International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 8; Issue 02(D); February 2019; Page No.17346-17348
DOI: http://dx.doi.org/10.24327/ijcar.2019. 17348.3287

Research Article

CYTOKERATIN-15 EXPRESSION IN NORMAL ORAL MUCOSAL TISSUE, ORAL SUBMUCOUS FIBROSIS AND ORAL SQUAMOUS CELL CARCINOMA: AN IMMNOHISTO CHEMICAL STUDY

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A R T I C L E I N F O

Article History:
Received 10th November, 2018
Received in revised form 2nd December, 2018
Accepted 26th January, 2018
Published online 28th February, 2019

Key words:
Cytokeratin 15, oral submucous fibrosis (OSF), immunohistochemistry (IHC), squamous cell carcinoma (SCC), normal oralmucosa (NOM)

A B S T R A C T

Background: Oral submucous fibrosis (OSF) is a pre malignant condition caused by habitual use of areca nut, Cytokeratin (CK) are epithelia specific intermediate filament proteins, the expression of which is altered in precancerous and cancerous lesions the objective of this study was to evaluate the expression of Cytokeratin-15 profile in normal oral mucosal tissue (NOM), OSF and oral squamous cell carcinoma (SCC) and analyze the difference in the expression of cytokeratin-15, in these cases.
Materials and Methods: Immunohistochemical analysis of 10 NOM cases, 35 OSF and 10 oral SCC taken from patients and archives was done and the data were correlated.
Results: Seventeen% (17%) of OSF cases, 10% of SCC and 10% of NOM tissue were negative for CK-15 staining. 22.8% of OSF cases, 0% of SCC and 0% of NOM tissue showed mild staining. 34.2% of OSF cases, 40% of SCC and 30% of NOM tissue showed moderate staining and 26% of OSF cases, 60% of SCC and 10% of NOM tissue showed intense staining. Since the P value is 0.105ns, there is no significant difference between the intensity levels and were not considered statistically significant.
Conclusion: This study shows that CK15 profile alone cannot be used to ascertain if it could be used as a surrogate marker for malignant transformation.

INTRODUCTION

OSF is a premalignant condition (MohdKhairi *et al*. 2009) caused by habitual use of areca nut, affecting the oropharynx and characterized by progressive fibrosis, however very little is known of CK-15 alteration in OSF. The present study is carried out to characterize the CK15 profile in OSF and ascertain if this could be used as a surrogate marker for malignant transformation.

MATERIALS AND METHODS

Fifty five (55) cases, 10 of normal oral mucosa tissue, 35 of oral sub mucous fibrosis and 10 of oral squamous cell carcinoma had been taken for the study. Oral submucous fibrosis cases for this study had been collected from the patients attending the department of oral medicine, oral surgery and other departments of Century dental college and local clinics. Oral submucos fibrosis tissue sections had also been used from the archive of department of oral pathology, Century dental College, India, for which approval from the ethical committee was taken. Normal oral mucosa tissue samples were obtained from 10 patients during surgical removal of the third molar tooth. Ten paraffin embedded blocks or sections of already diagnosed cases of oral squamous cell carcinoma were obtained from the archives. Histologically confirmed cases of oral sub mucous fibrosis and oral squamous cell carcinoma were included and sections of size less than 2 mm were excluded.

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Collection of Data

Sections of 3 μm thickness were prepared from the formalin fixed, paraffin embedded tissue blocks. The sections were mounted on poly L-lysine coated slides for Cytokeratin 14 expression the staining was done immunohisto chemically using polymerase technique, the primary and secondary antibody for the study were obtained from Biogenex, Bangalore. All the sections were coded before staining for CK-15, Evaluation of cytokeratin 15 was done under light microscope under 10x objective and the intensity of staining of epithelium were assessed as (-) negative, (+) mild, (++) moderate, (+++) intense, the sections were decoded and results tabulated. The intensity of staining was analyzed by the percentage of tissue section stained per slide.

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

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 analyzed. A total of 55 sections altogether were analyzed. The chi-square test was used to analyze the difference between the intensity levels and percentage positivity in normal, OSF and OSCC for CK15. Differences with a probability value < 0.05 were considered statistically significant. The results were analyzed using chi-square statistical test for significance. Photomicrographs were obtained using CX-(Olympus) microscope.

RESULTS

Out of 35 cases of OSF, 29 cases of OSF showed +ve staining, 10 cases of squamous carcinoma showed +ve staining & 9 cases of normal oral mucosal tissue showed positive staining out of 10 cases. A total of 48 cases were positively stained in this study. 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, 6 cases were negative, 8 cases showed mild staining, 12 cases showed moderate staining and 9 cases showed intense staining. Out of 10 cases of squamous cell carcinoma none of them were negative or mild but 4 of the cases showed moderate staining and 6 of cases showed intense staining. Out of 10 cases of normal oral mucosal tissue 1 case stained negative, none of the cases were mild, 3 of the cases showed moderate staining and 6 cases showed intense staining. A total of 7 negative, 8 mild, 19 moderate and 21 intense positions for CK15 was seen in 55 cases. , 17% of OSF cases, 0% of squamous cell carcinoma and 10% of normal oral mucosa tissue were negative for CK-15 staining. 22.8% of OSF cases, 0% of squamous cell carcinoma & 0% of normal oral mucosal tissue showed mild staining.34.2% of OSF cases, 40% of squamous cell carcinoma and 30% of normal mucosa tissue showed moderate staining and 26% of OSF cases, 60% of squamous cell carcinoma and 10% of normal mucosal tissue showed intense staining. The χ 2 value was 10.504 and the P value = 0.105ns. Since the P value is 0.105ns, there is no significant difference between the intensity levels and were not considered statistically significant.

