Myofibroblasts in Oral submucous fibrosis, Oral squamous cell carcinoma and Normal mucosa- an Immunohistochemical Analysis

Sivachandran Annamalai

SRM University

Shaik Mohamed Shamsudeen (✉ sshahul@kku.edu.sa)

King Khalid University <https://orcid.org/0000-0002-8222-6139>

Rooban thavarajah

Ragas Dental College and Hospital

Elizabeth jousha

Ragas Dental College and Hospital

Uma devi k Rao

Ragas Dental College and Hospital

Ranganathan Kannan

Ragas Dental College and Hospital

Research article

Keywords: Myofibroblasts, Alpha smooth muscle actin, Staining intensity, Oral Submucous Fibrosis, Oral Squamous Cell Carcinoma

Posted Date: December 2nd, 2019

DOI: <https://doi.org/10.21203/rs.2.17936/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Myofibroblasts are spindle shaped smooth muscle like fibroblast involved in the physiological repair of tissue structure can also cause pathological remodeling of tissue by forming fibrosis. They express smooth muscle actin which can be evaluated for their biological behavior in the physiological and pathological conditions.

Aim: To evaluate the expression of Myofibroblasts in oral submucous fibrosis, oral squamous cell carcinoma and normal mucosa by Immunohistochemical analysis. **Methods:** It is a cross-sectional study done to evaluate the expression of Myofibroblasts in oral submucous fibrosis (Group-1), oral squamous cell carcinoma (Group-2) and normal buccal mucosa (Group-3) using immunohistochemistry using alpha smooth muscle actin in formalin fixed, paraffin embedded tissue specimens. The localization of stain, nature of stain, intensity of stain and the percentage of cells stained among the three groups were studied and compared.

Results: The gender distributions in the study were in favor of males. It was observed that Staining Intensity was seen more in group I (30%) than in group II (20%) and progressively increased from early (16.5%) to advance stage(50%) of oral submucous fibrosis. Higher staining intensity was observed in well differentiated oral squamous cell carcinoma.

Conclusion: Myofibroblasts are important in normal healing process, but can induce fibrosis. In oral submucous fibrosis, they can be used to assess the severity of the lesion by their increased expression of alpha smooth muscle actin. In oral carcinogenesis increase in the number of alpha smooth muscle actin can change the distribution pattern results in tumor invasive characteristics. **Key Words:** Myofibroblasts, Alpha smooth muscle actin, Staining intensity, Oral Submucous Fibrosis, Oral Squamous Cell Carcinoma.

Background

Myofibroblasts are spindle shaped cells which produce collagen and having contractile properties like smooth-muscle, hence known as smooth muscle like fibroblast. They express alpha smooth muscle actin (α SMA)[1]. They primarily involved in wound healing by forming fibrous tissue and secrete extracellular matrixes helps in the repair process by reducing the physical size of the damage by their contractility properties. Once the tissue is repaired they disappear by apoptosis. Apart from the physiological repair process, they are involved in the pathological remodeling of the tissue in which they persist and form tissue deformation seen as hypertrophic scars [2].

Fibrosis is a progressive disease characterized by accumulation of scarring extracellular matrix proteins, which disrupt normal tissue architecture [2]. Oral Submucous Fibrosis (OSF) is one such disorder characterized by exuberant deposition of sub epithelial collagen in response to chronic areca nut chewing resulting in mucosal rigidity that leads to limitation in mouth opening. In various fibrotic disorders, the key cellular mediator of fibrosis is myofibroblasts, which when activated serves as primary collagen-producing cell [3, 4].

Myofibroblasts are also involved in the cancer progression by stimulating the microenvironment stromal cells. The activated Myofibroblasts express α -smooth muscle actin which represents the majority of tumor stromal cells [5]. Thus, it is necessary to understand the expression of myofibroblast in the molecular mechanism of oral submucous fibrosis and oral cancer progression.

