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Histomorphometric Analysis of Epithelial Thickness and Mucosal Vasculature in Oral Submucous Fibrosis

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Abstract: ***Aim: Morphometric image analysis was performed on H and E stained sections for evaluation of epithelial thickness, vascular density and vascular area percentage in different grades of oral submucous fibrosis. Materials and Methods: Twenty oral submucous fibrosis patients and ten controls comprised the study group. The H and E stained sections were examined and quantified using Optimas ver 6.0 software for the above parameters. Statistics: ANOVA was used to test the equality of means between epithelial thickness, vascular density and vascular area percentage. Pearson's correlation was used to find the correlation between these variables. Results: The present study shows consistent decrease in the epithelial thickness with increasing grades of the disease and the difference is found to be statistically significant. The mean vascular density is found to be almost of the same value in the three groups and the difference is found to be statistically insignificant. Vascular area percentage significantly increased in advanced oral submucous fibrosis relative to early submucous fibrosis. Conclusion: The present study shows that ischemia may not play a significant role in the etiopathogenesis of oral submucous fibrosis. Increased vascular area percentage seen in advanced stages could be an attempt of the tissue to reciprocate the stromal modifications seen in oral submucous fibrosis.***

Keywords: Epithelial thickness, oral submucous fibrosis, vascular density, vascular area percentage, image analysis

1. Introduction

Oral submucous fibrosis is a potentially malignant disease predominantly seen in people of Asian descent. It is a chronic progressive disorder and its clinical features depend on the stage of the disease. The histopathological hallmark of the disease is fibrosis that affects most parts of the oral cavity [1]. It is conventionally accepted that atrophic epithelium in oral submucous fibrosis is a result of decreased vascularity in the underlying connective tissue stroma. Consequent lack of tissue perfusion is believed to trigger the ischemic atrophy of the epithelium and thereby making it vulnerable to the effects of oral carcinogens [2]. There are many studies with diverse findings published regarding the epithelial thickness and degree of vascularity of oral submucous fibrosis. Against this backdrop, we carried out a methodology to assess the relationship between epithelial atrophy and vascularity by image analysis using Optimas ver6.0 software.

2. Materials and Methods

Twenty cases of oral submucous fibrosis diagnosed clinically and confirmed histopathologically comprised the studygroup. Ten age and sex matched healthy individuals comprised the control group. Staging of the disease and grading of biopsies were performed based on established criteria [3]. Twelve advanced and eight early cases of oral submucous fibrosis were included in the study. The biopsy samples were fixed in formalin and subsequently stained. A trinocular Nikon Fluorescence microscope (Eclipse E 600 Japan) attached with the DM 1200 F Nikon digital camera was used to capture the bright field images in blind conditions. Images at 400x magnification view fields covering the entire available area of the epithelium and underlying connective tissue were captured and enhanced with Adobe Photoshop (ver 7.0). They were later quantified in an image analyzer (Optimas ver 6.0) for epithelial thickness, vascular density and vascular area percentage using an area morphometric tool. The area of 400xview fields used to capture images was calculated by capturing a 1mm stage micrometer scale (100 divisions) at 400x magnification and calibrating it with the help of an image analyzer.

Statistical Analysis

ANOVA was used to test the equality of several means without effecting Type 1 error. Only if ANOVA shows significant difference, pairwise comparisons were made. Pairwise comparisons were made using "t" test for independent samples. Finally, the correlations between the three parameters were done using Karl Pearson's coefficient of correlation.

3. Results

Epithelial thickness

Epithelial atrophy is said to be one of the important features in oral submucous fibrosis [4]. The present study shows consistent decrease in the epithelial thickness with increasing grades of the disease and the difference is found to be statistically significant. To identify the significant difference, pairwise comparison was made using 't' test. The difference in epithelial thickness between early and advanced oral submucous fibrosis; control group and advanced oral submucous fibrosis was found to be statistically significant.

Vascular Density

The mean vascular density is found to be almost of the same value in the three groups and the difference is found to be statistically insignificant.

Vascular area percentage

Vascular area percentage shows an increasing trend as the disease progresses and the difference is found to be statistically significant. The difference between the control and early oral submucous fibrosis, early and advanced oral submucous fibrosis and control and advanced oral submucous fibrosis were found to be statistically significant.

Statistical analysis for correlation of epithelial thickness, vascular density and vascular area percentage showed that there exists a significant negative correlation between epithelial thickness and vascular area percentage.

