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Evaluation of Mast Cells, Vascular Luminal Diameter and Epithelial Thickness in Different Grades of Oral Submucous Fibrosis

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Abstract: ***Aim: To evaluate the implication of mast cell role in the changes of the vascular luminal diameter along with the epithelial thickness of oral submucous fibrosis. Materials And Methods: Patients with histological diagnosis of oral submucousfibrosis and healthy volunteers comprised the study group. The parameters, epithelial thickness and vascular luminal diameter were quantified using image analysis software. Enumeration of intact and degranulated mast cells were performed with the microscope fitted with a calibrated mechanical stage. Statistics: ANOVA was used to test the equality of means between epithelial thickness, vascular luminal diameter and mast cell count. Pearson's correlation was used to find the correlation between these variables. Results: The present study shows an inverse relation between epithelial thickness and vascular luminal diameter and the result was found to be statistically significant. The mast cell count showed a decreasing trend as the disease progresses. Conclusion: The study concluded that there was significant correlation between epithelial thickness and vascular luminal diameter, which is in agreement that hypoxia due to the increased fibrosis induces dilatation of vessels. Although presence of mast cells plays a significant role in early oral submucous fibrosis, their role in modulation of angiogenesis in later stages may be insignificant.***

Keywords: oral submucous fibrosis, image analysis, epithelial thickness, vascular luminal diameter, mast cells

1. Introduction

"Atrophicaidiopathica (trophica) mucosae" was the first name given to oral submucous fibrosis [1, 2]. The condition was also named as "diffuse oral submucous fibrosis" (3). Since then it has remained an enigma in spite of innumerable studies over the past five decades. This condition occurs predominantly among Indians and to a much lesser extent in other Asian populations. The prevalence rate of oral submucous fibrosis in India is approximately 1.2%. Interestingly, the prevalence and the incidence rates are high in South India where the incidence of oral cancer is also high [3]. In spite of extensive studies, the pathogenesis and other aspects of oral submucous fibrosis remain unclear till today [4].

Mast cells are found in human tissues, particularly in association with structures such as blood vessels and nerves. Mast cells are bone marrow derived and particularly depend on stem cell factor (SCF). Mast cells which have been shown to accumulate near sites of new capillaries have been implicated in angiogenesis. Mast cells can produce, store and release many kinds of chemical mediators, including histamine, tryptase, kinase, heparin and cytokines. However, role of mast cells in angiogenesis is not fully understood [5]. In the present study, we evaluate the implication of mast cells' role in the variations of the vascular luminal diameter along with the epithelial thickness in oral submucous fibrosis.

2. Materials and Methods

Twenty cases of oral submucous fibrosis diagnosed clinically and confirmed histopathologically comprised the study group. Ten age and sex matched healthy individuals comprised the control. Staging of the disease and grading of biopsies were performed based on established criteria [7]. 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 field covering the entire available area of the epithelium and underlying connective tissue were captured and enhanced with Adobe Photoshop (ver7). They were later quantified in an image analyzer (Optimas ver6) for epithelial thickness, vascular density and vascular area percentage using an area morphometric tool. The area of 400x view field 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.

Mast cell counting

Acidified toluidine blue was used as it gives rapid, crisp metachromatic staining of mast cells. The cytoplasm of the mast cells stained purple and the nuclei blue. Enumerations of intact and degranulated mast cells were performed with the microscope fitted with a calibrated mechanical stage, 10x and 100x objective lenses. The area encompassed by the eye piece graticule was designated as a microscopic field (MF) for counting purpose. This counts were converted to mean values of intact and degranulated mast cells and were compared with the control using students 't' test. The significant difference was set as $p < 0.05$.

Statistical Analysis

ANOVA was used to test 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 correlation between the epithelial thickness, vascular luminal diameter and mast cell count was 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 [6]. 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 submucousfibrosis; control group and advanced oral submucous fibrosis were found to be statistically significant.

Vascular Luminal Diameter

The mean vascular diameter shows an increasing trend as the disease progresses and the difference is found to be statistically significant. Pairwise comparison was made using 't'test to identify significant difference. The difference between the control and early oralsubmucous fibrosis; early and advanced oral submucous fibrosis; control and advanced oral submucous fibrosis were found to be statistically significant.

Mast Cell Count

The mast cell count shows significant difference between control and early oral submucous fibrosis and also early and advanced oral submucous fibrosis. Statistical analysis for correlation of epithelial thickness, mast cell count and vascular luminal diameter showed that there exists a significant negative correlation between epithelial thickness and vascular luminal diameter. It was also found that there was no significant correlation between mast cell count and vascular luminal diameter.