Methods

The study material comprised of 50 formalin fixed, paraffin embedded tissue specimens from archival blocks. The samples are divided into 3 groups namely: Group I- Oral submucous fibrosis (20 samples), Group II-Oral squamous cell carcinoma (20 samples) and Group III – normal buccal mucosa (10 samples) taken as control group.

Tissue samples were taken from archival blocks. The tissue processing was done with routine haematoxylin and eosin staining (H & E). Immunohistochemical staining was done with α -smooth muscle actin as primary antibody (Biogenex –synthetic NH₂ terminal decapeptide of α -smooth muscle actin, Mouse monoclonal category and IgG2a immunoglobulin) and Secondary antibody used were Biogenex-super sensitive IHC detection system kit (Poly Horse Radish Peroxidase –pretitrated anti-species immunoglobulin labeled with enzyme polymer, super enhancer reagent, anti-mouse monoclonal negative control serum, and liquid DAB- Diamino-benzidine-chromogen) The technique for Immunohistochemical analysis was done as per the standardized method [Fig-1].

STAINING CRITERIA AND STAINING INDEX CALCULATION METHODS

Calculation of staining intensity (SI)

The calculation of staining intensity was considered as no stain visible as 0 (SI 0), if the staining visible only under 40X is calculated as 1 (SI 1), if staining visible under 10X as 2(SI 2) and staining visible even at 4X considered as 3 (SI 3).

Calculation of percentage of cells (Labeling Index-LI)

The calculation of percentage of cells labeling index was done as if there is no positive cells it was considered as 0 (LI 0), if 1-25% positive cells was seen it was calculated as 1 (LI 1), if 25-50% positive cells was seen calculated as 2 (LI 2) and 50-100% positive cells was seen as 3 (LI 3).

Calculation of staining index

It is derived from multiplication of staining intensity and percentage of cells. (SI x LI)

The final score is grouped into no stain if the score is 0, mild if the score is 1-2, moderate if the score is 3-4 and intense categories if the score is 6-9.

Statistical analysis:

Statistical analysis was done using SPSS TM software (version 11.5). $p \leq 0.05$ was considered to be statistically significant.

- Kruskal Wallis and Mann-Whitney test was done to compare tissue localization of stain, cellular location, and nature of stain, intensity of stain and the percentage of cells stained among the three study groups.
- The inter-observer variability for the intensity of stain and percentage of cells stained was assessed using kappa statistics.

Results

Twenty cases of OSF (Group I), 20 cases of OSCC (Group II) and 10 cases of clinically appearing normal mucosa (Group III) were analyzed for immune reactivity of myofibroblasts. All the samples in groups were taken from the buccal mucosa.

DISTRIBUTION OF GENDER AMONG GROUPS

The males were predominant in the study comprising 95% in group I, 75% in group II and 80% in group III.

The age groups were divided into 20- 40 years, 41 - 60 years and 61+ years. In Group I, 65% belonged to age Group 20-40 years and 35% belonged to age Group 41-60 years. In Group II, 10% belonged to age Group 20-40 years and 65% belonged to age Group 61+ years. In Group III 90% belonged to age Group 20-40 years and 10% belonged to age Group 41-60 years

DISTRIBUTION OF HABITS AMONG GROUP I (OSF) AND GROUP II (OSCC)

In group I & II, 31 had habits of tobacco and rest of them had no habits. Within those who had habits 9.7 % had no stain, 51.6 % had mild staining, 22.6 % had moderate staining and 16.1% had intense staining. Those who are taking tobacco had no staining 55.6% had mild staining 33.3% and 11.1% had moderate staining.

I. STAINING INTENSITY

Distribution of Staining Intensity (SI) Of α -SMA (Alpha Smooth Muscle Actin) Among 3 Groups:

α -SMA revealed positivity in group I, II and III. In Group I and Group II cases showed 85% staining for α -SMA, whereas in Group III, positive staining was observed 30%.

