

IMMUNOHISTOCHEMICAL ANALYSIS OF MYOFIBROBLASTS USING α -SMA IN ORAL SUBMUCOUS FIBROSIS TURNING INTO MALIGNANCY- A CASE REPORT AND REVIEW

ABSTRACT:

Oral submucous fibrosis is a premalignant condition, characterized by fibrosis in oral mucosa causing stiffness of mucosa and leading to reduced mouth opening. The malignant transformation of oral submucous fibrosis is very high among the rest of the premalignant conditions. Chronic chewing of arecanut causes production of myofibroblasts. These myofibroblasts play an important role in neoangiogenesis as well as disintegration of the basement membrane at the tumor tumor invasive front leading to carcinoma. Here, we are doing an Immunohistochemical study using alpha SMA for proper microscopic visualizations of myofibroblastic activity in aOSFpatient, encompassing the pathogenesis and malignant potentiality of the disease.

Key words: *Arecanut, malignant transformation, oral cancer, α -SMA, oral submucous fibrosis, myofibroblast.*

INTRODUCTION

Oral submucous fibrosis is an insidious chronic disease characterized by juxta-epithelial hyalinization leading to muscle stiffness and rigidity causing difficulty in eating, speaking and swallowing. The condition was first reported by Schwartz (1952) in the name of "Atrophidiopathica (tropica) mucosae oris". The present name oral submucous fibrosis was coined by Dr Joshi in the year 1953¹. It is seen primarily in the Indian subcontinent, Southeast Asia, Taiwan, southern China, Polynesia, and Micronesia. The condition affects more than 5 million people in India alone. Cases among Asian communities in North America, Europe, and Africa also have been reported². A malignant transformation rate of 7.6% over a period of 10years has been documented³. It has been reported that activity of myofibroblasts are responsible for this malignant transformation⁴.

The importance of stromal myofibroblast in tumor angiogenesis is further highlighted in a study demonstrating that recruited myofibroblasts act as a secondary source of VEGF and compensate for the loss of VEGF in tumor cells⁵. Fibroblasts are widely recognized as a critical cell types involved in wound healing and tissue repair⁴ but TGF β -1 secreted by flavinoids induces oral fibroblast transdifferentiation into myofibroblast due to its ability to modify the extracellular matrix; myofibroblasts plays an important role in tumor invasion and metastasis⁶.

Myofibroblasts were first identified in granulation tissue; they are capable of speeding wound repair by contracting the edges of the wound. There are many possible ways of myofibroblast development:

1. Partial smooth muscle differentiation of a fibroblastic cell
2. Activation of a stellate cell (e.g. hepatic Ito cells or pancreatic stellate cells).
3. Loss of contractile phenotype (or acquisition of "synthetic phenotype") of a smooth muscle cell.
4. Direct myofibroblastic differentiation of a progenitor cell resident in a stromal tissue.
5. Homing and recruitment of a circulating mesenchymal precursor which can directly differentiate as above or indirectly differentiate through the other cell types as intermediates.
6. Epithelial to mesenchymal transdifferentiation (EMT) of an epithelial cell.

Myofibroblast cells in particular plays a crucial role in tumor progression, neoangiogenesis and tumor metastasis.

CASE REPORT

A 45-year-old male patient reported to the Department of Oral pathology and Microbiology with a chief complaint of reduced mouth opening and difficulty in eating accompanied by burning sensation. (Fig:1) Patient was histopathologically diagnosed as Oral sub-mucous fibrosis, by gold standard H&E stain (Fig:2,3) and Immunohistochemistry was performed to see the myofibroblastic activity (Fig:4). Patient's habit counseling was done as well as medications were given along with exercises were advised. Interestingly the patient again came back after 3 months

with the complaint of severe pain and non-healing ulcer in the right buccal mucosa with mouth opening now severely compromised (Fig: 5). He had habit of chewing tobacco, 4-5 packets/day, and alcohol consumption on daily basis since for last 25 years, which even after habit counseling he did not quit.

Extraoral examination revealed facial asymmetry, bilaterally palpable, fixed and tender submandibular lymph nodes.(Fig: 5). Intraoral examination revealed ulcero-proliferative growth on the right buccal mucosa involving the vestibular region, measuring approximately 3 × 2 cm extending from 45 to 48 region on buccal mucosa and the buccal vestibule. Lesion appeared to be blanched and was covered with areas of slough.(Fig:6). On palpation, findings revealed induration and tenderness. There was reduced mouth opening measuring approximately 18 cm.(Intercanine distance). The tongue appeared whitish in diffused areas with few petechial spots. Radiographic features revealed an ill-defined radiolucency with vertical bone loss with respect to 45, 46, 47, and 48 region(Fig: 7).

