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Study of Correlation Between Clinical Staging & Predictive Molecular Markers in Oral Sub Mucous Fibrosis

Lt Col (Dr) Sanjiv Baveja[1], Lt Col (Dr) Puneet Baveja[2]
Dept of Oral & Maxillofacial surgery, Military Dental Centre Base Hospital, New Delhi[1] & Dept of Pathology, R & R Hospital, New Delhi[2]
Corresponding author - Lt Col (Dr) Puneet Baveja

Abstract: *A prospective study of clinically diagnosed 50 cases of oral submucous fibrosis was done in which biopsies of all patients were subjected to routine histopathology and immunohistochemistry (IHC) studies to molecular markers such as proliferating cell nuclear antigen (PCNA) and p53 which is a tumor suppresser gene. All patients were subdivided according to age, sex, and habits and were clinically staged. After confirming the diagnosis on histopathology, IHC studies were done to see the expression of PCNA and p53 in the formalin fixed slides. Patients were in the age group of 21-60 years, 39 (78%) were males and 11(22%) were females. In the clinical stage wise distribution, there were 9 cases in stage I, 14 in stage II, 17 in stage III, 10 in stage IV. On correlating the stage with PCNA IHC grade, it was found that percentage of cases showing PCNA positivity increased as the clinical stage increased - 1 in stage I (11.11%), 3 in stage II (21.4%), 5 in stage III (29.4%), 5 in stage IV (50%). Similarly increased number of cases showed p53 positivity as the clinical stage of OSMF increased - 3 in stage I (33.3%), 7 in stage II (50%), 8 in stage III (47.5%), 7 in stage IV (70%). However, no definite correlation could be established between stage and IHC grading scale which showed variable results, but by enlarge, increased grading of markers was seen in advanced stage (Grade 2 and 3 positivity was seen only in stage 3 and 4 and not in stage I of OSMF.* ***Conclusion:*** *Immunohistochemistry using molecular markers p53, PCNA is useful in stratifying the risk of malignant transformation in OSMF. It is recommended that this panel of these antibodies be used for prognostication as cases which demonstrate a higher degree of expression of the aforementioned panel of molecular markers are more likely to progress to malignancy,*

Keywords: *Oral sumucous fibrosis (OSMF), Oral Cancer, p53, Proliferating cell nuclear antigen (PCNA), Immunohistochemistry (IHC)*

I. Background

In the Indian subcontinent, the use of smokeless tobacco in various forms is very popular. This habit, which usually involves the chewing of a betel quid (combined areca nut, betel leaf, tobacco and slack lime), has led to the development, in a large proportion of users, of a unique generalized fibrosis of the oral soft tissues, called ***oral submucous fibrosis***. JJ Pindborg[1] (1966) defined it as "an insidious chronic condition affecting any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by and/or associated with vesicle formation, it is always associated with a juxta-epithelial inflammatory reaction followed by a fibroelastic change of the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus and inability to eat. It is estimated that the prevalence in India has increased over past 04 decades from 0.03% to 6.42%.[2] Multifactorial etiology in the causation of OSMF has been postulated which includes capsaicin, betel nut alkaloids, hypersensitivity, autoimmunity, genetic predisposition, malnutrition. chronic iron deficiency. However, the role of betel nut chewing in one form or another remains a common denominator. Histological examination of the lesions of OSMF usually reveals nonspecific inflammatory infiltrate usually containing a number of eosinophils, cells which are otherwise seldom found in oral inflammation. Older lesions demonstrate reduced vascularity, reduced numbers of inflammatory cells along with dense bundles and sheets of collagen in the subepithelial region. This thick band of hyalinized subepithelial collagen typically shows varying extension into sub mucosal tissues replacing the fatty or fibrovascular tissues normal to the site. Epidemiological studies have shown that as many as 10% of OSMF patients develop an oral carcinoma. As the impetus for screening increases, recognition of a true pre malignant condition becomes exceedingly important to recognize oral carcinoma at an early stage. Although there are clinical methods for early and reliable detection of premalignant tissue changes which help to identify “suspicious" area for representative biopsy along with the conventional histopathology, they cannot determine the progression to oral cancer with certainty. The data from various sources suggest that cellular proliferation is one important indicator of the biologic aggressiveness of a malignant lesion. It is a proven fact now that uncontrolled proliferation may be an important change that signals premalignant transformation. Many molecular markers have been studied to provide information on differentiation, proliferation and prognosis of malignant lesions. Amongst these, proliferating

cell nuclear antigen (PCNA) and p53 (a tumor suppressor gene) have been shown to fluctuate during the cell cycle and to dramatically increase in late G1 and S phases and is considered to be well correlated with cell proliferation[7,8,9]. It is believed that if premalignant lesions are detected at an early stage, risk factor management i.e. cessation of smoking and chewing betel nut can be attempted along with use of chemo-preventive techniques, process of progression of these lesions to malignancy can be halted or even reversed. Therefore, keeping these views in mind, the present study was undertaken to study the role of predictive molecular markers in prognostication and progression of OSMF so that the follow -up and treatment decisions are rational.

