International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Correlation of Elevated Mast Cell Density with Microvessel Density in Various Grades of Oral Submucous Fibrosis

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Abstract: ***Aim: To correlate the elevated mast cell density with the microvessel density in various grades of oral submucous fibrosis. Materials and Methods: H and E stained sections of oral submucous fibrosis (OSMF) were examined and microvessel density (MVD) quantified using an image analyzer. Mast cell density (MCD) calculated using a microscope fitted with a calibrated mechanical stage. Statistics: ANOVA was used to test the equality of means between MVD and MCD. Results: Mast cell density showed a consistent increase with Microvessel density in different grades of OSMF although the correlation was statistically insignificant. Discussion: Mast cells play a significant role in inducing angiogenesis in OSMF and modalities to control mast cell mediators would in turn improve the prognosis of OSMF patients.***

Keywords: mast cell density, microvessel density, image analysis, oral submucous fibrosis

1.Introduction

Oral submucous fibrosis (OSMF) is a premalignant condition of questionable pathogenesis characterized by inflammation and progressive mucosal fibrosis. Mast cells have been associated with variety of inflammatory and fibrotic conditions and their role in OSMF is unknown. OSMF is a premalignant condition where the MCD increase with the disease progression.[1] numerous quantitative studies have shown that angiogenesis plays an important role in malignant transformation of tumours. Recent evidence indicate that mast cells might induce tumour progression by stimulating angiogenesis.[2]

2.Materials and Methods

OSMF cases which were diagnosed histologically using H and E staining were selected as the study group. A total of 20 cases were selected in which 8 were of early and 12 were of advanced OSMF. The control group comprised of 10 cases of age and sex matched healthy individuals. The biopsy sample were fixed in formalin and stained with H and E. 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 microvessel density (MVD) 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 was done using Acidified toluidine blue 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. These counts were converted to mean values of intact and degranulated mast cells and were compared with the controls 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 mast cell density (MCD) and microvessel density (MVD) was done using Karl Pearson's coefficient of correlation.

3.Results

Mast cell density

The mean mast cell densities were 0.164, 0.322 and 1.034 respectively in control, early and advanced cases. The differences between controls and early, control and advanced and early and advanced OSMF cases were all statistically significant with P value of 0.000. The present study showed increased mast cell density as the disease progressed.

Microvascular density

MVD increased from early to advanced stages of OSMF. A statistical significance was seen only between the control and early cases of OSMF where as the difference was statistically insignificant when the advanced cases were compared with the control and early.

Table 1: Comparison of mast cell density (MCD) and microvessel density (MVD) between different groups

	Group	N	Mean	Std. Deviation	F	p value

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Mast cell density	**Control**	10	0.164000	0.0302581	**171.493**	**.000**
	Early	8	0.322500	0.0446414		
	Advanced	12	1.034167	0.1781449		
	Total	30	0.554333	0.4188546		
Microvessel density	**Control**	10	0.002100	0.0003162	**2.344**	**0.115**
	Early	8	0.001763	0.0003335		
	Advanced	12	0.001808	0.0004337		
	Total	30	0.001893	0.0003895		

Table 2: Pairwise comparison of mast cell density (MCD) and microvessel density (MVD) between control and early

	Group	N	Mean	Std. Deviation	t	p value
Mast cell density	**Control**	10	0.164000	0.0302581	**8.973**	**.000**
	Early	8	0.322500	0.0446414		
Microvessel density	**Control**	10	0.002100	0.0003162	**2.197**	**.043**
	Early	8	0.001763	0.0003335		

Table 3: Pairwise comparison of mast cell density (MCD) and microvessel density (MVD) between control and advanced

	Group	N	Mean	Std. Deviation	t	p value
Mast cell density	**Control**	10	0.164000	0.0302581	**15.204**	**.000**
	Advanced	12	1.034167	0.1781449		
Microvessel density	**Control**	10	0.002100	0.0003162	**1.768**	**.092**
	Advanced	12	0.001808	0.0004337		

Table 4: Pairwise comparison of mast cell density (MCD) and microvessel density (MVD) between early and advanced

	Group	N	Mean	Std. Deviation	t	p value
Mast cell density	**Early**	8	0.322500	0.0446414	**10.979**	**.000**
	Advanced	12	1.034167	0.1781449		
Microvessel density	**Early**	8	0.001763	0.0003335	**0.252**	**.804**
	Advanced	12	0.001808	0.0004337		

Table 5: Correlation

	r	P value	
Mast cell density	**-.200**	**.289**	**P> 0.05**
Microvessel density			

4.Discussion

Tumor microenvironment affects several cellular events such as neoplastic growth, differentiation, migration and metastases. The role of various cells like mast cells is very important in the formation of this microenvironment. Recent studies report that mast cells play a key role in tumor angiogenesis. Several angiogenesis factors like VEGF have been identified[7].The present study showed insignificant correlation between mast cell density and microvessel density in various grades of OSMF.

Although in the present study, there was no significant correlation between MCD and MVD, the mean MCD and MVD increased as the disease progressed. It was consistent with the results of the study by Lamaroon et al., which showed a significant relation between both parameters[3]. Study by Sharma et al., also showed a significant correlation between MCD and MVD. A chemical mediator tryptase released by mast cells act as a potent proangiogenic factor and leads to extracellular degradation and angiogenesis stimulation (Toda et al., 1999). There was a statistically significant increase of intact mast cells from normal mucosa to the advanced cases of OSMF but the degranulated mast cells increased only in the early stages of OSMF. MVD was increased from early to advanced stages and this increase may be due to the angiogenic factors such as nitric oxide, VEGF, vFGF, angiopoietin 1 and 2, other than the mast cells. The increase in MCD and MVD revealed that both have a significant role in the etiopathogenesis of OSMF.

5.Conclusion

This study suggest that mast cells might play a key role in modulation of angiogenesis by secreting angiogenic factors and can be used as an indicator of disease progression in OSMF. The limitations of this study can be overcome by a large sample size and a multicentric trial.

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