International Journal of Enhanced Research in Medicines & Dental Care (IJERMDC),
ISSN: 2349-1590, Vol. 5 Issue 2, February-2018, Impact Factor: 1.338

ORIGINAL RESEARCH

Expression of collagen III in Oral Submucous Fibrosis: An immunohistochemical study

Dr. Abhay Kumar[1], Dr. Daya Shankar[2], Dr. Sobhana Chandra[3]

[1,2]Senior Resident, Department of Dentistry, Patna Medical College & Hospital, Patna-4, Bihar
[3]Professor, Department of Conservative & Endodontics, Purvanchal Institute of Dental Sciences, Gorakhpur, U.P, India.

ABSTRACT

Background: Oral submucous fibrosis is a precancerous, collagen metabolic disorder caused due to fibrosis in oral mucosa. It is linked with the habit of chewing betel quid and areca nut.

Aim& Objective: the present study was planned for identification and expression of collagen III in different grades of Oral Submucous Fibrosis by immunohistochemistry and compared with normal mucosa.

Materials and Method: Paraffin sections of 80 cases of different grades of Oral Submucous Fibrosis and 10 cases of normal mucosa as control were stained with H&E and with antibody of collagen III. The identification and expression of collagen was evaluated on IHC stained sections and assessed by light microscope along with image analyzer software. The obtained data was analyzed statistically. Result: Collage III expression increment was statistically significant in submucosa region , for all groups but in muscle region Collagen III expression was statistically insignificant.

Conclusion: Collagen III expression was found to increase in grade I oral submucous fibrosis but subsequently expressed lesser as severity of submucous fibrosis increased. Thus collagen III increase seen as supplemental naïve.

Keywords: OSMF, COLLAGEN III, Immunohistochemistry.

INTRODUCTION

Oral Submucous Fibrosis (OSMF) is a premalignant condition , which has been even mentioned in ancient medical literatures **"Vidari"** under mouth and throat diseases by **Shrusrutha** in 2500 B.C[1]

OSMF is a well recognized as chronic, insidious high risk potentially malignant disease , usually attributed to the habits of consuming arecanuts. Due to unique in presentation, OSMF has recently drawn attention of researches worldwide. Collagen are most abundant, insoluble, extracellular, glycoproteins the human body, thus contributing to the stability of the tissues and organs and helping in the maintainace of their structural integrity in the body[2]

Adult oral mucosa comprises of 10-15% of type III collagen but majority by Collagen type I and others in lesser amount. Collagen type III (COL III) is predominant in fetal oral mucosa , thus is suggestive for developmental alteration in the synthesis and degradation of the relative other collagen types , as maturity happens in mucosal structure[3]

COL III is a homotrimer, consisted of three identical alpha I chains. Its encoded by COL III alpha I gene present on chromosome 2. Col III is frequently present in association with other collagens, especially Collagen I[4]. Direct involvement of collagens in the pathophysiology of various disease process has been established , so its important that the expression and role of collagens in OSMF should be assessed to understand the pathogenesis of the lesion. Thus this study aims to identify and quantify the collagen type III in OSMF using monoclonal COL III antibody and findings compared with those of normal oral mucosal tissues.

MATERIAL & METHODS

Paraffin embedded formalin fixed (FFPE) tissue sections of 4µ thickness were taken from 80 cases of OSMF and 10 cases of normal buccal mucosa for immunohistochemical staining & assessment of COL III expression.

Histopathological grading of OSMF was done according to **Pindborg and Sirsat** classification criteria on haemotoxylin and eosin (H&E) stained sections[5].

Immunostaining of sections was done by primary COL III and polymer HRP detection system provided by Biogenex Laboratories, CA, USA and procedure followed as directed by manufacturer.further the stained sections were evaluated for the intensity of staining by light microscope and was graded based on their staging intensity as 1 (for mild), 2 (for moderate) and 3 (for intense/deep). Collagen expression was evaluated by image analysis software and the final data obtained were tabulated and statistical analysis was done.

RESULT

The staining intensity for COL 3 in normal buccal mucosa and in Grade II and Grade III OSMF cases revealed to be moderate (score 2) but in Grade I cases of OSMF showed intense staining (score 3).(figure 1-3 & Table 1).

