Cytokeratin -19 Expression in normal oral mucosal tissue, oral sub mucous fibrosis and oral squamous cell carcinoma-An immunohistochemical study

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Abstract: Objective*: The present study is carried out to characterize the CK19 profile in Normal oral mucosa, OSF and Oral Squamous cell carcinoma and ascertain if this could be used as a surrogate marker for malignant transformation.* ***Materials and Methods****: Immunohistochemical analysis of 10 NOM, 35 OSF and 10 oral SCC cases taken from patients and archives using cytokeratin 19 was done and the data were correlated.* ***Results****: Fourteen% (14%) of OSF cases, 0% of SCC and 20% of NOM tissue were negative for CK-14 staining. 31% of OSF cases, 10% of SCC and 10% of NOM tissue showed mild staining. 37% of OSF cases, 40% of SCC and 20% of NOM tissue showed moderate staining and 17% of OSF cases, 50% of SCC and 50% of NOM tissue showed intense staining. Since the P value is 0.138ns, there is no significant difference between the intensity levels and were not considered statistically significant.* ***Conclusion****: This study shows that CK19 profile alone cannot be used to ascertain if it could be used as a surrogate marker for malignant transformation.*

Key words: *Cytokeratin 19(CK19), oral submucous fibrosis (OSF), immunohistochemistry (IHC), squamous cell carcinoma (SCC), normal oral Mucosa (NOM)*

1. INTRODUCTION:

OSF is one of the most commonest potentially malignant disorders, previously called as premalignant condition .The present study is carried out to characterize the CK19 profile in OSF and ascertain if this could be used as a surrogate marker for malignant transformation and if the results hold positive then cytokeratin 19 can be the ideal surrogate marker that can be used to analyze and characterize the rate and conversion of OSF to oral squamous cell carcinoma or any malignant lesion.

Recently, various Immunohistochemical markers are showing great promise in helping to predict prognosis and response therapy, even at a very early point of tumor development or may it be a premalignant condition like OSF[1].

Alteration of CK19 expression has been documented in leukoplakia and oral cancer[2]. The study provides a detailed interaction between OSF and cytokeratin 19 and analyses its surrogate marker potential

2. MATERIALS AND METHODS:

2.1 Hypotheses to be tested:

Whether cytokeratin-19 can be used as a surrogate marker for malignant transformation of oral submucous fibrosis

2.2 Source of data:

35 cases of oral submucos fibrosis, 10 normal oral mucosal tissue and 10 of oral squamous cell carcinoma were taken for the study.

Confirmed Oral submucos fibrosis progressing cases to oral squamous cell carcinoma were taken for this study and were collected from department archives and the patients attending the department of oral medicine, oral surgery and other departments of Yenepoya Dental College, Mangalore,Karnataka,India after the taking informed patients concern.

Normal oral mucosa tissue samples were obtained from 10 patients during surgical removal of the third molar tooth after taking informed patients concern.

Ten paraffin embedded blocks or sections of already diagnosed cases of oral squamous cell carcinoma which were progressed from oral submous fibrosis were obtained from the archives.

2.3 Sample Size

55 cases.

- 10 of Normal oral mucosa tissue
- 35 of Oral sub mucous fibrosis

- 10 of Oral squamous cell carcinoma

Inclusion: Histologically confirmed cases of oral sub mucous fibrosis and oral squamous cell carcinoma were included

Exclusion: Sections of size less than 2µm were excluded.

2.4 Collection of data

Sections of 3µm thickness were prepared from the formalin fixed, paraffin embedded tissue blocks.

The sections were mounted on poly L-lysine coated slides for cytokeratin 19 expression the staining was done immunohistochemically using polymerase technique , the primary and secondary antibody for the study was obtained from Biogenex ,Bangalore.

All the sections were coded before staining for CK-19, Evaluation of cytokeratin 19 was done under light microscope under 10× objective and the intensity of staining of epithelium was assessed as (-) negative, (+) mild, (++) moderate, (+++) intense, the sections was decoded and results tabulated[1].

The intensity of staining was analyzed by the percentage of tissue section stained per slide.

If no tissue is stained -Negative
If 1/3 of the epithelium tissue is stained (approximately 33%) - Mild
If 2/3 of the epithelium tissue is stained (approximately 66%) - Moderate
If more than 2/3 of the epithelium tissue is stained (above 66%) -Intense[1].

Two independent observers evaluated the slides when discrepancy existed a third pathologist was asked to evaluate the slide to arrive at the consensus conclusion, 35 cases of oral submucos fibrosis section,10 cases of squamous cell carcinoma sections and 10 cases of normal oral mucosal sections were analysed. A total of 55 sections altogether were analysed.

3. STATISTICAL ANALYSIS:

The results were calculated using Fisher's exact test.All statistical analyses were done using STATA 14. P value less than 0.05 was considered statistically significant. Photomicrographs were obtained using CX-(Olympus) microscope with attachable camera.