In Group I out of 20 OSF cases 30 % had a score of 3 (SI3), 20 % had a score of 2 (SI2) (**Fig-2**), 35 % had a score of 1 (SI1) (**Fig-3**) and 15% had a score of 0 (SI0). Group II out of 20 cases 20 % had a score of 3 (SI3), 30 % had a score of 2 (SI2) (**Fig-4**), 35 % had a score of 1 (SI1) (**Fig-5**) and 15% had a score of 0 (SI0). In Group III out of 10 normal cases 30 % had a score of 0 (SI0) and (**Fig -6**) 70 % had a score of 1 (SI1) (**Fig-7A**)

Distribution of Staining Intensity among Different OSF Grading:

In 20 cases of OSF, 6 cases belonged to Grade I, 10 cases belonged to Grade II and 4 cases belonged to Grade III. In Grade I 16.7% had a score of 0 (SI0), 50% had a score of 1 (SI1), 16.7 % had a score of 2 (SI2) and 16.7 % had a score of 3 (SI3). In Grade II 40 % had a score of 1 (SI1), 30 % had a score of 2 (SI2), and 30% had a score of 3 (SI3), In Grade III 50 % had a score of 0 (SI0) and 50 % had a score of 3 (SI3) (**Fig-7B**)

Distribution of Staining Intensity among Different OSCC Grading:

In 20 cases of OSCC, 13 cases belonged to well differentiated group, 3 cases belonged to moderately differentiated and 4 cases belonged to poorly differentiated. In well differentiated group, 23.1% had a score of 0 (SI0), 23.1% had a score of 1 (SI1), 23.1 % had a score of 2 (SI2) and 30.8 % had a score of 3 (SI3). In moderately differentiated group 66.7 % had a score of 1 (SI1) and 33.3 % had a score of 2 (SI2). In poorly differentiated group 50 % had a score of 1 (SI1) and 50 % had a score of 2 (SI2) (**Fig-7C**)

KAPPA STATISTIC VALUE

The inter-observer agreement for the staining intensity of stain for all the 3 groups was arrived using kappa statistics and kappa value is **0.689**.

1. PERCENTAGE OF IMMUNOPOSITIVE CELLS- LABELING INDEX (LI)

Distribution of Labeling Index (Li) Of α -SMA (Alpha Smooth Muscle Actin) Among 3 Groups:

α -SMA revealed positivity in group I, II and III. In Group I and Group II cases showed 85% staining for **α -SMA**, whereas in Group III, positive staining was observed 40%.

In Group I out of 20 OSF cases 5 % had a score of 3 (LI3), 40 % had a score of 2 (LI2), 40 % had a score of 1 (LI1) and 15% had a score of 0 (LI0). Group II out of 20 cases 15 % had a score of 0 (LI0) and 85 % had a score of 1 (LI1). In Group III out of 10 normal cases 60 % had a score of 0 (LI0) and 40 % had a score of 1 (LI1) (**Fig-8A**)

Distribution of Labeling Index (Li) Among Different OSF Grading:

Total 20 cases of OSF, 6 cases belonged to Grade I, 10 cases belonged to Grade II and 4 cases belonged to Grade III. In Grade I 16.7% have a score of 0 (LI0), 50% had a score of 1 (LI1) and 33.3 % had a score of

2 (LI2). In Grade II 50 % had a score of 1 (LI1), 40 % had a score of 2 (LI2) and 10% had a score of 3 (LI3). In Grade III 50 % had a score of 0 (LI0) and 50 % had a score of 2 (LI2) **(Fig-8B)**

Distribution of Labeling Index (Li) Among Different OSCC Grading:

Total 20 cases of OSCC, 13 cases belonged to well differentiated group, 3 cases belonged to moderately differentiated and 4 cases belonged to poorly differentiated. In well differentiated group, 23.1% had a score of 0 (LI0) and 76.9 % had a score of 1 (LI1). In moderately and poorly differentiated group all had a score of 1 (LI1) **(Fig-8C)**.