II. Material & Methods

50 patients of OSMF were selected from the OPD of dental centre of a tertiary care hospital and were subjected to biopsy. A detailed history was taken from each patient including data on medical, dental & dietary background with special emphasis on habits like tobacco, smoking & betel nut / leaves chewing. A history regarding chili consumption was also elicited. All patients were subdivided according to age, sex, habits and clinical staging. Patients with a history of prior management of oral submucous fibrosis (either conservative or surgical) were excluded from the study. Patients having frank oral malignancies were excluded from the study. Patients selected for the study were grouped into four categories based on clinical findings (Khanna and Andrade 1995)[3] as: Very early (Stage-I), early (Stage-II), moderately advanced (Stage-III), advanced (Stage-IV). Diagnosis of OSMF was confirmed on histopathological examination and histological staging was assigned as per the laid down criteria[3]. Serial sections of these cases were subjected to immunohistochemical staining with peroxidase-labeled streptavidin-biotin technique, using monoclonal antibodies supplied by Dako Cytomation (Dako Labs, USA) targeted against PCNA and p53.The immunohistochemistry slides were examined for positivity of cells as indicated by yellow to brown discrete epithelial membrane or nuclear staining as seen under light microscopy. Markers were graded as

0 - Absent immunostaining
1 - Immunostaining limited to basal & Para basal layers of the epithelium
2 - Immunostaining of at least half the thickness of the epithelium
3 - Full thickness immunostaining

The correlation of both the proliferative markers with the clinical stage of oral submucous fibrosis was then assessed by an appropriate statistical analysis.

III. Results

In this study, 50 patients of oral submucous fibrosis were selected as per the laid down criteria. On observing the age wise distribution of the patients it was seen that out of the total 50 patients, 10 (20%) were in the age group of 21–30 years, 22 (44%) in 31–40 years, 13 (26%) in 41–50 years, and 3 (6%) patients were in 51-60 years and 2(4%) patients were >60 years in age. Out of total number of 50 patients, 39 (78%) were females and 11 (22%) were males.

All the patients selected for the study gave a history of chewing areca nut/ pan/ gutkha/tobacco. 32 (64%) patients in addition gave a history of smoking (Biddi/ cigarette), and 15 (30%) patients had indulged in multivariate habits like chewing, smoking and alcohol consumption. Also 10 (20%) patients gave a history of excessive consumption of chilies since childhood. Out of 50 patients it was observed that 12 patients indulged exclusively in chewing habits, 17 patients had chewing and smoking habits, 12 patients had chewing, smoking and alcohol consumption habit, 7 patients had chewing and excessive chili consumption habit, and 2 patients had chewing, smoking and excessive chili consumption habit.

Out of the total of 50 patients 9 (18%) were placed in stage I, 14 (28%) in stage II, 17 (34%) in stage III, and 10 (20%) in stage IV.

All 50 patients of oral submucous fibrosis had burning sensation with mouth, which was aggravated on eating spicy food. 15 patients had ulcerations with the oral mucosa. 10 patients complained of excessive salivation and 39 patients had difficulty in mouth opening. None of the stage-I patients had difficulty in mouth opening. Blanching of the oral mucosa in all 50 patients was observed 4 of the stage-I patients had vesicles on soft palate. Papillary atrophy of tongue in 8 patients and restricted tongue movements in 5 patients of stage-IV was observed. In 8 patients of stage-IV, movements of the soft palate were absent. 4 patients in stage-IV also had associated leukoplakia. In the stage-I patients only the soft palate and faucial pillars were involved. Whereas in all stage-II patients and 11 patients of stage-III the soft palate, faucial pillars and the buccal mucosa were found involved. In 6 of stage-III and all stage-IV patients, the uvula and lips were also involved in addition to the above sites. All the stage-I patients had a mouth opening (Interincisal distance) of more than 35 mm. Mouth opening was ranging from 26-35 mm in stage-II patients, 16-25 mm in stage-III patients and 15 mm or less in stage-IV patients. No palpable fibrous bands were present in the stage-I patients. Palpable fibrous bands in the buccal mucosa were present from the retromolar area to the 2nd molar region in stage-II patients, from the

