

## EFFECT OF SMOKING ON ORAL EPITHELIUM IN ORAL SUBMUCOUS FIBROSIS PATIENTS IN LOCAL POPULATION

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### ABSTRACT

*Oral Submucous Fibrosis (OSF) is a pre-cancerous condition that has ability to get malignant transformation. The main causative factors of OSF are smoking, betel nut, pan masala, gutka, naswar, chalia etc. The chemicals used in all these cause dysplastic changes into the oral mucosa epithelium and eventually leads to malignant transformation into Oral Squamous Cell Carcinoma (OSCC). The aim of the present study was to observe the dysplastic changes in buccal mucosa associated with smoking in OSF patients.*

*Forty patients of all age groups and both genders were evaluated as per inclusion and exclusion criteria and written informed consent was taken. The study was done at the Department of Pathology (Histopathology Section), Postgraduate Medical Institute, Lahore. Biopsy specimens from study participants were obtained from Punjab Dental Hospital, Lahore.*

*Punch biopsy was performed under local anesthesia, and the sample size measuring 5mm was taken from the buccal mucosa. Staining with Hematoxylin and eosin (H&E) was done after tissue specimens were fixed and processed. The prepared slides were seen under light microscope to record the dysplastic changes in epithelium.*

*Statistical significance was evaluated by  $\chi^2$  analysis and showed significant association of Age with history of smoking and duration of smoking with the value  $p=0.001$  &  $p=0.002$  respectively. Also the significant association was found between Dysplastic changes in OSF patients with age, history of smoking and duration of smoking ( $p= 0.05$ ,  $p=0.00$ ,  $p=0.03$  respectively). This suggests that patients who are 31-45 years of age with more than 10 years of smoking history showed moderate dysplastic changes than patients age range 18-30 with 5 years or less than 10 years of smoking history showed mild dysplastic changes.*

*In local population no such related study has been done so study must be conducted to rule out the dysplastic changes exhibiting in buccal mucosa of OSF patients with smoking history.*

**Key Words:** Oral Submucous Fibrosis, Smoking, Dysplasia in epithelium.

### INTRODUCTION

Oral cancer comprises such malignancies that arises from oral tissues. It is one of the major global threats to public health, including Pakistan. It has been persisted

as lethal disease over the years because of the fact that approximately 50% annually diagnosed cases were in progressive stages at the time of identification. Oral Squamous Cell Carcinoma (OSCC) is among the first eight most common cancers all over the world in both sexes.<sup>1</sup>

Oral cancer prevalence is comparatively lesser in developed countries as compared to developing countries. It has been characterized by a variable degree of exposure to the different etiological and risk factors.<sup>2</sup> A survey in 2005 declared Pakistan as a high risk country in oral cancer and ranked as the second most common reporting cancer representing 8.8% in both men and women.<sup>2</sup>

OSCC is a malignancy associated with multifactorial causative agent, combination of various factors like environmental, viral infections and genetic alter-

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ation which altogether results in a malignant lesion. Commonly OSCC occurs in the presence of common premalignant conditions as Oral Leukoplakia, Oral Lichen Planus and Oral Submucous Fibrosis.<sup>1</sup> Other contributing factors are alcohol and tobacco usage associated with viruses like papilloma viruses, Epstein-Barr virus, herpes simplex virus.<sup>3</sup>

OSF is a pre-cancerous condition; its ability to get malignant was first discovered in 1950s and was most prevalent in Asians. The presenting complaints include rigidity of lips, tongue and palate and intolerance to spicy food. This stiffness eventually leads to less mouth opening and difficulty eating, talking, chewing and even swallowing.<sup>4,5</sup>

Some previous literature showed high association of smoking, alcohol consumption and betel quid chewing with oral cancer<sup>6-8</sup>, and added an evidence that tobacco smoking and alcohol abuse play a key role in the pathogenesis of OSCC.<sup>8</sup>

A global survey reported in 2005 that more than billion individuals use cigarettes every day. Hence smoking is accountable for about 30% of totally cancer-associated losses in United States.<sup>9</sup> Tobacco associated sources causes various malignant diseases as cancers of the lung, oral cavity, pharynx, esophagus, stomach, liver, pancreas, kidney, bladder, and cervix.<sup>10</sup> Above 60 malignancies were reported in conventional cigarette smoke and few of them were also reported in side stream smoke.<sup>11</sup> Moreover smoking as a key risk factor of OSF which ends into OSCC, alter the activity of chemo preventive mediators<sup>12</sup>, increases the clearance of particular targeted anticancer treatments<sup>13</sup>, decreases the efficiency of cancer treatment<sup>6-8</sup> and causes metastasis.<sup>14</sup>