III. STAINING INDEX

Distribution of Staining Index among the Groups.

Using Kruskal-Wallis test the comparison among groups derived, there is a significant difference in the expression of myofibroblasts. **(Fig-9A)**

Comparison Of Staining Index Between Group I And Group III

In OSF, 15% of the cases showed no staining while 40% mild staining, 20% moderate staining and 25% of the cases had intense staining in connective tissue. In normal mucosa, 40% exhibited mild staining and no staining was seen in 60% of the cases. The result is statistically significant.

Comparison Of Staining Index Between Group II And Group III

In OSCC, 15% of the cases showed no staining while 65% mild staining and 20% moderate staining in connective tissue. In normal mucosa, 40% exhibited mild staining and no staining was seen in 60% of the cases. The result is statistically significant.

Comparison Of Staining Intensity Between Group I And Group II

In OSF group, 15% of the cases showed no staining while 40% mild staining, 20% moderate staining, and 25% of the cases had intense staining in connective tissue. In OSCC group, 15% of the cases showed no staining while 65% mild staining and 20% moderate staining in connective tissue. As both the lesions expressing myofibroblasts, the result is statistically not significant.

Comparison of Staining In Different OSF Grading

In Grade I, 16.7% showed no stain, 50% showed mild stain 16.7% moderate and 16.7 % showed intense staining. In Grade II 50 % showed mild staining 20 % showed moderate and 30% showed intense staining. In Grade III 50 % showed no stain, 25% showed moderate and 25 % showed intense staining. **(Fig-9B)**

Comparison of Staining In Different OSCC Grading

In well differentiated group 23.1% showed no stain, 46.2% showed mild stain and 30.8% showed moderate staining. In OSCC moderately differentiated 100% showed mild staining. In OSCC poorly differentiated 100% showed mild staining (**Fig-9C**).

Discussion

Myofibroblasts are smooth muscle like fibroblasts and one of the phenotypic heterogeneity among the fibroblasts. They can alter the inflammatory response by secreting the soluble mediators of inflammation. Furthermore myofibroblasts is suggested to be induced by TGF β , a potent pro-inflammatory and pro-fibrotic cytokine related to imbalance between collagen deposition and degradation in Oral Submucous Fibrosis [6]. Hence the role of myofibroblasts in the repair process has varying functions in the physiological and pathological conditions

The distribution of genders in the study groups ranged from a ratio 4:1 in favor of Males: Females in the control group and 19:1 in the OSF group. The male preponderance among OSF has been documented in several studies and can be due to the habit of using tobacco [7].

The age distribution of OSF is higher between 20-40 years as similar to other studies reported in the literature [7]. Similarly the occurrence of OSCC commonly occurs among older age groups as reflected in our study. As normal controls were obtained during impaction procedures 90% of they were obtained from 20-40 age groups.

Staining intensity:

Staining intensity between the three study groups was not statistically significant and the expression was seen more in group I than in group II (20%). The staining intensity progressively increased from early OSF (16.5%) to advance OSF (grade III 50%). This indicates that OSF represents a failed wound healing process of the oral mucosa after chronic sustained injury resulting in scarring and fibrosis. This could be also in response to the hypersensitivity caused by arecoline and the resultant persistent (juxtra epithelial inflammatory response) in OSF, which acts as an initiating factor leading to a defective inflammatory response and activation of fibroblasts culminating in fibrosis [8].

The well differentiated OSCC showed higher staining intensity indicating that myofibroblasts are stimulated during the repair of extra cellular matrix and are capable of augmenting and down-regulating the inflammatory response by secretion of these soluble mediators of inflammation [9].