The gold standard biopsy was performed again. The Hematoxylin&Eosin Stained section reveals the presence of an ulcerated surface epithelium showing dysplastic changes throughout. The dysplastic features include nuclear hyperchromatism, nuclear and cellular pleomorphism (presence of most of atypical cells seems to be spindle shaped), increased nuclear cytoplasmic ratio, increased and abnormal mitotic figures (Fig:9). There was presence of actively proliferating, neoplastic stratified squamous epithelial cells seen breaching the basement membrane and invading deep into the underlying connective tissue stroma. The fibrovascular dense connective tissue stroma characterized by the presence of hyalinization and homogenization of the collagen fibers along with perivascular fibrosis and perineural invasion(Fig.9). Histopathologically it was reported to be a case of moderately differentiated squamous cell carcinoma. For academic interest we also performed the Immunohistochemistry which was suggestive of increased myofibroblastic activity along with neo-angiogenesis. This was a significant finding to show the malignant transformation within the lesional tissue.

DISCUSSION

Oral submucous fibrosis, considered as a potentially malignant disorder with the malignant transformation rate as 2.3-7.6% in the Indian context⁶. The main etiology of the OSF is said to be

betel nut either the ripened fruit or the fresh green fruit that is taken by people all around the world. It is the by-product of the areca nut namely alkaloids¹, flavonoids and trace elements⁷. The alkaloids present are arecaidine, arecoline, guvacin and guvacoline, considered to be most potent cause along with flavonoid component including tannins and catechins. which causes collagen disturbances as well as enhanced myofibroblastic activity⁴. Myofibroblasts secrete cytokines and matrix metalloproteinases which in turn contribute to the destruction of extracellular matrix and cause tumor growth⁸.

The pathogenesis of OSF includes genetic predisposition including polymorphism of genes such as cytochrome P450 3A, along with this it also lies in increased collagen production followed by cross-linking of the produced collagen as well as less collagen destruction. Both OSF- and normal cells produce ~85% type I collagen and ~15% type III collagen. In OSF cells, however, the ratio of the $\alpha 1(I)$ to $\alpha 2(I)$ chains of type I collagen is ~3:1 whereas in normal cells it is ~2:1⁹ all these ultimately results in increased collagen in the stroma of mucosa. Other factors such as iron and nutritional deficiencies of other micronutrients, chronic candidiasis, genetic abnormalities, herpes simplex virus, human papilloma virus, autoimmunity⁹, etc., have been postulated and are known to have either direct effect in causing the disease or an indirect effect by mediating the immune system which is compromised in these precancerous conditions.

As the patient continues the intake of areca nut, along with collagen discrepancies, the Zn/Fe ratio gets altered leading decreased Zn amount which is a free radical scavenger and increased Fe content which increases the tendency of collagen formation, more of copper release which increases the action of lysyl oxidase activity which catalyses the conversion of lysine molecules into highly reactive aldehydes that forms cross-links in extracellular matrix protein that is collagen.

Oral submucous fibrosis often manifests in young adult betel quid users. Typical chief complaints include an inability to open the mouth (trismus) and stomatopyrosis with intolerance to spicy foods¹¹. An interincisal distance of less than 20 mm is considered to be in severe stage²; in advanced cases, the jaws may be inseparable leading to decreased food uptake which additionally leads nutritional deficiency. As the individual continues the habit the energy demand remains same or increases for that matter, but due to nutritional deficiency muscles get fatigued leading to reduced mouth opening. Additionally if the habit continues atrophy of the mucosa takes

place making the patient experience stomatopyrosis, these small cuts acts as the gate way for other carcinogens to reach the basal cells of the mucosa⁴ commencing malignant transformation to squamous cell carcinoma⁹. Squamous cell carcinoma accounts for more than 90% of oral malignancies. During betel nut chewing, the thick fibers injure the oral mucosa which causes inflammation of epidermal cells and activates macrophages to secrete cytokines. Transforming growth factor- β (TGF- β) is a major cytokine involved in OSF progression. It regulates the expression of α -SMA and type 1 collagen in myofibroblasts¹⁰ (Figure:10).

