinogenesis in the long run.

MN is a recently updated topic in oral cancer field; it comes from fragments of chromosomes or whole chromosomes, which lag behind at anaphase during period of nuclear division. It can be said that micronuclei provides a specific site for early genotoxic event for exfoliated oral epithelial cells which are produced by carcinogenic agents. In various studies, the correlation between frequency of micronuclei and severity of this genotoxic damage has been well established<sup>15</sup> (Sivasankari *et al.*, 2008). Micronuclei are mostly seen in exfoliated epithelial cells like buccal mucosa and wall of urinary bladder at the time of precancerous and cancerous condition<sup>16</sup> (Jadhav *et al.*, 2011).

Micronuclei are stimulated by different types of carcinogenic and genotoxic agents like tobacco, Supari, & alcohol in oral epithelium. It's seen in different conditions like chronic renal diseases, chronic tonsillitis & rheumatoid arthritis<sup>17,18,19</sup> (Unal *et al.*, Roth *et al.*, Ramos- Remus *et al.*).

In betel, the components used like nut, tobacco & calcium hydroxide are known for the promotion of reactive oxygen species causing further DNA damage<sup>20</sup>.

Increase in the percentage of micronuclei count potentially increases the risk of malignant disorder like oral submucous fibrosis, leukoplakia, erythroplakia, lichen planus & squamous cell carcinoma<sup>21,22</sup>.

The chewers of tobacco and Supari from long time may cause oral cancer, Trismus (opening of mouth become less, would be less than four it would indicate submucous fibrosis).

For micronucleus analysis buccal sample taken from patient and make slide and observed under microscope to see the genomic damage in panmasala and areca nut chewers by counting the frequency of micronucleus in the cell.

## 2. Conclusion

Most of these studies explored established the fact that the chewing of all varieties of tobacco and *Supari* indeed has strong association with development of oral sub-mucous fibrosis (precancerous condition). After some time, it may get converted into cancerous condition and one of the possible mechanisms could be micronucleus development. Long time chewing habit increases the frequency of micronucleus in buccal cells, which is an indicator of damage of cell and accumulation of such mitotic errors and genetic damage can later convert into potentially malignant disorders and oral cancer.

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