Labeling index:

The labeling index indicates the more percentage of cells stain with α SMA in group I than in group II or III and can be due to chronic inflammation and continuous tissue remodeling in OSF as well as chronic

sustaining injury to factors such as arecoline, micro trauma could cause the transient or continuous differentiation of fibroblast from undifferentiated stem cell or a differentiated fibroblast pool [10].

The labeling index among the various grades of OSF shows an increase from grade 1 to III. This indicates with increase of stimulus and disease grade, more amount of myofibroblasts differentiation occurs [11].

However the difference between the grades were not statistically significant in OSCC with varying grades of differentiation, no significant difference could be found in the labeling index. Hence the expression of α SMA indicates that differentiation of tumor does not appear to influence the differentiation of myofibroblasts [11].

Staining index:

There was statistical significant difference in the pattern of staining index among the study group. Intense staining of α SMA was observed only in group I while moderate staining was seen in group I and II only. Group II had only the effect of inflammation and hence had a mild staining index in 40% of cases while no staining was seen in 60% of cases. The intense staining in group I was similar to the findings in the literature [13]. Though 65% cases of group II showed a mild SI and 20% cases had moderate SI, none had intense SI. The absence of intense SI in group II needs to further analyzed.

On comparing the Staining Index between OSF and normal tissue, it is observed that there was a wide difference between the expressions of SI using α SMA. The uniform mild expression in both cases probably is a result of the mild continuous inflammation in both the condition [14, 15].

On comparing the group II and group III, it is observed that there is only a mild difference between the grades of Staining Index. However it was statistically significant. The uniformity in expression probably relates to the chronic inflammation that exists between both the conditions [15].

There is no significant difference between group I and II ($p=0.157$) However 24% of cases exhibited intense staining in group I while no cases of group II had intense SI. This indicates that OSF had more myofibroblasts expression than OSCC [16, 17].

The staining index did not considerably vary within the grades of the OSF. This indicates that the staining intensity as well as labeling index is crucial factor for the staining index. In this cross sectional study the expression of SI 3 increased with increasing grades of OSF. While no such observation was seen in labeling index. Low number of cells expressed SMA positivity while the staining intensity varied. This indicates that with grades of OSF increasing only a small subset of fibroblast population expressed intensive staining while the number of cells expressing α SMA never altered [18,19].

Conclusions

Persistent, chronic inflammation leads to formation of a subset of fibroblastic population-myofibroblasts. The progressive increase of myofibroblasts in the Oral submucous fibrosis can be used as a marker in evaluating their severity. Myofibroblasts stimulate tumor development and constitute the tumor stroma

and their presence in the invasion front of the tumor suggests they are associated with tumorigenesis. Clinical evidence suggests that the presence of myofibroblasts is associated with a poor prognosis have to be evaluated.

Abbreviations

α SMA- alpha smooth muscle actin

OSF- Oral submucous fibrosis

OSCC- Oral squamous cell carcinoma

SI- staining intensity

LI – labeling index

TGF β -transforming growth factor beta

Declarations

Ethics approval and Consent to participate:

The study protocol was evaluated and approved by the Institutional Ethics Committee of the Ragas Dental College and Hospital, Chennai.

Consent for Publication: Not applicable

Availability of data and material: The data and material can be available on request.

Competing interests: The authors declare that they have no competing interests.

Funding: The research did not receive any financial support from funding agencies.

Affiliations:

Department of Oral pathology, SRM University, College Dentistry,kattankulathur,Chennai,India

Dr. Sivachandran Annamalai

Department of dental diagnostic Science & Oral Biology, College Of Dentistry, King Khalid University ,Abha,Saudi Arabia

Dr. Shaik Mohamed Shamsudeen

Dr. Rooban T, Dr. Elizabeth Joshua, Dr. Umadevi K Rao Dr. Ranganathan Kannan

Author's contribution:

Conception , Design the study (SA, EJ, UR, RK),Acquisition of data and biological samples(SA,SM), Data analysis and interpretation (EJ, UR, RK, SA,RT,SM)Manuscript drafting and editing (SM,SA),Final approval of manuscript (EJ, UR, RK, SA,RT,SM).