Histopathological features of Oral submucous fibrosis is characterized by juxtaepithelial and submucosal deposition of densely collagenized the collagen arrangement gets more organized with advancing histological grade, hypovascular connective tissue with variable numbers and type of chronic inflammatory cells². Epithelial changes include hyperkeratosis with marked epithelial atrophy in older lesions rarely ulcerations may also be seen in some cases. Epithelial dysplasia is found in 10% to 15% of cases submitted for biopsy, and carcinoma is found in at least 6% of sampled cases among them we will be discussing one of the case². Dysplasia leads to Carcinoma-in-situ further to breach in basement membrane leading to oral squamous cell carcinoma.⁸ Oral carcinogenesis is a multistage process, which often simultaneously involves precancerous lesions, progresses further with invasion and metastasis. Oral squamous cell carcinoma (OSCC) is one of the most common malignancies worldwide. It is 95% of all forms of head and neck cancer and over the last decade its incidence has increased by 50%. Its pathogenesis is multifactorial, predominantly associated with smoking, alcohol and snuff, as well as the involvement of human papilloma virus infection¹². OSCC is the most common malignant neoplasm derived from the stratified squamous epithelium of the oral mucosa. According to the International Histological Classification of Tumors; histological characteristic of the lesions is based on the degree of tumor differentiation, which include well-differentiated tumors, moderately-differentiated tumors and the undifferentiated (anaplastic) tumors. The degree of differentiation is essential to evaluate the tumor's growth rate and ability to metastasize¹².

Histopathologic features of a squamous cell carcinoma is well known, where the malignant neoplastic epithelial cells shows abundant eosinophilic cytoplasm with large, often darkly staining (hyperchromatic) nuclei along with an increased nuclear-to-cytoplasmic ratio, with varying degrees of cellular and nuclear pleomorphism². Stroma seems be highly vascular with

neo-angiogenesis, presence of malignant neoplastic epithelial cells along with trans-differentiated fibroblasts, namely the myfibroblasts. Stromal myofibroblasts were heterogeneously detected in OSCC and its presence was higher in tumors with a more diffuse histological pattern of invasion¹³. These myofibroblasts can be identified by certain characteristic features of the cytoskeleton, particularly by the expression of α -smooth muscle actin (SMA)¹³ (Fig. 10).

CONCLUSION

OSF is considered as a potentially malignant disorder and this potentiality is significantly attributed by the myofibroblasts, which not only induces carcinogenesis but also causes tumor progression. A very faster rate of malignant transformation is evident where there is increased myofibroblastic activity which is thought to be due to continuation of the persistent deleterious habit of the patient which we evaluated by α -SMA IHC staining. Hence, to prevent the risk of malignant transformation, habit counseling needs to be done aggressively irrespective of age, community and socio-economic status along with urgent regulatory actions are therefore needed to control the manufacture, marketing and the consumption of products that contain carcinogens.⁵

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Figure 1: Extraoral photograph showing reduced mouth opening.



Figure 2: Photomicrograph shows parakeratinized stratified squamous epithelium along with hyalinized fibrovascular stroma .(H and E 10X).

