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CORRELATION OF INFLAMMATION AND VASCULARIZATION WITH ORAL LEUKOPLAKIA SEVERITY

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Abstract:

Background and purpose: *Variation in sub-mucosal vascularization and inflammatory changes detected with immunohistochemical staining have been shown to be associated with the development of dysplasia and invasiveness of epithelial cells in precancerous and malignant lesions. This study looked at changes in areas routinely stained with hematoxylin and eosin (H&E) during oral leukoplakia and progression of squamous cell carcinoma (SCC).). The aim of the study was to determine whether changes in H&E routine color change in submucosal vascularization and leukoplakia inflammatory infiltration could contribute to assessing the severity of the lesion.*

Place and Duration: *In the Department of Oral, Dental, Maxillofacial and Pathology Mayo Hospital Lahore for one year duration from March 2018 to March 2019.*

Methods: *In this cross-sectional, descriptive and comparative study, total of 125 sample which includes 35 cases of mild to moderate dysplasia, 38 severe dysplasia and 52 cancer in situ cases and were examined, stained with H and E. To analyze the data, the chi-square test, Mann-Whitney test, Kruskal-Wallis test and cumulative sequential logistic regression were performed.*

Results: *Patients with severe dysplasia, cancer in situ and SCC had significantly higher vascular density than patients with mild to moderate dysplasia ($p < 0.0001$). However, the difference in vascularization was not statistically significant between severe dysplasia, cancer in situ and SCC ($p = 0.78$). The inflammatory cell infiltration intensity in underlying connective tissue was significantly different between the three groups ($P < 0.0001$), and the density of inflammatory cell infiltration was maximum in the SCC group.*

Conclusion: *Increased inflammatory cell infiltration and submucosal vascularization may additionally subsidize to the prognosis of more aggressive epithelial dysplasia.*

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INTRODUCTION:

Leukoplakia is clinically present as a white plaque that is not recognized as another lesion known on the oral mucosa. Histological changes include hyperkeratosis, dysplasia, and cancer in situ after invasive squamous cell carcinoma (SCC). About 15% of epithelial dysplasia passes to SCC[1-3]. This is a common oral lesion for pathologists for classification and diagnosis. The classification system is based on dysplastic changes in the epithelial cell layer; however, changes in sub-mucosal vascularization (3–10) and inflammation (11–15) are associated with dysplasia and the development of invasive epithelial cells[4-6]. In precancerous and malignant lesions. Several studies have shown an increase in vascularity in the early stages of dysplastic transformation and there is no significant difference between severe dysplasia, cancer in situ and SCC[7-9]. All of these studies used immune-histochemical staining to investigate the intensity of these submucosal lesions, but these changes are by Jalayer et al. and Gommes et al. This study examined whether changes in inflammatory infiltration and submucosal vascularization of leukoplakia in routine sections of H&E could contribute to assessing the severity of the lesion.

MATERIAL AND METHODS:

125 samples were taken from Department of Oral, Dental, Maxillofacial and Pathology Mayo Hospital Lahore for one year duration from March 2018 to March 2019. Histo-pathologically; all samples were diagnosed based on squamous cell carcinoma or oral dysplasia. Age and gender data were obtained from patients' medical records. In general, according to the WHO classification system, two experienced and qualified oral pathologists observed H&E stained fragments of all cases using an optical microscope (Olympus, CH-2, and Japan). Patients with insufficient connective tissue bases and patients with suspected diagnosis, such as secondary atypia and lichenoid dysplasia, were omitted from the study. Vascularity was then evaluated in areas where basal connective tissue was not inflamed around the epithelial lesion and the invasive edge of SCC. 40 times all sections were scanned (objective lens 4 times and lens 10 times) in a microscopic field to determine the highest density (hot spots) of blood vessels. Then, the average number of blood vessels with a diameter of μ0.5 μm was determined in five areas of hot spots in the 400X microscopic field (17). In addition, the severity of inflammation was assessed as mild (≤ 25 inflammatory cells), moderate (25-125 inflammatory cells) and severe (inflammatory cells above 125). Data analysis was done by Mann-Whitney test, chi-square test, cumulative sequential logistics regression was performed and Kruskal-Wallis test. In addition, cumulative logistic regression was used to model the impact of age and inflammation on the size of the lesion.

RESULTS:

Patients and tissue features were examined in 125 sample consisting of three groups: i) mild and moderate dysplasia (35 cases), ii) severe dysplasia, squamous cell carcinoma (52 cases) and cancer (38 cases); 53.4 ± 11.7, 59, 93 ± 13.01 was the mean SD and 63.56 ± 14.20 was the average age. The mean age of patients was significantly different in the three groups (p = 0.005); in post hoc comparisons, a significant difference was found between the group with mild and moderate dysplasia and the group with SCC in terms of age (p = 0.003). In addition, 42.9% (n = 15) of patients with mild to moderate dysplasia, 71.4% (n = 27) and 50% (n = 26) of patients with severe dysplasia are SCC.