Acknowledgment: NIL

References

1. Punnya V. Angadi, Alka D. Kale, Seema Hallikerimath. Evaluation of myofibroblasts in oral submucous fibrosis: correlation with disease severity. *Journal of Oral Pathology & Medicine* 2011; 40(3) : 208–213.
2. Steven E. Mutsaers, Jill E. Bishop, Gus Mcgrouter, Geoffrey J. Laurent. Mechanisms of Tissue Repair: from Wound Healing to Fibrosis. *J. Biochem. Cell Bid.*1997; 29: 5-17.
3. Kundendu AryaBishen, Raghu Radhakrishnan, Kapaettu Satyamoorthy. The role of basic fibroblast growth factor in oral submucous fibrosis pathogenesis. *J Oral Pathol Med* 2008; 37: 402–411.
4. Tilakaratne WM, Klinikowski M.F, Takashi Saku, Peters TJ, SamanWarnakulasuriya. Oral submucous fibrosis: Review on aetiology and pathogenesis. *Oral Oncology* 2006; 42:561– 568.
5. Hu Yanjia, JianXinchun. The role of epithelial–mesenchymal transition in oral squamous cell carcinoma and oral submucous fibrosis. *Clinica Chimica Acta* 2007; 383: 51–566.
6. Mithani SK, Mydlarz WK, Grumbine FL, Smith IM, Califano JA. Molecular genetics of premalignant oral lesions. *Oral Dis* 2007; 13:126-33.
7. Ranganathan K, Devi UM , Joshua E, Kirankumar K, SaraswathiR. Oral submucous fibrosis: a case-control study in Chennai, South India.. *J Oral Pathol Med* 2004; 33:274–277
8. Rajalalitha, S. Vali. Molecular pathogenesis of oral submucous fibrosis – a collagen metabolic disorder. *J Oral Pathol Med.* 2005; 34:321-8.
9. Beacham DA, Cukierman E. Stromagenesis: the changing face of fibroblastic microenvironments during tumor progression. *Semin Cancer Biol.* 2005; 15:329–41.
10. Xouri G, Christian S. Origin and function of tumor stromafibroblasts. *Semin Cell Dev Biol* 2010; 21:40-6.
11. Wynn TA. Cellular and molecular mechanisms of fibrosis. *J Pathol* 2008; 214:199-210.
12. Lúcio PS, Cavalcanti AL, Alves PM, Godoy GP, Nonaka CF. Myofibroblasts and their relationship with oral squamous cell carcinoma. *Braz J Otorhinolaryngol* 2013; 79:112-

13. Philip T, Kumar TD, Rajkumar K, Karthik KR, Priyadharsini N, Kumar AR. Immunohistochemical evaluation of myofibroblasts using alpha-smooth muscle actin in oral submucous fibrosis. *SRM J Res Dent Sci* 2014; 4:243-7.
14. Utsunomiya H, Tilakaratne WM, Oshiro K, Maruyama S, Suzuki M, Ida-Yonemochi H. Extracellular matrix remodeling in oral submucous fibrosis: Its stage-specific modes revealed by immunohistochemistry and in situ J *Oral Pathol Med* 2005;34:498-507.
15. Darby I, Skalli O, Gabbiani G. Alpha-smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. *Lab Invest* 1990; 63:21-9.
16. Chaudhary M, Gadgil AR, Vidhale G, Mankar Gadgil MP, Gondivkar SM, Gawande M. Comparison of myofibroblasts expression in oral squamous cell carcinoma, verrucous carcinoma, high risk epithelial dysplasia, low risk epithelial dysplasia and normal oral mucosa. *Head Neck Pathol* 2012; 6:305-
17. Kellermann MG, Sobral LM, Da Silva SD, et al. Myofibroblasts in the stroma of oral squamous cell carcinoma is associated with poor prognosis. *Histopathology* 2007; 51: 849–53.
18. Desmoulière A, Guyot C, Gabbiani G. The stroma reaction myofibroblast: A key player in the control of tumor cell behavior. *Int J Dev Biol* 2004; 48:509-17.
19. Gupta MK, Mhaske S, Ragavendra R, Imtiyaz. Oral submucous fibrosis – Current Concepts in etiopathogenesis. *Peoples J Sci Res* 2008; 1:39-44.

