ORIGINAL RESEARCH

A Comparative Study on Vascularity as a Prognostic Marker in Various Stages of Oral SubMucous Fibrosis Patients

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ABSTRACT

Background: Oral Sub Mucous Fibrosis (OSMF) being categorized as a potentially malignant disorder, due to its insidious chewing habits of areca nut which causes fibrotic changes in the mucosa and depletion of microvasculature ensuing towards carcinoma.The study was performed to assess and analyse the mucosal vasculature with regard to the progression of the disease from early to advanced stages of OSMF.

Materials and Methods: The study sample consists of 40 clinically and histopathologically proven OSMF cases. They were divided into four groups based on clinical staging and grading given by Pindborg and Sirsat. Paraffin embedded sections of 3-4 microns thickness tissue were stained with Masson's trichome. Morphometric analysis was performed for measuring the mean blood vessel area (MVA) using image analysis software 6.0.

Results: Comparison of vascular area between the four stages of OSMF showed highly significant P value of 0.0035 by Kruskal Wallis ANOVA and Pair wise comparison showed significant P value of 0.0019, 0.0041 between stage I & II, stage I &III respectively by Mann-Whitney U test.

Conclusion: The mean blood vessel area was found to be distinctly increased in stage III and decreased in stage I. This suggests, that the altered vascularity is seen in the progressive stages of OSMF. Thus, MVA is one of the adjuvant to assess vascularity and detect the prognosis of OSMF patients.

Keywords: Fibrosis, oral cavity, prognosis, trismus, atrophy, burning pain, vessel, blood

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INTRODUCTION

Oral Submucous fibrosis (OSMF) is a well recognized fibrotic disorder of the oral mucosa and categorised to be as potentially malignant. It is an insidious, chronic disease affecting any part of the oral cavity and sometimes the pharynx and oesophagus. It is characterized by a of varying mucosal changes due to the fibroelastic changes of the juxtaepithelial layer, resulting in a retreat inability to open the mouth.[1] Aetiopathogenesis of this condition is multifactorial. It is well correlated with the intake of spicy food, consumption of areca nut, strong genetic susceptibility and immunological aspects are consider in pathogenesis of OSMF.[2,3] The prevalence of OSMF in India on an increasing trend for past few years. It varies between 0.03% and 3.2% according to various studies.[4] This is due to the heave of commercial products into the market like gutkha, pan masala, supari, mavva etc. Systematic evaluation from various studies have substantiated that this disorder could advance into carcinoma. The rate of malignant transformation was nearly 7 to 15 % in the Indian subcontinent.[5,6,7]

Early stages of OSMF revealed a significant amount of vasculature and inflammation due to the cytokines. Advanced stages show dilated and condensed capillaries in the same region along with atrophic epithelium. Altered state of vasculature is always an inquisitive for the researchers and investigators, as this disorder differs in each geographic region.[3]

The present study evaluates the area of vasculature, by histomorphometric analysis that can be an adequate tool to assess its level in various stages of OSMF.

MATERIALS AND METHODS

Clinically diagnosed 40 cases of OSMF were subject-

ed to incisional biopsy as a part of routine histological examination for confirmation. Informed consent from patients and approval from IRB (Institutional Review Board) for the study was obtained. Patients with symptoms of trismus, burning sensation, mucosal fibrous bands were included and patients with inability to open their mouth due to trauma were excluded. Paraffin embedded sections of 3-4 microns of each case were divided into two sets. One set of each case was stained with hematoxlyin and eosin, other set was stained with Masson's trichome which highlights the endothelial cells which is the area of interest in the present study. Examination of the slides and evaluation of the microphotographs was done with usage of Jenoptik camera 3.0 and image analysis software (image express pro 6.0). Scanner (4X) was used to identify areas of collagen bundles and vasculature. High power (40X) was used to capture exactly fifty blood capillaries for each case. All these microphotographs were adjusted such that the areas of vasculature were clearly observed and were made convenient for measurement of blood vessel area by Image Pro express 6.0. Data obtained was transferred to an excel sheet, to analyze the mean vessel area by using the Kruskal Wallis ANOVA and Mann- Whitney U test .

RESULTS

The grading and staging criteria proposed by Pindborg and Sirsat was followed. There were 40 cases of OSMF which were divided evenly into 10 cases in each stage from stage I to IV. The mean vessel area was highest in stage III (5156.45) and least in stage I (1237.34). Kruskal Wallis ANOVA was used to analyze the means between the four stages of OSMF which showed high statistical significance with a P-value of 0.0035. (Table- 1) and with log showed a P-value of 0.009. Mann Whitney U test was performed for a pair wise comparison between the means of each stage. High statistical significance was obtained between stage I and II with a P-value of 0.0019 and 0.0041 between stage I and III.(Table- 2) Log with Tukeys multiple posthoc procedures showed a P-value of 0.0012 and 0.0050 in stage II and stage III respectively.