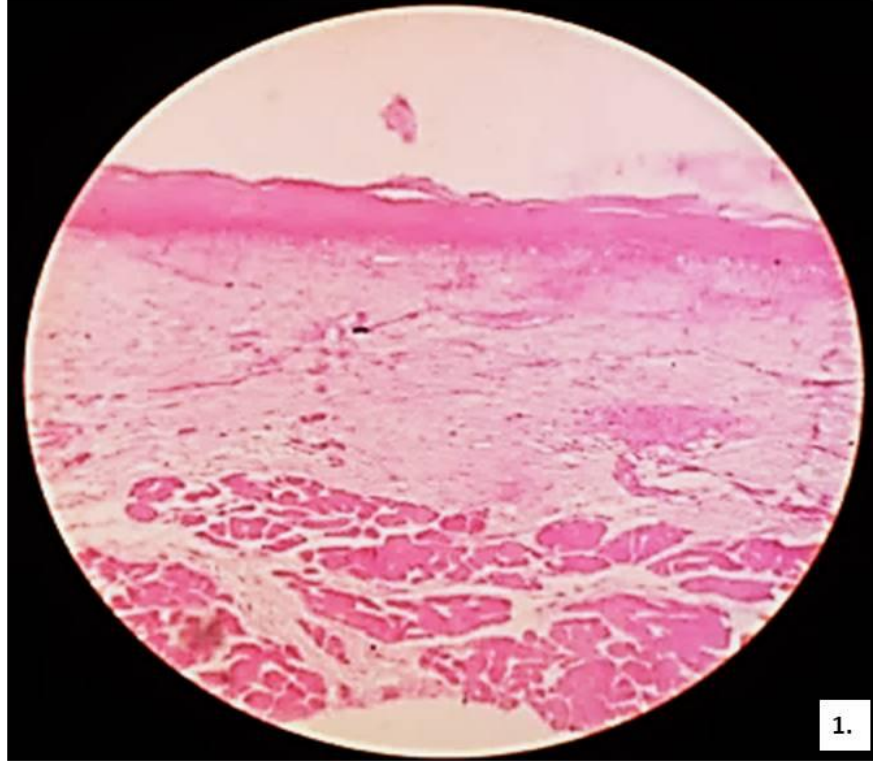


Figure 3: Photomicrograph showing hyalinized stroma(H& E 40X).

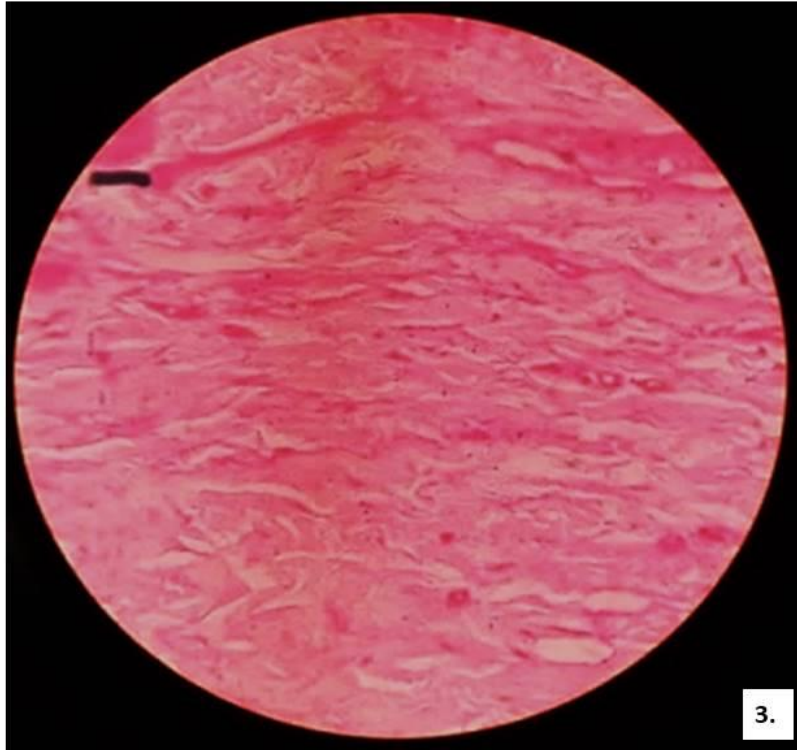


Figure 4: Photomicrograph showing IHC with alpha- SMA of the same section (40X).