Women have been reported to carry an increased risk of lung, oral and oropharyngeal cancer compared with men who had similar cigarette smoking exposure levels.<sup>15-17</sup> The underlying theory of this gender-dependent risk is poorly unstated.<sup>17</sup>

The malignant transformation of OSF ranges from 7-13%.<sup>18</sup> Changes in epithelium observed in the patients with OSF shows mostly epithelial atrophy, or epithelial hyperplasia, keratosis with either hyper orthokeratosis or hyper parakeratosis, pyknosis in basal layer nuclei, lack of glycogen in severe Grade III scenarios, intercellular edema, hyalinization occurring close to epithelium and collagen with diverse thickness<sup>19</sup> vacuolization of prickle-cell layer, amplified mitotic activity, Increased levels of pro-inflammatory cytokines and reduced antifibrotic interferon have also been demonstrated in patients with OSF and atypical epithelium changes<sup>20</sup> with moderate epithelial hyperplasia eventually resulting in squamous cell carcinoma.<sup>21</sup>

Under the light of above mentioned literature a need to work on the OSF patients and observe the risk factor (smoking) which causes dysplastic changes of oral mucosa. These changes further transform OSF into malignancy. In local population no such relevant study has been done on this objective, so the present study was conducted to rule out the dysplastic changes exhibiting in buccal mucosa of OSF patients having smoking history.

## METHODOLOGY

Forty patients according to inclusion criteria that was, only OSF patients having two age groups 18-30 years and 31-45 years of both gender were selected by convenience sampling. Written consent was obtained and signed from all enrolled patients prior to the study. Subjects having any pre-malignant lesion other than OSF was not the part of the study. Forty diagnosed OSF cases were selected without OSCC or other neoplastic disease.

Punch biopsy was performed under local anesthesia to collect tissue specimen from buccal mucosa at the time of surgical resection at Punjab Dental Hospital, Lahore. The sample size measuring 5mm was taken from the affected buccal mucosa. Cautious resection of OSF tissue was taken from buccal mucosa. Specimens were fixed in 10% buffered formalin solution. After processing, three sections each of 3 to 4 microns thickness were cut from each selected block using a rotary microtome. Two slides were made from one tissue specimen for Haematoxylin and Eosin staining.<sup>15</sup>

The prepared slides were seen under light microscope for the confirmatory diagnosis of OSF and the findings were noted down in proforma. The findings in epithelium like presence of keratinization (non-keratinized/keratinized), thickness (atrophic/hyperplastic), dysplastic changes (mild/moderate/severe), inflammatory cells (acute/chronic), pleomorphism, mitotic figures and hyperchromatism were recorded.

Apart from these the presence of fibrous tissue, hyalinization and inflammatory cells in the connective tissue was also noted and entered in the proforma. The readings were then entered into the proforma of each subject, previously having their bio data. Statistical analysis was done with Statistical Package for Social Sciences (SPSS) software, version 20.0.  $\chi^2$  analysis was applied to observe the association of Age with History of smoking and duration of smoking, also observed association of age, history of smoking and Duration of smoking with Dysplasia in Epithelium.

## RESULTS

A total number of 40 samples from the buccal mucosa of the patients with OSF were taken. Age range

TABLE 1: ASSOCIATION OF AGE WITH HISTORY OF SMOKING (YEARS)

Age	History of Smoking	
	Present	Absent
18-30	5	21
31-45	7	7
Total	12	28

P=0.043\*

TABLE 2: ASSOCIATION OF AGE WITH DURATION OF SMOKING (YEARS)

Age	Duration of Smoking in years			
	Absent	1-5	6-10	>10
18-30	21	5	0	0
31-45	7	1	3	3
Total	28	6	3	3

P=0.004\*

TABLE 3: ASSOCIATION BETWEEN DYSPLASTIC CHANGES IN ORAL EPITHELIUM AND AGE

Dysplasia in epithelium	Age		Total
	18-30	31-45	
Absent	10	2	12
Mild	11	3	14
Moderate	5	9	14
Total	26	14	40

P=0.017\*

TABLE 4: ASSOCIATION BETWEEN DYSPLASTIC CHANGES IN ORAL EPITHELIUM AND HISTORY OF SMOKING (YEARS)

Dysplasia in epithelium	History of Smoking	
	Absent	Present
Absent	9	2
Mild	11	2
Moderate	8	7
Severe	0	1
Total	28	12

P=0.04\*

of the subjects was divided into two groups i.e. 18-30 years and 31-45 years. Both genders were included in the study.  $\chi^2$  analysis was applied to observe the association between history and duration of smoking with age groups, suggesting significant relation showing the value p=0.001 and p=0.002 respectively. Also the

TABLE 5: ASSOCIATION BETWEEN DYSPLASTIC CHANGES IN ORAL EPITHELIUM AND DURATION OF SMOKING (YEARS)

Dysplasia in epithelium	Duration of Smoking in years				Total
	Absent	1-5	6-10	>10	
Absent	9	2	0	0	11
Mild	11	0	0	2	13
Moderate	8	4	2	1	15
Severe	0	0	1	0	1
Total	28	6	3	3	40

P=0.01\*

significant association was found between Dysplastic changes in OSF patients with age, history of smoking and duration of smoking (p= 0.05, p=0.00, p=0.03 respectively).