DISCUSSION

Oral Submucous fibrosis is chronic debilitating disease and is considerately included in the group of potentially malignant disorders.[8] Pindborg and Sirsat were first to describe OSMF as a habit based condition insidious in

Stage	Mean	Std.Dev.	Sum of ranks	H-value	P-value
Stage I	1237.34	1857.10	103.00	13.6000	0.0035*
Stage II	3759.58	1198.80	278.00		
Stage III	5156.45	7209.89	238.00		
Stage IV	2515.82	1519.43	201.00		
*p<0.05					

Table-1: Comparison of Four Stages with area of blood vessels by Kruskal Wallis ANOVA

Stage	Means	Std.Dev.	SE	Sum of ranks	U-value	Z-value	P-value
Stage I	1237.34	1857.10	587.27	64.00	9.00	-3.0993	0.0019*
Stage II	3759.58	1198.80	379.09	146.00			
Stage I	1237.34	1857.10	587.27	67.00	12.00	-2.8725	0.0041*
Stage III	5156.45	7209.89	2279.97	143.00			
Stage I	1237.34	1857.10	587.27	82.00	27.00	-1.7386	0.0821
stage IV	2515.82	1519.43	480.48	128.00			
Stage II	3759.58	1198.80	379.09	118.00	37.00	-0.9827	0.3258
stage III	5156.45	7209.89	2279.97	92.00			
Stage II	3759.58	1198.80	379.09	124.00	31.00	-1.4363	0.1509
Stage IV	2515.82	1519.43	480.48	86.00			
Stage III	5156.45	7209.89	2279.97	113.00	42.00	-0.6047	0.5454
Stage IV	2515.82	1519.43	480.48	97.00			

Table-2: Pair wise comparisons of four stages with area of blood vessels by Mann-Whitney U test

onset, affects any portion of the oral cavity and oesopharynx. The epithelial changes mainly include early stage hyperplasia & late stage atrophy of the surface epithelium due to altered vascularity and decreased perfusion. The changes in the connective tissue vary from dense fibrosis to hyalinization.[9] Along with the distinctive histopathological features of OSMF, vascularity should be analyzed as it has a prognostic value to detect tumour development. Decreased vascularity and lack of perfusion is seen the progressing stages of OSMF, the later is reduced due the stromal hyalinization. Constant deposition of carcinogens from tobacco and areca nut in the sub-epithelial region leads to a dysplastic epithelium. There are many studies which indicated that vascularity and associated angiogenesis in OSMF can serve as an alternate marker for tumor development.[10,11,12]

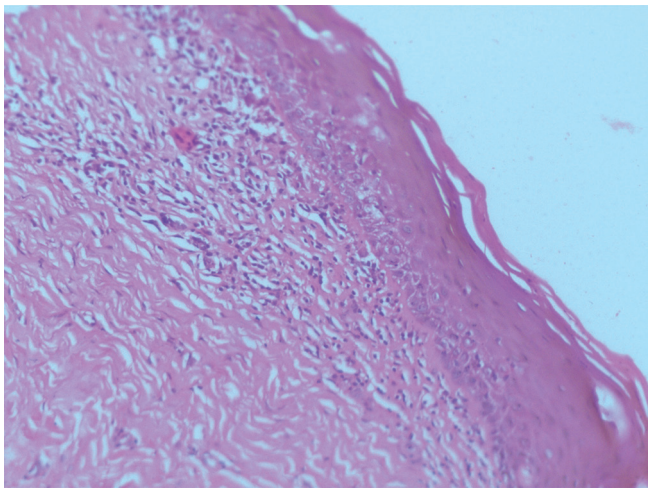

Figure-1: 10 X view of a H&E stained section of OSMF stage -2

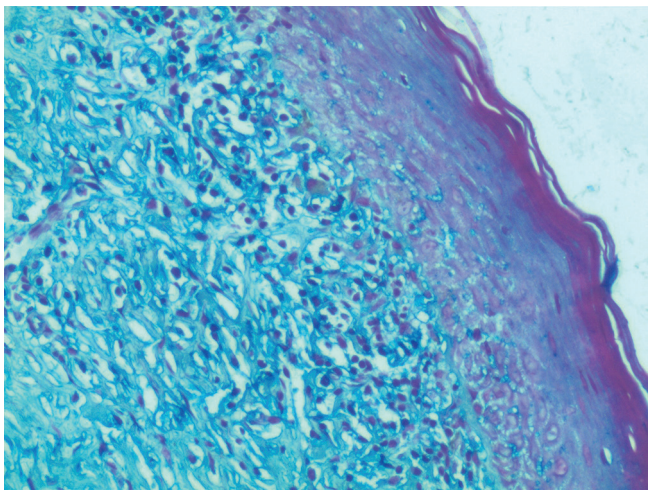

Figure-2: 20X view of a masson's trichome stained section of OSMF stage – 2.

The present study is to analyze the vasculature in various stages of OSMF and to determine the mean vessel area with emphasis of Masson's trichome stain on endothelial cells and blood vessels. The mean vessel area was highest in stage III OSMF cases which was in accordance with the study of Rajendran et.al and Fang et.al who showed that the mean vascular area increases with the progression of the disease. This occurs as compensation to the tissue hypoxia in OSMF cases[13,14] Murgod V.V et.al, Debnath S et.al consolidated that the vascular area was increased in early stages than in advanced stages of OSMF.[15,16] Mamta Singh et.al described in her study that mean blood vessel area showed marked increase in stage II and significant decrease in stage IV.[17] The morphometrical analysis of mean vascular area in above mentioned studies showed marked increase in stage II and marked decrease in stage IV but our study proposes that there is marked increase in mean vascular area till stage III and decrease in stage IV. This could be attributed to the geographic distribution of the sample and nutrition status of the individuals included in the study sample.

CONCLUSION

Angiogenesis can serve as marker to detect tumor progression. Altered state of vascularity is seen in progressing stages of OSMF. Mean vessel area is one of the potential quantitative methods to determine the prognosis of these patients. Our study showed marked increase in mean vessel area in stage III, suggesting that it can be used as an surrogate prognostic marker. Larger sample size and qualitative analysis can be helpful in detecting the correlation between vasculature and disease progression.

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