Figures

IHC PROCEDURE FLOW CHART

- APES coated slides with 2 paraffin embedded tissues
- Placed in xylene thrice (5 minutes each)
- Placed in 100% isopropanol (5 minutes)
- Placed in 70% isopropanol (5 minutes)
- Washed in distilled water thrice (5 minutes each)
- Placed in 3% hydrogen peroxide (20 minutes)
- Kept in citrate buffer at pH 6 and autoclaved for antigen retrieval and bench cooled for 40 minutes
- Washed in distilled water thrice (5 minutes)
- Protein blocking serum added and incubated for one hour
- Primary antibody added to the specimen and incubated for one hour
- Washed in PBS thrice (5 minutes each)
- Secondary antibody added and incubated in an enclosed hydrated container (30 minutes)
- Washed in PBS thrice (5 minutes each)
- Avidin biotin enzyme reagent added and incubated (30 minutes)
- Washed in PBS thrice (5 minutes each)
- DAB added and incubated in an enclosed hydrated container (5 minutes)
- Washed in PBS thrice (5 minutes each)
- Stained with haematoxylin (20 seconds)
- Washed in tap water
- Placed in 70% isopropanol (1 minute)
- Placed in 90% isopropanol (1 minute)
- Placed in 100% isopropanol (1 minute)
- Placed in xylene (1 dip)
- Slides were mounted using DPX
- Slides were observed under the LM and graded.

Figure 1

Immunohistochemistry protocol

Figure 2

Immunohistochemical stained section showed myofibroblast in OSF-10x

Figure 3

Immunohistochemical stained section showed myofibroblast in OSF -40x

Figure 4

Immunohistochemical stained section showed myofibroblast in OSCC-10x

Figure 5

Immunohistochemical stained section showed myofibroblast in OSCC -40x

Figure 6

Immunohistochemical stained section showed myofibroblast in normal tissue-10x

Fig-7A : DISTRIBUTION OF STAINING INTENSITY AMONG THE GROUPS

Fig 7B: DISTRIBUTION OF STAINING INTENSITY AMONG DIFFERENT OSF GRADINGS

Fig-7C: DISTRIBUTION OF STAINING INTENSITY AMONG DIFFERENT OSCC GRADINGS

Figure 7

A: Distribution of staining intensity among the groups B: Distribution of staining intensity among different OSF gradings C: Distribution of staining intensity among different OSCC gradings

Fig-8A -DISTRIBUTION OF PERCENTAGE OF CELLS AMONG THE GROUPS

Fig-8B: DISTRIBUTION OF PERCENTAGE OF CELLS AMONG DIFFERENT OSF GRADINGS

Fig 8C: DISTRIBUTION OF PERCENTAGE OF CELLS AMONG DIFFERENT OSCC GRADINGS

Figure 8

A: Distribution of percentage of cells among the groups B: Distribution of percentage of cells among different OSF gradings C: Distribution of percentage of cells among different OSCC gradings

Fig-9A : DISTRIBUTION OF STAINING INDEX AMONG THE GROUPS

Fig 9B: DISTRIBUTION OF STAINING INDEX AMONG DIFFERENT OSF GRADINGS

Fig 9C: DISTRIBUTION OF STAINING INDEX AMONG DIFFERENT OSCC GRADINGS

Figure 9

A: Distribution of staining index among the groups B: distribution of staining index among different OSF gradings C: distribution of staining index among different OSCC gradings