## DISCUSSION

Ninety percent of the head and neck carcinomas are oral squamous cell carcinomas globally. Approximately 35,000 new cases are recorded annually in the US.<sup>23</sup> 40,000 new cases are recorded in the EU and 10,915 new cases in Japan.<sup>24</sup> While oral cancer records for 40% of all cancers in the Indian sub-continent.<sup>25</sup> Globally, OSF is found in 2.5 million people mostly from Southern India and ranges from 0.2-2.3% in males and 1.2-4.57% in females.<sup>26</sup>

Unfortunately due to late diagnosis of oral cancers the survival frequency of oral cancer persistently remained unaffected over the former three eras.<sup>27</sup> Diagnosis of an oral pre-cancerous lesion or oral cancer has remained a challenge for the dental practitioners, mainly in diagnosis, assessment and treatment of a disease at initial stage.<sup>28</sup> OSF has high morbidity because it causes inability to open mouth, trouble eating and eventually leading to nutritional deficits. Significant death rate since it can transform into OSCC.<sup>31</sup>

Oral sub mucous fibrosis has a tendency toward malignancy since it is characterized as a pre malignant condition usually associated with betel quid chewing, smoking and spicy food. Microscopically it is characterized by epithelial atrophy, basal cell hyperplasia, excessive deposition of collagen fibers in connective tissue and surface keratinization. Malignant transformation rate of oral sub mucous fibrosis into squamous cell carcinoma is 7.6%.<sup>32</sup> OSCC is most dominant carcinoma, accounting for more than eighty percent of head and neck cancers.<sup>29</sup>

Oral premalignant lesions and OSCC are mainly treated on the basis of histological features, site involved, and stage of the disease. However, Oral health

personnel should be aware of all the recent advances so the accurate, less time consuming diagnosis of oral premalignant lesions or OSCC can be made so eventually the patient will benefit from them.<sup>30</sup>

Globally 274,000 individuals have been affected with oral cancers yearly, and its incidence have been ranked on the basis of smoked tobacco consumption all over the world.<sup>16,42</sup> It has been recognized that relationship between the quantity of tobacco product consumed and progress of oral cancer is a dose-response relationship.<sup>33,34</sup>

All sites of the oral mucosa are prone to progress into malignancy due to tobacco associated smoking, together with lips, tongue, palate, gums, and buccal mucosa.<sup>34</sup> It has been surveyed that about 30-80% of oral mucosa cancers originates from already existing premalignant lesions mainly Leukoplakia, Erythroplakia, and OSF.<sup>35,36</sup>

The present study results were in similarity with few studies conducted in past had played a noteworthy role, to show the significant association among the frequency of buccal changes and smoking frequency, few of them observed no association.<sup>52</sup> In a study of 120 subjects, Konopacka reported in 2003 that the incidence of oral epithelial cells was three times increase in smokers ( $n = 50$ ) than nonsmokers ( $n = 70$ ) by showing the significant buccal cell frequency. Microscopically, buccal cells are recognized as comparatively increase in size, flat pancake-like form, nongranular cytoplasm, centrally placed nucleus, and increase nucleus-cytoplasm proportion.<sup>53</sup>

## CONCLUSION

Present study showed a significant association between smoking and dysplastic changes occurring in OSF patients. So the results of present study add on evidence on the light of previous reported literature, that patients who are 31-45 years of age with more than 10 years of smoking history showed moderate dysplastic changes than patients age range<sup>18-30</sup> with 5 years or less than 10 years of smoking history showed mild dysplastic changes. Therefore they are more prone to have a malignancy because of greater dysplastic changes reported.

## SUGGESTION

Hence OSF is a lifestyle-associated disease where smoking counts as a main causative factor. It can transform into malignancy, as the result of an old lesions turned into carcinoma due to dysplastic changes occur in oral epithelium. So there is a need to do a constant counseling of a smokers to stop smoking and provide the education and awareness to such individuals.

## DISCLOSURE STATEMENT

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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### CONTRIBUTION BY AUTHORS

- Khola Ahmad Khan:** The idea was generated by her. Along with the sample collection and staining procedure, all work was done by her. Write up, microscopy and analysis of results was also done by her..
- Mariyah Javed:** She assisted during data entry and statistical analysis using spss version 20.
- Ahmad Waqas Javed:** He assisted during the sample collection and being a surgical trainee punch biopsies were taken with his help along with sample preservation..