Table 1: Comparison of Vascular luminal diameter, Intact mast cell count, Degranulated mast cell count and Epithelial thickness between different groups

		N	Mean	Std. Deviation	F	P value
Vascular luminal diameter	Control	10	14.930	1.5798	197.28	.000
	Early	8	21.200	3.7891		
	Advanced	12	39.267	3.2726		
	Total	30	26.337	11.3854		
Intact mast cell count	Control	10	.60	.516	18.26	.000
	Early	8	2.38	.916		
	Advanced	12	.75	.622		
	Total	30	1.13	1.008		
Degranulated mast cell count	Control	10	.20	.422	41.41	.000
	Early	8	2.75	1.035		
	Advanced	12	.33	.492		
	Total	30	.93	1.285		
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		

Table 2: Pairwise comparison of Vascular Luminal Diameter, Intact Mass Cell Count, Degranulated Mast Cell Count and Epithelial Thickness between control and early groups

	Group	N	Mean	Std. Deviation	T	P Value
Vascular luminal diameter	Control	10	14.930	1.5798	4.76	.000
	Early	8	21.200	3.7891		
Intact mast cell count	Control	10	.60	.516	5.20	.000
	Early	8	2.38	.916		
Degranulated mast cell count	Control	10	.20	.422	7.12	.000
	Early	8	2.75	1.035		
Epithelial thickness	Control	10	1688.30	222.196	1.40	.179
	Early	8	1561.75	136.793		

Table 3: Pairwise comparison of Vascular Luminal Diameter, Intact Mass Cell Count, Degranulated Mast Cell Count and Epithelial Thickness between control and advanced groups.

Group Statistics						
	Group	N	Mean	Std. Deviation	t	P
Vascular luminal diameter (A)	Control	10	14.930	1.5798	21.46	.000
	Advanced	12	39.267	3.2726		
Intact mast cell count (B)	Control	10	.60	.516	.608	.550
	Advanced	12	.75	.622		
DE granulated mast cell count (F)	Control	10	.20	.422	.674	.508
	Advanced	12	.33	.492		
Epithelial thickness	Control	10	1688.30	222.196	9.97	.000
	Advanced	12	984.75	99.749		

Table 4: Pairwise comparison of Vascular Luminal Diameter, Intact Mass Cell Count, Degranulated Mast Cell Count and Epithelial Thickness between early and advanced groups

Group Statistics						
	Group	N	Mean	Std. Deviation	t	P
Vascular luminal diameter	Early	8	1561.75	136.793	11.36	.000
	Advanced	12	984.75	99.749		
Intact mast cell count	Early	8	21.200	3.7891	4.74	.000
	Advanced	12	39.267	3.2726		
Degranulated mast cell count	Early	8	2.38	.916	7.04	.000
	Advanced	12	.75	.622		
Epithelial thickness	Early	8	1561.75	136.793	10.93	.000
	Advanced	12	984.75	99.749		

Table 5: Correlation between Epithelial thickness, Vascular luminal diameter and Mast cell count

	r	P value	
Epithelial thickness Vascular luminal diameter	-.858	.000	P<0.05
Epithelial thickness Intact mast cell count	.264	.159	P>0.05
Epithelial thickness Degranulated mast cell count	-.200	.289	p>0.05
Vascular luminal diameter Intact mast cell count	-.198	.294	p>0.05
Vascular luminal diameter Degranulated mast cell count	-.221	.240	p>0.05
Intact mast cell count Degranulated mast cell count	.726*	.000	P<0.05

4. Discussion

Oral submucous fibrosis is an insidious, precancerous, chronic disease that may affect the entire oral cavity and sometimes extent to the pharynx. It is occasionally preceded by the formation of vesicles and is mostly associated with a subepithelial inflammatory reaction followed by fibroelastic changes of the lamina propria, accompanied by epithelial atrophy [7]. Hence, we studied the epithelial thickness, vascular luminal diameter and mast cell density in various grades of oral submucous fibrosis and also the correlation between these factors.

In literature, Rajendran et al. reported that thickness of epitheliumalter in all the grades of oral submucous fibrosis. The irritating agent exacerbates the fibrosis in the submucosa initiated by chronic inflammatory cells which promote proliferation of fibroblasts and also by stabilizing the collagen that they produce [8]. Later, he also suggested that excessive fibrosis in the connective tissue seems to be the primary pathology in oral submucous fibrosis. The atrophic changes in the epithelium are secondary [9, 10].

In the present study vascular luminal diameter showed a consistent increase as the disease progressed. This result was contradictory to the findings of Sirsat et al. and Fang et al. which suggested dilatation and congestion of the blood vessels in the early stages and constriction to obliteration at the later stages [11, 12].

Tilakaratne et al. concluded that the mean vascular dilatation occurred as a result of adaptive response to compensate for tissue ischemia/hypoxia [13].The diminished perfusion of the epithelium caused by defective vascularization of subjacent connective tissue due to physical constraints of fibrosis is generally considered to lead to epithelial atrophy as disease progresses [14]. This is in accordance with our study, which showed increased epithelial atrophy in advanced stages.

The correlation between vascular luminal diameter and the mast cell density show an inverse relation, i.e., the mast cell density tends to decrease as the disease progress. This suggests that the usual tissue reaction in response to ischemia or hypoxia does not appear to operate in oral submucous fibrosis. In this situation where new vascular

formations are not possible, vasodilatation remains the only alternative [15].

In the early stages of oral submucous fibrosis, mast cells and their derivatives, namely histamine and bradykinin along with prostaglandins have a significant role in angiogenesis. As the disease progress, these mediators' role seem to diminish and are taken over by other factors. Etiopathogenesis of epithelial atrophy based on a state of persistent ischemia need further objective elucidation with a larger number of patients, possibly in a multi-institutional setting to help better define the clinical implications of these alterations.

5. Conclusion

This study suggests an inverse relationship between the epithelial thickness and vascular luminal diameter and is established by Pearson's correlation analysis. The mast cell count tends to play a minor role in advanced stages of oral submucous fibrosis. This shows that other factors may be involved in the modulation of angiogenesis and may be used as indicators of the disease progression in future.

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