Table 1: Comparison of Epithelial Thickness, Vascular Density and Vascular Area percentage between different groups

		N	Mean	Std. Deviation	F	p
Epithelial thickness	Control	10	1688.30	222.196	60.87	.000
	Early	8	1561.75	136.793		
	Advanced	12	984.75	99.749		
	Total	30	1373.13	360.688		
Vascular density	Control	10	.002100	.0003162	2.34	.115
	Early	8	.001763	.0003335		
	Advanced	12	.001808	.0004337		
	Total	30	.001893	.0003895		
Vascular area percentage	Control	10	.1640	.03026	171.49	.000
	Early	8	.3225	.04464		
	Advanced	12	1.0342	.17814		
	Total	30	.5543	.41885		

Table 2: Pairwise comparison of Epithelial Thickness, Vascular Density and Vascular Area percentage between control group and early groups

	Group	N	Mean	Std. Deviation	t	p
Epithelial thickness	Control	10	1688.30	222.196	1.47	.179
	Early	8	1561.75	136.793		
Vascular density	Control	10	.002100	.0003162	2.19	.043
	Early	8	.001763	.0003335		
Vascular area percentage	Control	10	.1640	.03026	8.97	.000
	Early	8	.3225	.04464		

Table 3: Pairwise comparison of Epithelial Thickness, Vascular Density and Vascular Area percentage between control group and advanced groups

	Group	N	Mean	Std. Deviation	T	p
Epithelial thickness	Control	10	1688.30	222.196	9.87	.000
	Advanced	12	984.75	99.749		
Vascular density	Control	10	.002100	.0003162	1.76	.092
	Advanced	12	.001808	.0004337		
Vascular area percentage	Control	10	.1640	.03026	15.20	.000
	Advanced	12	1.0342	.17814		

Table 4: Pairwise comparison of Epithelial Thickness, Vascular Density and Vascular Area percentage between early and advanced groups

	Group	N	Mean	Std. Deviation	T	p
Epithelial thickness	Early	8	1561.75	136.793	10.93	.000
	Advanced	12	984.75	99.749		
Vascular density	Early	8	.001763	.0003335	.252	.804
	Advanced	12	.001808	.0004337		
Vascular area percentage	Early	8	.3225	.04464	10.97	.000
	Advanced	12	1.0342	.17814		

Table 5: Correlation between Epithelial thickness, Vascular density and Vascular area percentage

PARAMETERS	R	P	
Epithelial thickness Vascular density	.289	.121	p>0.05
Epithelial thickness Vascular area percentage	-.886	.000	P<0.05
Vascular density Vascular area percentage	-.200	.289	p>0.05

4. Discussion

Oral submucous fibrosis is a known premalignant condition predominantly seen among people of Indian origin. Epidemiological studies have suggested the habit of areca quid chewing as a major etiological factor. Its malignant potential has been exclusively studied for the last three decades but many aspects of etiology and pathogenesis still remain an enigma [5]. Oral submucous fibrosis is characterised by inflammation and progressive mucosal fibrosis. Epithelial changes include hyperplasia in the early stage and atrophy in the later stage. The changes in connective tissue vary from fibrosis to hyalinization [6]. The morphometric analysis of the epithelial thickness and the vasculature in normal oral mucosa, early and advanced oral submucous fibrosis were conducted to validate these points. The parameters used to determine the vasculature in the mucosa were vascular density and vascular area percentage.

In the present study, vascular density was more or less the same in both the test and the control groups in confirmation with the study conducted by Rajendran et al [7]. In contrast, the study by Sabarinath et al, showed that vascular density had a tendency to increase as the disease progressed, although the increase among the various stages of oral submucous fibrosis was not statistically significant [6]. Singh et al [11] and Fang et al [10] also found a significant increase of vascular density in the early stage of oral submucous fibrosis in comparison with the other stages.

An exponential increase in the mean vascular area percentage was noted with the disease progression. Similar results have been obtained in previous studies where the tumour micro vessel area was found to be higher in tumours than in normal mucosa [8].In the present study there was an inverse relationship between vascular area percentage and epithelial thickness. Since the vascular density is more or less the same in the test and the control groups, the increase in vascular area percentage as the disease progresses might be due to an increase in the vascular luminal diameter. Study by Rajendran et al also showed that mean vascular area percentage tended to increase in oral submucous fibrosis as the disease progressed. They suggested that the usual tissue reaction in response to ischaemia or hypoxia did not appear to operate in OSMF. In this situation, where neoangiogenesis or vasculogenesis is not feasible, vasodilation remains the only alternative [7].

The reasons behind the thinning of the epithelium may be due to other factors like the reduced cell division in the progenitor compartment (hypoproliferation), accelerated tissue turnover time, actual change in cell morphology of the individual cells of specific compartment or all compartments which need to be further explored. Thus present study demonstrated that there is a highly significant inverse correlation between the thickness of the epithelium and the vascular area percentage.

5. Conclusion

A negative correlation was found between epithelial thickness and vascular area percentage, which is statistically significant. Contrary to the conventional views, the present study shows thatischemia may not play a significant role in the etiopathogenesis of oral submucous fibrosis.The results of the study need to be evaluated using a larger sample size to establish the true relationship between these parameters in oral submucous fibrosis.

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Author Profile

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