retromolar area to the 1st premolar region in stage-III patients, and from the retromolar area to the angle of the mouth in stage-IV patients. Circular fibrous bands with the lips were also present in stage-IV patients. On correlating the stage with PCNA IHC grade, it was found that percentage of cases showing PCNA positivity increased as the clinical stage increased - 1 in stage I (11.11%), 3 in stage II (21.4%), 5 in stage III (29.4%), 5 in stage IV (50%). Similarly increased number of cases showed p53 positivity as the clinical stage of OSMF increased - 3 in stage I (33.3%), 7 in stage II (50%), 8 in stage III (47.5%), 7 in stage IV (70%). However, no definite correlation could be established between stage and IHC grading of both the cases which showed variable results.

Table 18 P53 positivity as per clinical stage of oral submucous fibrosis

CLINICAL STAGE	NUMBER OF PATIENTS	p 53 positive	p53 positivity %
STAGE I	09	03	33.3%
STAGE II	14	07	50%
STAGE III	17	08	47.5%
STAGE IV	10	07	70%

Table 19

Correlations

		HIST	P53	CLINICAL	PCNA
HIST	Pearson Correlation	1.000	.160	.972**	.314*
	Sig. (2-tailed)	.	.267	.000	.027
	N	50	50	50	50
P53	Pearson Correlation	.160	1.000	.216	.618**
	Sig. (2-tailed)	.267	.	.131	.000
	N	50	50	50	50
CLINICAL	Pearson Correlation	.972**	.216	1.000	.352*
	Sig. (2-tailed)	.000	.131	.	.012
	N	50	50	50	50
PCNA	Pearson Correlation	.314*	.618**	.352*	1.000
	Sig. (2-tailed)	.027	.000	.012	.
	N	50	50	50	50

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 20(a) Correlation of IHC grade with clinical stages of oral submucous fibrosis

IHC GRADE	CLINICAL STAGE				TOTAL
	STAGE I	STAGE II	STAGE III	STAGE IV	
PCNA positivity	01	03	05	05	14
p53 positivity	03	07	08	07	25
TOTAL	04	10	13	12	39

Table 20(b) Correlation of IHC grade with histological stages of oral submucous fibrosis

IHC GRADE	HISTOLOGICAL STAGE				TOTAL
	STAGE I	STAGE II	STAGE III	STAGE IV	
PCNA Positivity	01	04	05	10	20
p53 Positivity	05	08	08	09	30
TOTAL	06	12	13	19	50

IV. Discussion

Oral submucous fibrosis is a chronic condition of the oral mucosa which was first described among five East African women of Indian origin under the term, *'atrophia idiopathica (tropica) mucosae oris'*. Since then this condition has also been described by various authors as, *'idiopathic scleroderma of the mouth', 'idiopathic palatal fibrosis* and *'sclerosing stomatitis'*. In India this condition was first described as *'diffuse oral*

submucous fibrosis' and as *'submucous fibrosis of the palate and pillars'*. OSMF is defined as an insidious, chronic disease affecting most part of the oral cavity, sometimes the pharynx, and seldom the larynx. Although occasionally preceded by and/or associated with vesicle formation, it is always associated with a juxta-epithelial inflammatory reaction followed by a fibroelastic changes and progressive hyalinization of the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus and inability to eat. Patients with submucous fibrosis generally complain of a burning sensation of the oral mucosa, aggravated by spicy food.

A serious implication of this condition is the risk of the development of oral cancer. The precancerous nature of oral submucous fibrosis was first mentioned by Paymaster[4] who observed the development of slowly growing squamous cell carcinoma in one-third of his OSMF patients. The precancerous potential of OSMF has been emphasized by various other authors. It has been reported that patients suffering from OSMF in India have a higher incidence of leukoplakia and carcinoma than those without this disease. The precancerous nature of this disease is further supported by higher prevalence of leukoplakia among submucous fibrosis patients, high frequency of epithelial dysplasia in these lesions, concurrent finding of OSMF in oral cancer patients and increased incidence of oral cancer in patients having preexisting submucous fibrosis. Areca nut (Areca catechu, betel nut) chewing (5, 6), use of chilies, and nutritional deficiencies has been thought to play an etiological role in OSMF. The role of autoimmunity and genetic predisposition as a cause of OSMF has also been implicated.