Expression of CK19 in NOM, OSF and OSCC

Lesions/control	CK-19 EXPRESSION				Total
	negative	Mild	moderate	Intense	
OSF(35)	5(14%)	11(31%)	13(37%)	6(17%)	35(100%)
Squamous Cell carcinoma(10)	0	1(10%)	4(40%)	5(50%)	10(100%)
Normal oral mucosa tissue(10)	2(20%)	1(10%)	2(20%)	5(50%)	10(100%)
Total 55	7(12.7%)	13(23.6%)	19(34.5%)	16(29%)	55(100%)

*** P-value=0.138 TABLE 1: Expression of CK19 in NOM, OSF and OSCC**

4. RESULTS:

The comparison of Cytokeratin-19 expression between oral submucos fibrosis, normal oral mucosal tissue and oral squamous cell carcinoma is given in Table 1, Figure 1, supported by Figure 2, 3. Out of 35 cases of OSF, all 30 cases of OSF showed +ve staining, 10 cases of squamous carcinoma showed +ve staining & 8 cases of normal oral mucosal tissue showed positive staining . A total of 55 cases were positively stained in this study. Table 1 shows the difference between the intensity levels and percentage positivity in normal Oral mucosal tissue, oral submucos fibrosis and oral squamous cell carcinoma.

Out of 35 cases of OSF, 5 cases were negative, 11 cases showed mild staining, 13 cases showed moderate staining and 6 cases showed intense staining. Out of 10 cases of squamous cell carcinoma none of them were negative, 1case showed mild staining, 4 of the cases showed moderate staining and 5 of cases showed intense staining. Out of 10 cases of normal oral mucosal tissue 2 cases stained negative, 1 case showed mild staining, 2 of the cases showed moderate staining and 5 cases showed intense staining.

A total of 7 negative, 13 mild, 19 moderate and 16 intense positions for CK19 was seen in 55 cases. Table 1 showed, 14% of OSF cases, 0% of squamous cell carcinoma and 20% of normal oral mucosa tissue were negative for CK-19 staining. 31% of OSF cases, 10% of squamous cell carcinoma & 10% of normal oral mucosal tissue showed mild staining.37% of OSF cases, 40% of squamous cell carcinoma and 20% of normal mucosa tissue showed

moderate staining and 17% of OSF cases, 50% of squamous cell carcinoma and 50% of normal mucosal tissue showed intense staining.

After Fisher's Exact test The P value = 0.138ns. Since the P value is 0.138ns, there is no significant difference between the intensity levels and were not considered statistically significant.

5. DISCUSSION:

Oral potentially malignant disorder (OPMD) is a common term suggested to replace oral pre-cancer, Including both oral precancerous lesions and oral Precancerous conditions. All oral lesions that carry a risk of malignant transformation are included under this term. Oral submucos fibrosis in of the oral potentially malignant disorders[3].

Oral sub mucous fibrosis (OSF) is a chronic, progressive scarring disease, which predominantly affects the people of south- East Asian origin[4].

The early signs and symptoms of this disorder are intolerance to spicy food, altered salivation, ulceration, vesiculation, pigmentation changes, recurrent stomatitis and petechiaes. Extra orally, there may be loss of pigmentation or excessive pigmentation of vermilion borders in some of the cases[5].

The buccal mucosa, retro molar area and soft palate are the most commonly affected sites. The mucosa in this region develops a blotchy, marble – like pallor and a progressive stiffness of sub epithelial tissues[6]. Most of the OSF tissues used in our study were from buccal mucosa region

Clinically OSF cases can be categorized into three clinical stages according to their ability to open the mouth, as given below.

Stage I – Mouth opening ≥45 mm
Stage II – Restricted mouth opening 20- 44mm
Stage III – mouth opening ≤ 20mm[7]

A vivo study on mouse model for study of development of oral sub mucous fibrosis by Sumetha Perera showed the areca-nut treated oral epithelium showed progressive changes in epithelial thickness leading to atrophy, increased cellularity of fibroblasts, fibrosis of connective tissue , focal infiltration of inflammatory cells and muscle atrophy[8]

Histologically OSF can be grouped into four clearly definable stages: very early, early, moderately advanced and advanced. These stages are based on nature of the sub epithelial collagen, presence or absence of edema, physical state of the mucosal collagen, overall fibroblastic response state of blood vessels and predominant cell type in the inflammatory exudate. The connective tissue in advanced state is characterized by the sub mucosal deposition of extremely dense and a vascular collagenous tissue with variable numbers of chronic inflammatory cells[4].

Kiran Kumar et al (2007) proposed histological grading as follows:

Grade 1:
Loose, thick and thin fibers
Grade II:
Loose or thick fibers with partial hyalinization.
Grade III:
Complete hyalinization [9]

All epithelial tissues have cytokeratin in them and potentially malignant disorders like OSF have epithelial component.Cytokeratin that form the cytoskeletons of the epithelial cells are of several molecular types. The patterns of expression of these different types of CKs vary depending upon the type of epithelial cells and hence, they may be used as potential markers of cell differentiation and malignant transformation. CK 19, a type I (acidic) keratin, is the smallest keratin and is unique in that it lacks the Carboxyterminal, non - α - helical tail domain, which is typical for all other keratins[10].