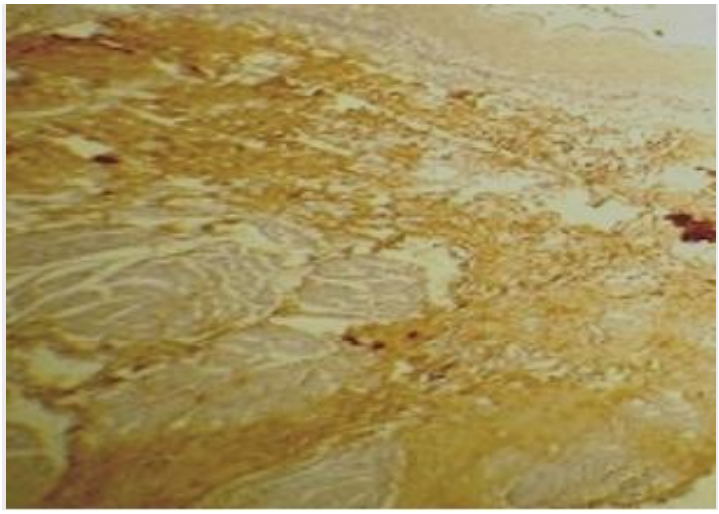


Fig.1: Grade I OSF case showing expression of collagen I antibody expression. Note the depth and intensity of the expression (×10)

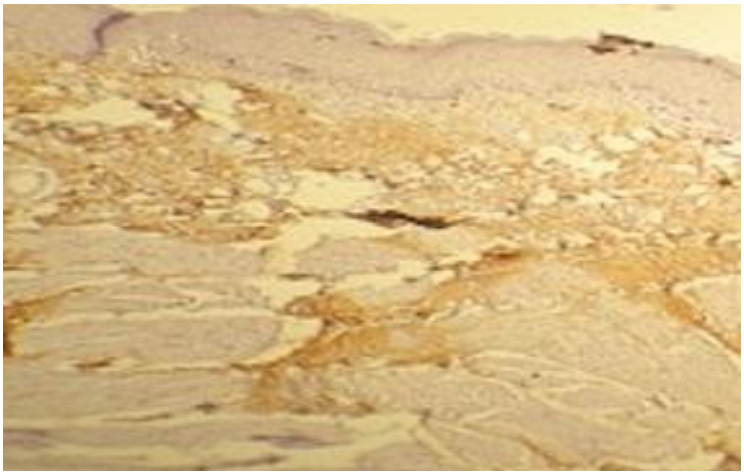


Fig.2: Grade I OSF case showing expression of collagen III antibody expression. Note the decreased intensity and decreased footprint of the antibody expression (×10)

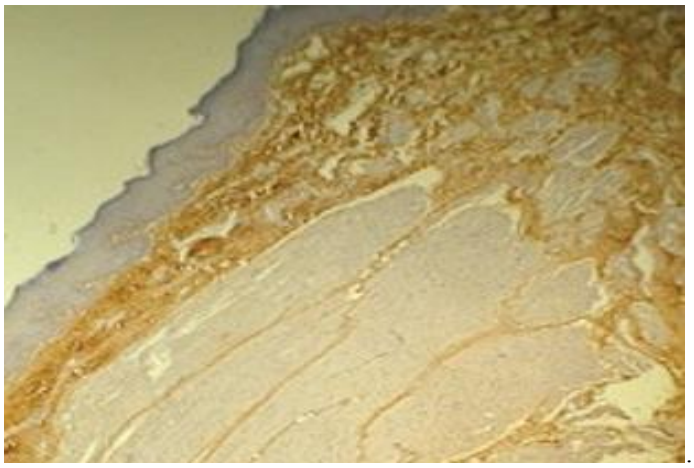


Fig. 3: Grade III OSF case showing expression of collagen I antibody expression. Note the increased intensity especially in the sub-epithelial areas where hyalinization and density of collagen are usually seen (×10)

Table 1:- Staining intensity of COL III (AS observed under light microscope)

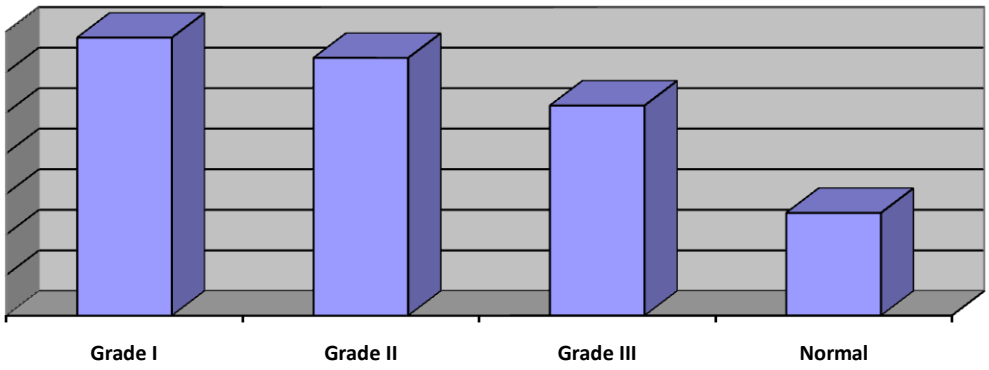
Staining Intensity of Col III (AS observed under light microscope)	
Groups	Staining intensity of col 3
Grade I OSMF (n=19)	3
Grade II OSMF (n=30)	2
Grade III OSMF (n=31)	2
Normal Buccal Mucosa (n=10)	2