Oral submucous fibrosis affects both sexes. A predominance of women was observed in several studies[12]. In the present study also, there were 39 females out of 50 randomly selected patients reporting to dental OPD & only 11 were males. Most of the patients with this condition are in the 31 to 40 year age group. In the present study, we had 48 patients in 20–60 years range and 02 patients were >60 years old. The youngest patient was 23 years old and the oldest patient was 65 years old.

The patients selected for the study were grouped into four categories based on clinical findings as: Stage-I (Very early), Stage-II (early), Stage-III (moderately advanced), and Stage-IV (advanced). The most frequently affected locations in oral submucous fibrosis are the buccal mucosa and the retromolar area. It also commonly involves the soft palate, palatal fauces, uvula, tongue, and labial mucosa. OSMF generally originates from the posterior part of the oral cavity and subsequently involves the anterior locations[3]. We, too in our study, found OSMF to start in the posterior parts of the oral cavity and later on involve the anterior parts. In the stage-I case only the soft palate and faucial pillars were involved. In stage-II & Stage-III the soft palate, faucial pillars and the buccal mucosa were involved. In 6 of the stage-III and all the stage-IV cases the lips were also involved. The onset of the disease is insidious and is often of 2 to 5 years duration. The most common initial symptom is burning sensation of the oral mucosa, aggravated by spicy food. Vesiculation, excessive salivation, ulceration, altered pigmentation, recurrent stomatitis, defective gustatory sensation, and dryness of the mouth have also been indicated as early symptoms. In the present study, all the patients presented with burning sensation of the mouth, 15 patients had ulcerations of the oral mucosa, out of which three were in stage-I, one in stage-II, two in stage-III and two in stage-IV. 10 patients complained of excessive salivation, out of which three were in stage-I and three in stage-II. In our study all the patients in stage-II, stage-III and stage-IV complained of difficulty in opening the mouth. All the patients in stage-III and stage-IV were unable to blow their cheeks and whistle. None of the patients in our study presented with pain in the ears or nasal voice.

All the patients, in our study, on examination revealed blanching of the mucosa. 4 patients in stage-I also revealed vesicles on the soft palate. As the disease progresses, the mucosa becomes stiff and vertical fibrous bands appear therein. The diagnosis of this disease must always be based on the presence of palpable fibrous bands. In our study all the patients in stage-II, stage-III and stage-IV had palpable fibrous bands. Palpable fibrous bands in stage-II patients extended from the retromolar area to 2nd molar region, in stage-III patients extended from the retromolar area to the 1st premolar region, and in stage-IV patients from the retromolar area to the angle of the mouth. Circular fibrous bands in the lips were present in all stage-IV cases. None of the cases in stage-I had fibrous bands but had presented with blanching of the mucosa, ulcerations, vesicles, excessive salivation, and burning sensation of the mouth. In the present study 8 patients in stage-IV showed no mobility of the soft palate. In stage-IV 5 patients showed restricted mobility of the tongue and in five patient's papillary atrophy of the tongue was seen. A reduction in mouth opening takes place as the severity of the disease increases. In the present study the mouth opening of stage-I cases was normal, of stage-II cases ranged from 26 mm to 35 mm, of stage-III cases ranged from 16 mm to 25 mm, and that of stage-IV cases was less than 15 mm.

Chiang CP, Lang MJ, Liu BY et al. (2000) observed that aberrant expression of p53 protein was found in all types of precancerous oral lesion studied. Positive p53 staining was most common in epithelial dysplasia (70% of specimens), followed by oral sub mucous fibrosis (60%) and epithelial hyperplasia (40%). Furthermore, the percentage of keratinocytes with positive p53 staining was higher in epithelial dysplasia than in oral sub mucous fibrosis or epithelial hyperplasia specimens. They suggested that p53 expression increases with the morphologic transformation of normal-appearing epithelial cells into dysplastic epithelial cells, and that p53 may play an important role in oral carcinogenesis[7]. Trivedy C, Warnakulasuriya KA, Tavassoli M et al (1998)