UNDER PPL

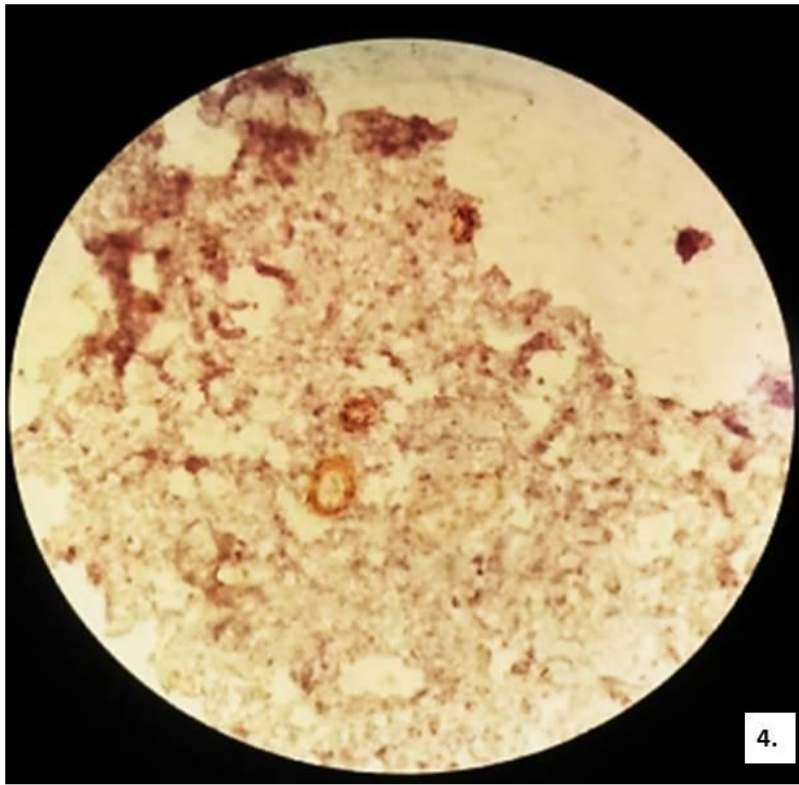


Figure 5: Extraoral photograph showing facial asymmetry.

UNDER PPL



Figure 6: Intraoral photograph showing an ulceroproliferative growth involving the buccal Mucosa.

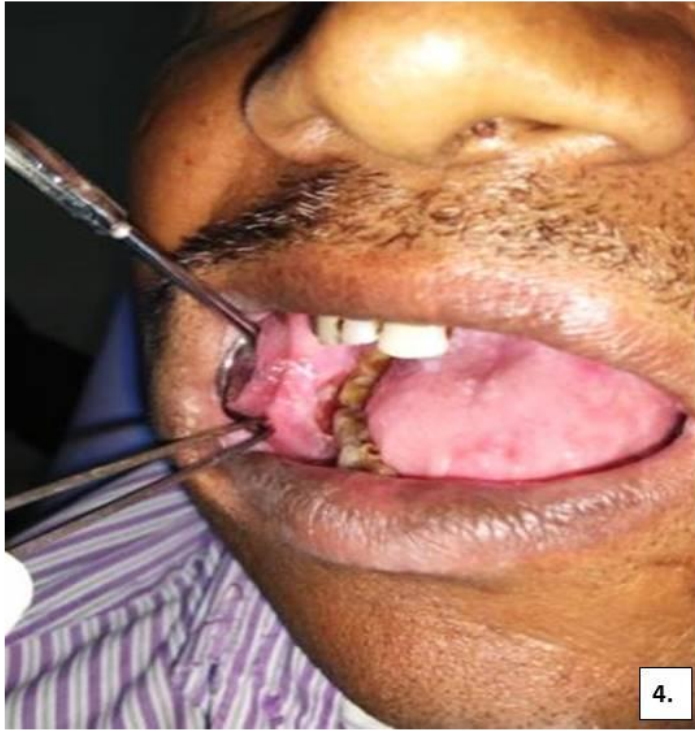


Figure7: OPG reveals an ill defined radiolucency in respect to 45,46, 47, 48.



Figure 8: Photomicrograph showing ulcerated epithelium with breach in the basement membrane(H and E 10X).

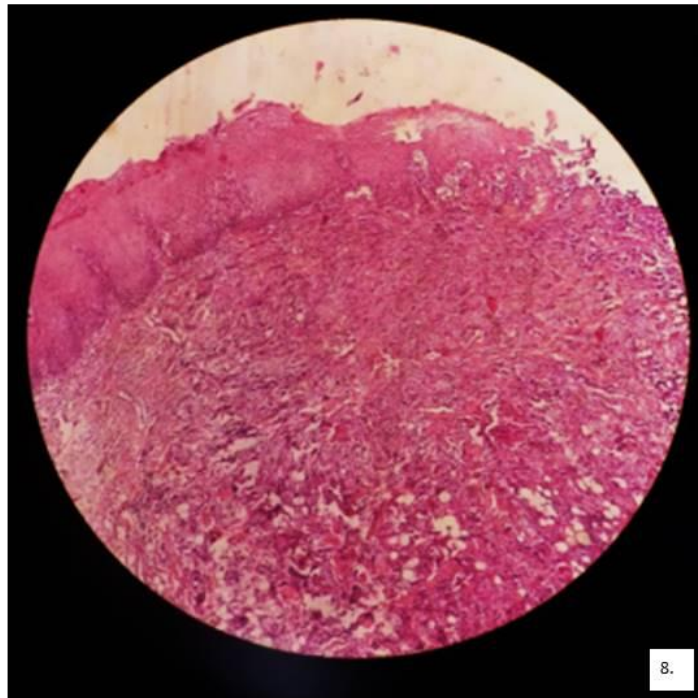


Figure 9: Photomicrograph showing the connective tissue having numerous neoplastic malignant epithelial cells within stroma (H and E 40X).