In a study done by Ali Yousif et al Expressional profile of both markers (p16 and CK19) was different in same tumour. CK19 positivity was associated with age whereas p16 showed insignificant expression. The expression of P16 decreased while CK19 increased with the tumour grade.

This finding also shows that progressive loss of p16 and high expression of CK19 according to tumor metastasis. When p16 and CK19 evaluated together may have more accurate prediction of clinical outcome/prognostic marker in human OSCC and important molecular event in pathogenesis of oral carcinoma[11]

Anand Lalli, in his IHC study observed an increase of KI and K10 in the suprabasal layer, induction of K6 in the basal layer and completes loss of K19 in the epithelium. In a subset of the most severe OSF cases (14%), K17 expression was completely lost in the basal layer which might define them to be at most risk to undergo malignant transformation. There was no detectable expression of K8, K18, K7 and K9 and the expression of K4, K13, K14, K15 and K16 did not change in OSF [12].

In an IHC study by W.M. Tilakaratne, the data indicated that (hypoxia inducible factor) HIF-1α is up regulated at both protein and mRNA levels in OSF and the correlation with dysplasia is statistically significant. HIF –

1α may play a role in malignant transformation of OSF. Further over expression of HIF -1α may contribute to the progression of fibrosis. It may be possible to use HIF – 1α as a marker for malignant transformation of OSF[13]

In an Immunohistochemical study on increased CK19 expression correlated with pathologic differentiation grade and prognosis in oral squamous cell carcinoma patients by Lai-ping zhong, showed that CK 19 protein expression and gene transcription in OSSC tissue correlate significantly with pathologic differentiation grade. Positive CK 19 expression in distant tissue suggests a higher tumor recurrence rate and a lower survival rate[14].

Rajendran R in his Immunohistochemical study showed significant increase in the levels of stromal expression of (matrix metalloproteinase) MM P-1, MMP- 2 and MMP- 9 and (tissue inhibitors of metalloproteinase) TIMP – 1and TIMP- 2 using monospecific antibodies reacting against tissue antigens [15].

Unlike our study, an IHC study by K. Ranganathan, Significant difference in the CK staining pattern was seen between Normal oral mucosa, OSF and Oral cancer. Significant changes in OSF included increased intensity of staining for (Pan Cytokeratin) panCK and (high molecular weight cytokeratin) HMWCk, aberrant expression of CK8 and decreased expression of CKs 5 and 14[2].

Our study showed a total of 7 negative, 13 mild, 19 moderate and 16 intense positions for CK19 was seen in 55 cases.14% of OSF cases, 0% of squamous cell carcinoma and 20% of normal oral mucosa tissue were negative for CK-19 staining. 31% of OSF cases, 10% of squamous cell carcinoma & 10% of normal oral mucosal tissue showed mild staining.37% of OSF cases, 40% of squamous cell carcinoma and 20% of normal mucosa tissue showed moderate staining and 17% of OSF cases, 50% of squamous cell carcinoma and 50% of normal mucosal tissue showed intense staining and analysis showed insignificant difference in the expression compared to a IHC study done by Ranganath (2006), on Cytokeratin expression in oral submucos fibrosis, where 50 cases of OSF 10 each of normal and oral cancer constituted the study material. Basal staining in OSF and Basal & supra basal staining in normal and Oral cancer was seen. The CK 14 staining was seen in two cases (20%) of normal, one case (2%) of OSF and seven cases (70%) of oral cancers. Mild staining was seen in normal and OSF, in Oral cancer six (60%) exhibited mild staining while one (10%) showed moderate staining, the expression in OSF was less than that in normal's and Oral cancer (P=0.00)[2]

In a study done by Alam[1] ,CK 14 also did not show a significant difference in expression in Normal oral mucosa, Oral sub mucous fibrosis and Oral squamous cell carcinoma which is similar to our study with CK 19 indicating the fact that when cytokeratin's are individually assessed for staining with NOM,OSF and OSSC Usually they do not show any potential difference in expression, hence cannot be used individually as surrogate marker for malignant transformation.

6. CONCLUSION:

Though few studies have shown that pancytokeratins, HIF – 1α [2,13] can be used as a surrogate marker, In our study, there was no significant difference between the intensity levels, between NOM, OSF, OSCC, hence, this study showed that CK19 profile alone cannot be used to ascertain if it could be used as a surrogate marker for malignant transformation of OSF. Hence our study says that that CK-19 does not play a significant role alone in the transformation of Oral Sub mucous fibrosis to oral squamous cell carcinoma. More IHC studies have to be done on different cytokeratin individually to explore a wider diagnostic usage in future and rule out the fact established by few studies[1] that Cytokeratin types when individually assessed do not show a significant difference in there expressions. Thereby increasing their diagnostic and prognostic implementations.

7. ACKNOWLEDGEMENT:

We would like to acknowledge all the non-authors who have technically supported the study.

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