The quantitative expression of COLIII in different study groups was analyzed by one way ANOVA test.
Mean distribution and quantitative expression of collagen III in submucosa of different grades of OSMF and controls (normal buccal mucosa).

Collagen III expression was found to be most in grade I OSMF in comaparision to othe study groups and the mean distribution was found to be statistically significant. (Table2 & Graph1)

Table 2:- Quantitative expression of COLIII in submucosa (One way Anova test)

Group	**Collagen III**		
	Mean ± SD	**F**	**P**
Grade I (n=19)	0.3426 ± 0.152	4.55	0.005 (S)
Grade II (n=30)	0.3175± 0.168		
Grade III (n=31)	0.2588±0.146		
Normal (n=10)	0.1265±0.068		



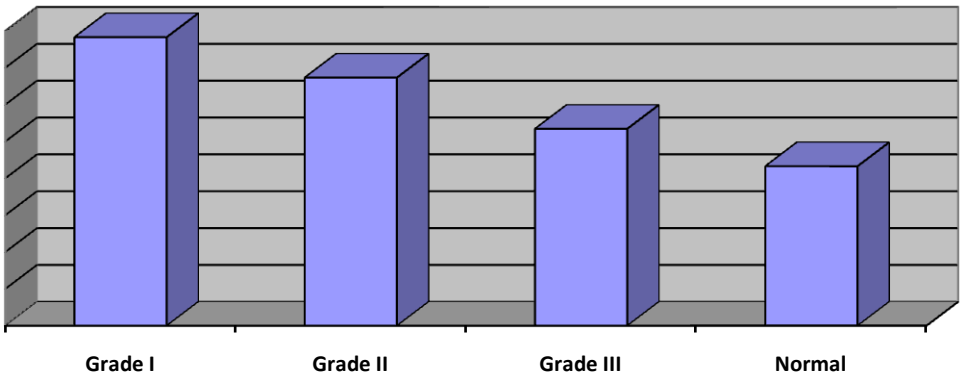
Graph 1:- Expression of COL III in Submucosa of OSMF

Mean distribution and quantitative expression of collagen III in muscle of different grades of OSMF and controls (normal buccal mucosa).

In grade I OSMF case, COL III expression was found to be more in comaparision to other grades of OSMF cases and in control(normal bucca mucosa). The mean distribution was found to be statistically insignificant.(Table 3 & Graph 2)

Table 3:- Quantitative expression of Collagen III in muscle area (One way Anova test)

Group	Collagen III Mean ± SD	F	P
Grade I (n=19)	.1565 ± .0403	0.41	0.567 (NS)
Grade II (n=30)	.1346± .0425		
Grade III (n=31)	.1068±.0368		
Normal (n=10)	.0865±.0463		



Graph 2:- Expression of COL III in muscle of OSMF

In OSMF tissues it seems to be trend of having increase in deposition of COL III, which in advance stages of OSMF gets replaced by other matured and more stable Collagens.

DISCUSSION

Oral Submucous Fibrosis is a condition caused due to over deposition of collagen fibers. Its pathognomic is directly linked to the clinical presentation of the pale oral mucosa, fibrotic bands and trismus and potentially malignant[6] researchers have been focusing on the type and pathogenesis of the fibrotic processes.

Collagen plays a vital role not only in maintaining structural integrity but also in determining tissue functions[7].
Though COL type I is present in most abundance but COL III is important for correlating for tissues extensibility[8].

Collagen type III plays an important role in the flexibility of the connective tissue of oral mucosa as well as in the regulation of the collagen fibril diameter in the tissue[9].

Utsunomiya et al reported abrupt increase of collagen 3 in grade I of OSMF. He noticed COL3 to be characteristically enhanced in the submucosa[10].