reported that 15 of 20 (75%) oral sub mucous fibrosis specimens from Pakistani patients had positive p53 staining. Cox and Walker also detected positive p53 staining in 15 of 20 (75%) oral sub mucous fibrosis specimens from Indian and Nepalese patients[8]. C.P. Chiang, M.J. Lang, B.Y. Liu et al. (2000), studied PCNA patterns in oral submucous fibrosis, epithelial hyperplasia, epithelial dysplasia and normal oral mucosa. The mean PCNA labeling indices in normal oral mucosa, oral submucous fibrosis, epithelial hyperplasia and epithelial dysplasia were 8.8±2.7%, 21.1 ±12.5%, 25.5±5.2% and 44.9± 15.4% respectively. The authors suggested that gradual increase of PCNA expression with the morphologic transformation of normal epithelial cells into dysplastic epithelial cells meant that there is increased proliferative activity in oral premalignant lesions with disease progression[10]. Cox SC. Walker DM (1996) showed that the percentage of PCNA-positive basal epithelial cells in oral submucous fibrosis (31.8%) is greater than that in normal oral mucosa (7.6%)[11].

In our study also, it was observed that the percentage positivity of PCNA & p53 increased with the advancement of stage of disease. It was noted that there was an increase in the number of cases showing IHC positivity with the clinical progression of OSMF from stage-I to stage-IV. Even though no definite correlationship between PCNA and p53 grading scale and clinical stages of oral submucous fibrosis could be established in the present study possibly due to small sample size. it was noted that by enlarge, increased grading of markers was seen in advanced stage (Grade 2 and 3 positivity was seen only in stage 3 and 4 and not in stage I of OSMF. Therefore, studies using a large sample size should be conducted in future to confirm the above findings as these markers have true potential to serve as a useful adjunct to clinical practice and these findings may be put to use in predicting the rapid progression and malignant transformation of OSMF.

V. Conclusion

Predictive molecular markers p53 and PCNA are a useful adjunct in stratifying the risk of malignant transformation in OSMF. It is recommended that this panel of these molecular markers be used for prognostication and follow up of cases of OSMF for early detection of oral cancer. .

References

[1]. Pindborg JJ, Sir sat SM. Oral submucous fibrosis. Oral Surg Oral Med Oral Pathol 1966; 22(6): 764-79.
[2]. Kiran Kumar K., Saraswathi T.R., Ranganathan K., Uma Devi M., Elizabeth J. Oral submucous fibrosis: a clinico-histopathological study in Chennai. Indian J Dent Res. 2007;18:106–111. [PubMed]
[3]. Khanna JN, Andrade NN. Oral submucous fibrosis: a new concept in surgical management. Report of 100 cases. Int J Oral Maxillofac Surg 1995; 24(6): 433-9.
[4]. Paymaster JC. Cancer of the buccal mucosa. I. Clinical study of 650 cases in Indian patients. Cancer 1956; 9: 431-5.
[5]. Lal D. Diffuse oral submucous fibrosis. Journal of the All India Dental Association 1953; 26: 1-3.
[6]. Canniff, J. P. and Harvey, W. The etiology of oral submucous fibrosis: the stimulation of collagen synthesis by extracts of areca nut. International Journal of Oral Surgery 1981; 10, 163-7.
[7]. Chiang CP, Lang MJ, Liu BY et al. Expression of p53 Protein in Oral Submucous Fibrosis, Oral Epithelial Hyperkeratosis, and Oral Epithelial Dysplasia J Formos Med Assoc 2000;99:229-234.
[8]. Trivedy C, Warnakulasuriya KA, Tavassoli M et al. p53 aberrations in oral submucous fibrosis and oral squamous cell carcinoma detected by immunocytochemistry and PCR-SS Trivedy C, Warnakulasuriya KA, Tavassoli M et al CP. J Oral Pathol Med 1998; 27:72-77.
[9]. Huang W, Coltrera M, Schubert M, Morton T, Truelove E. Histopathologic evaluation of proliferating cell nuclear antigen (PCNA) in oral epithelial hyperplasias and premalignant lesions. Oral Surgery Oral Medicine Oral Pathology 1994; 78:748-754.
[10]. C.P. Chiang, M.J. Lang, B.Y. Liu et al. Expression of proliferating cell nuclear antigen (PCNA) in oral submucous fibrosis, oral epithelial hyperkeratosis and oral epithelial dysplasia in Taiwan. Oral Oncol 2000; 36: 353-359.
[11]. Cox SC. Walker DM. Epithelial growth fraction and expression of p53 tumor suppressor gene in oral submucous fibrosis. Aust Dent J 1996; 41:91-96.
[12]. J. P., Harvey, W., and Harris, M. (1986). Oral submucous fibrosis: its pathogenesis and management. British Dental Journal, 160, 429-34.

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