DISCUSSION

Areca nut chewing is an important risk factor for the development of oral submucos fibrosis especially the psychotropic alkaloids arecoline and areca dine in it. The areacanut polyphenols, catechin and tannins stabilize the collagen structure and make them less susceptible to collagenase. OSF fibroblasts have been shown to synthesize stable collagen structure and more lysyl oxidase. This reinforces collagen cross- linkages. The OSF fibroblasts also have been found to secrete less amount of collagenase and have less collagen phagocytic activity when compared to normal fibroblasts. All these phenomena lead to increase in number of collagen and thereby resulting in fibrosis. Cytokeratin 15: Cytokeratin intermediate filaments are present in essentially all epithelial cells and in neoplasms derived from them. Cytokeratin positivity is therefore a sensitive marker for carcinoma. The Cytokeratin are divided into more than 20 subtypes. This subdivision is based on their isoelective pH as well as their molecular weight. The acidic group includes Cytokeratin 9-19 and tends to be of lower molecular weight. The basic group (1-8) is of higher molecular weight. The low molar weight (LMW)

keratins are seen in more simple non- stratified epithelia and tumors derived there from, on the other hand, the high molecular weight (HMW) keratin are seen in more complex stratified squamous epithelia and their corresponding tumors.

An IHC study was done by Su and Morgan (1996), on Cytokeratin 14 and 18 expressions in normal, dysplastic and malignant oral epithelia. In normal epithelia, CK14 mRNA and protein were present almost exclusively in the basal layer of non-cornified and in rete- processes of cornified sites. Dysplastic epithelium showed irregular extension of the CK14 transcript and protein into superficial cells. In squamous cell carcinoma (SCC), CK14 transcript was abundant in most samples whilst in one poorly differentiated carcinoma, mRNA but no protein was detected. These findings indicate difference in the control of expression of CK14 & CK18 normal epithelia and show that regulation is further disturbed during dysplastic change and malignancy (Su and Morgan, 1996). In an IHC study, by Kelen *et al*. (1999) on Cytokeratin expression in initial oral mucositis of head & neck irradiated patients Concluded that increased CK expression can be associated with the reactive proliferation of epithelium & increasing resistance of the oral mucosa during the initial phase of radiotherapy. In a IHC study by Lalli and Tilakaratne (2008) on altered keratinocyte phenotype in oral submucos fibrosis, correlation of Cytokeratin17 expression with disease severity showed increased CK1 and CK10 expression in supra basal layers, induction of CK6 in the basal layer and complete loss of CK19 in the epithelium, there was increased CK17 expression in the suprabasal layers, which correlated with disease severity. There was no detectable expression of the CK18, CK7 & CK8 and the expression of CK4, CK13, CK14, CK15 and CK16 did not change. In OSF, Cytokeratin profiles could be useful as histological diagnostic markers and provide important insight into the pathogenesis of the disease and its predisposition to malignancy (Lalli A, 2008).In an IHC study by Ranganath (2006), on Cytokeratin expression in oral submucos fibrosis, 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).

CONCLUSION

It was concluded that CK15 does show a statistically significant expression in the study suggest that in epithelium in OSF alteration of CKs occur similar to that seen in pre-cancerous lesions & oral cancers but, In our study 29 cases of OSF out of 35, 10 cases of squamous cell carcinoma out of 10 and 9 cases of normal oral mucosal tissue out of 10 showed positive staining. Mild staining was seen in 22.8% (8 cases) of OSF cases and 0% of squamous cell carcinoma and normal oral mucosa tissue. 34.2% (12 cases) of OSF cases, 40% (4 cases) of squamous cell carcinoma and 30%(3 cases) of normal oral mucosal tissue showed moderate staining and 26%

(9 cases) of OSF cases, 60% (6 cases) of squamous cell carcinoma and 60% (6 cases) of normal mucosal tissue showed intense staining In our study, there was no significant difference between the intensity levels, between NOMT, OSF, OSCC, hence, this study showed that CK15 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-15 does not play a significant role alone in the transformation of Oral Sub mucous fibrosis to oral Squamous cell carcinoma.

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How to cite this article:

Shan Nawaz Malik (2019) 'Cytokeratin-15 Expression in Normal Oral Mucosal Tissue, Oral Submucous Fibrosis and Oral Squamous Cell Carcinoma: an Immnohisto chemical Study', *International Journal of Current Advanced Research,* 08(02), pp.17346-17348. DOI: http://dx.doi.org/10.24327/ijcar.2019.17348.3287

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