De Waal et al mentioned that a shift towards F3 fibroblasts might take place in OSMF and F3 were responsible for producing larger quantities of collagen. Increase in COL III along with other collagen (primarily COL I) in the progressive grades of OSMF was seen DE waal et al[11] mentioned a fact that the collagen type III seen to be localized around salivary glands , muscle fibers and blood vessels. This could be well correlated by Reddi, who mentioned type III collagen to be present in variety of connective tissues such as blood vessel walls, skin, muscle and placenta[12]. So it proves that in normal condition COL III is mainly present between muscle fibers, which helps in their extensibility and maintains flexibility among muscle bundles.

Havoarova et al revealed that in the group of varicose (dilated) veins prevailed the presence of COL3 in superficial veins of lower limb of human[13].

Xu et al reported , increase in collagen III in different grades of OSMF with progression of disease , which was statistically significant as in line with findings of this study.(i.e. COL III do increase with advancing grade of OSMF[14].

In this study, Col III antibody expression was ubiquitous in all tissues , albeit with varying intensity and localization.in early grade of OSMF, increase in expression of COL III was noted.

Later, showed a drop/ decrease in grade II and grade III of OSMF, indicating a replacement of COL III. The present results are in concordance with previous studies. The use of monoclonal antibody reduces the possibility of cross-reactivity and increases the accuracy of the findings obtained.

Similar results have been expressed in previous studies where COLIII enhancement have been reported[10].

Rubis et al in recent study revealed that dynamics of ECM turnover in DCM (dilated Cardiomyopathy) is high, which is reflected by the increased levels of CTGF (connective tissue growth factor) and degradation enzymes, helping in increase of synthesis of COL III. Thus proving that the fibrosis of ECM is related to the duration of the disease but is unrelated to the dynamics of collagen metabolism[15].

CONCLUSION

COL III was found to be increased in all grades of OSMF with a peak in the Grade I OSMF as compared with normal buccal mucosa. The pattern of collagen deposition appeared to be rather complex in the disorder of OSMF the role of Col III may be important in increased fibrosis and it appeared to act synergistically with its counterpart as its proved by COL III subsequent shift or decrease along the advancement in grades of OSMF. The net result is more stable form of collagen causing increased fibrosis in the OSMF tissues, which is reflected in the clinical conditions of Oral Submucous fibrosis.

REFERENCES

[1] Yoshimura K , Dissanayake UB . Morphological changes in oral mucosa and their connective tissue core regarding oral submucous fibrosis. Arch Histol Cytol 2005 ;68(3):185-192
[2] Gelse K, Pöschl E, Aigner T. Collagens – Structure, function, and biosynthesis. Adv Drug Deliv Rev: 2003;55:1531-46.
[3] Bornstein P, Sage H. Structurally distinct collagen types. Annu Rev Biochem 1980; 49:957-1003.
[4] Von der Mark K. Localization of collagen types in tissues. Int Rev Connect Tissue Res 1981; 9:265-324.
[5] Binnie WH, Cawson RA. A new ultrastructural finding in oral submucous fibrosis. Br J Dermatol. 1972;86:286-90.
[6] Pindborg JJ, Sirsat SM. Oral submucous fibrosis. Oral Surg Oral Med Oral Pathol 1966;22:764-72
[7] Shu-Wei Chang, Sandra J. Shefelbine. Structural and Mechanical differences between Collagen Homo and Hetrotrimers: Relevance for the molecular Origin of Brittle Bone disease. J Biophysical 2012;102:640-648
[8] Tiedtke J , Marks O. Stimulation of collagen production in human fibroblasts. Cosmetic Science technology 2007.15-18
[9] Xu L X , Ohsaki Y . Immunohistochemical stdies on the distributions and age related changes of type I and III collagen in the oral mucosa of mice. J dent res 1993; 72(9):1336-43

[10] Utsunomiya H , wanninayake M. Extracellular matrix remodeling on oral submucous fibrosis : its specific modes revealedby immunohistochemistry and in situ hybridization. J oral path & med 2005; 34(8): 498-507
[11] De Waal J, Olivier A. the fibroblast population in oral submucous fibrosis. J oral path & med 1997; 26:69-74
[12] Reddi A H. Collagen and cell differentiation- In "Biochemistry of collagen" ed; Plenum Press 1976 ; 449-78
[13] Haviarova Z, Janega P. Comparison of collagen subtype I and III presence in varicose and non-varicose vein walls. Brastisl lek listy 2008;109(3):102-105
[14] Liu X, Shu-Fan L. The expression of collagen type I and type III in oral sbmucous fibrosis. J oral med 2000;A:16
[15] Rubis P et al . Fibrosis of extracellular matrix is related to the duration of the disease but is unrelated to the metabolism in dilated cardiomyopathy. Inflamm Res.2016.