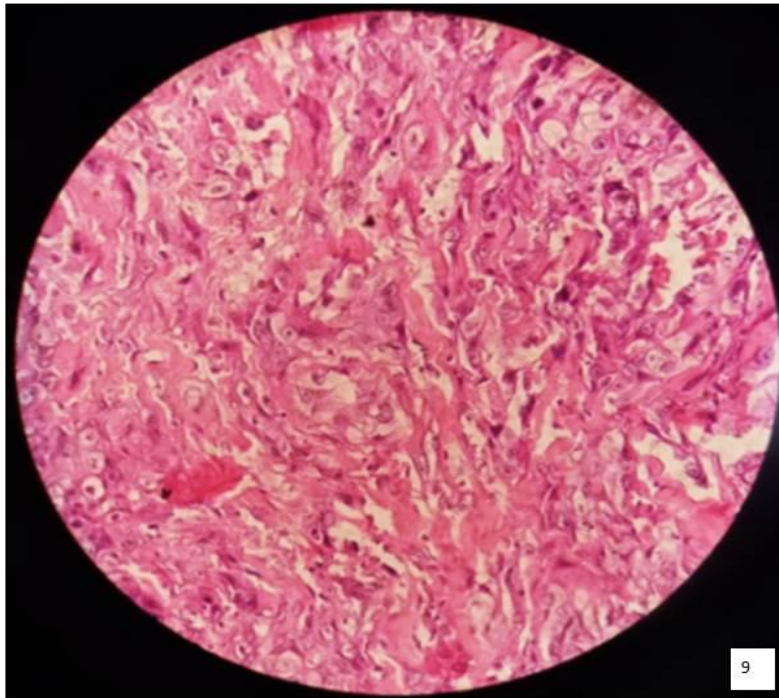
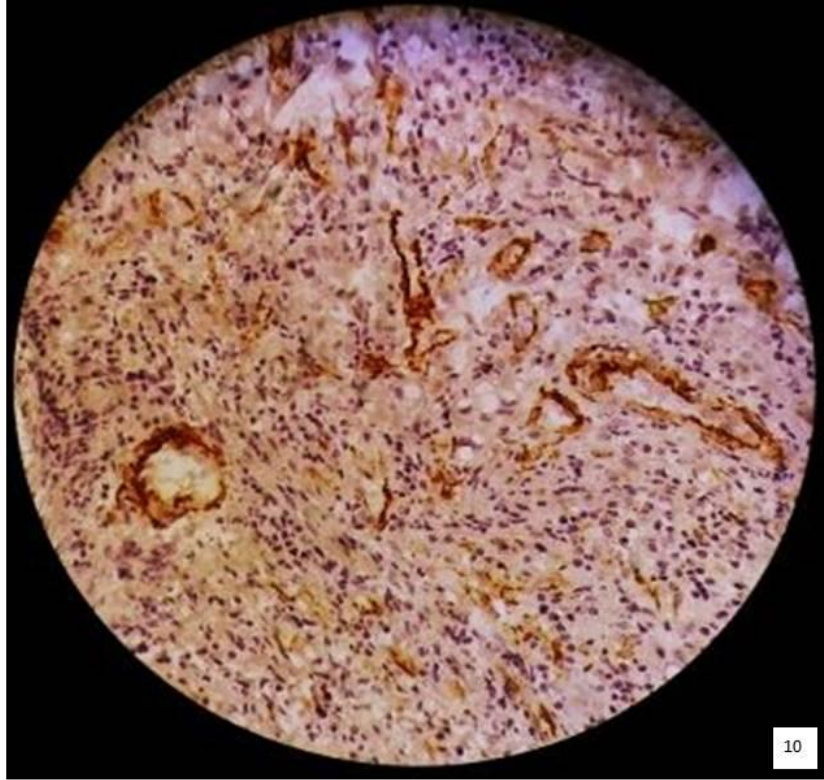


Figure 10: IHC of same section showing the aggressive myofibroblastic activity(40X).



UNDER PL