There was no significant relationship between sex and degree of injury (P = 0.22). The vessels in each tissue were counted. The mean ± SD values of vessels for mild to moderate dysplasia were 9.46 ± 5.53, 17.86 ± 6.36 for severe dysplasia and cancer in situ, and the number was recorded for SCC with an average of 19, 11 ± 6.50. Post hoc comparison of the number of vessels in different groups showed mild and moderate dysplasia in situ and significantly higher vascular density in severe dysplasia (p <0.0001), but severe vascular dysplasia and SCC in situ cancer. (P = 0.78) (Table 1).

Table 1. Mean Vascularity of the Studied Groups

Groups	Mean (SD)	*P* value
Mild to moderate dysplasia	9.46±5.53	
Severe dysplasia to carcinoma	17.86±6.36	0.000*
Squamous cell carcinoma	19.12±6.50	

* The vascularity of mild to moderate dysplasia group was significantly lower than the other groups (*P*<0.001)

Infiltration of inflammatory cells.

The infiltration density of inflammatory cells in underlying connective tissue in the study groups was significantly different (P <0.0001) (Table 2)

Table 2. Intensity of Inflammatory Cells Infiltration in the Studied Groups

Inflammation	Groups			P Value
	Mild to moderate dysplasia	Severe dysplasia to carcinoma	Squamous cell carcinoma	
Mild	18 (51.4%)	0 (0%)	5 (9.6%)	
Moderate	9 (25.7%)	11 (28.6%)	9 (17.3%)	0.000*
Severe	8 (22.9%)	27 (71.4%)	38 (73.1%)	

* There was a significant difference among the three groups (*P*<0.0001).

An ordered logistic regression was carried out to model the effect of age, vessel density and inflammation on damage. The results showed that the change in severe inflammation increased the likelihood of 4.42 times more serious damage compared to the change in mild inflammation (p = 0.03). In addition, the risk of serious injury increases with age 1.05 per year (p = 0.007), an increase in vascular density correlates positively with the most severe dysplasia (p <0.001) (Table 3).

Table 3. Ordinal Logistic Regression for Modeling the Effect of Age, Vascular Density, and Severity of Inflammation on the Grade of the Lesion

Variables		B	SE	p-value	OR
Intercept	**grade (mild to moderate dysplasia)**	4.99	1.25	<0.001	146.78
	grade (severe dysplasia to carcinoma)	5.92	1.29	<0.001	373.62
Inflammation (severe)		1.49	0.69	0.03*	4.42
Inflammation(Moderate)		0.49	0.73	0.55	1.55
Inflammation(Mild)		0	-	-	-
Age		0.05	0.02	0.007*	1.05
Vascularity		0.14	0.04	<0.001*	1.15

*The lesions with severe inflammation increased the odds of more severe lesion compared to lesions with mild inflammation (P=0.03). The risk of more severe lesions increases with age (P=0.007), while, increase in vascular density is positively related to severer dysplasia (P<0.001).

The relationship between vascularization and inflammation.

After infiltration of high density inflammatory cells in three groups, an increase in the number of vessels was observed. The Kruskal-Wallis test shows the average number of vessels in lesions with mild inflammatory cell density. The infiltration was much smaller than moderate to severe inflammatory cell infiltration (p <0.05); However, there was no significant difference in the average number of vessels between average infiltrates and intensive infiltration of inflammatory cells (Table 4).

Table 4. Comparison of Mean Vascularity at Different Inflammation Intensities

Group	Inflammation	Mean Number of Vessels ±SD	P-Value
Mild to moderate dysplasia	Mild	**7.11±3.60** [1]	**0.024** * (Kruskal- Wallis Test)
	Moderate	**11.89±5.67** [1]	
	Severe	**12.00±7.17** [1]	
Severe dysplasia to carcinoma	Mild	-----	**0.454** (Mann-Whitney Test)
	Moderate	**16.00±6.63**	
	Severe	**18.60±6.45**	
Squamous cell carcinoma	Mild	**12.40±3.78** [1]	**0.034** * (Kruskal Wallis Test)
	Moderate	**14.11±5.58** [1]	
	Severe	**20.00±6.56** [1]	

* *P*<0.05

[1] The mean vascularization of all groups with mild inflammation was significantly lower than those with moderate and severe inflammation.

DISCUSSION:

The results of this study regarding the density of vessels in H&E stained sections are consistent with several previous studies. Although fewer vessels were observed in H&E stained sections than in those immunohistochemically stained, there was a significant difference in vascularization between the three groups[10-11]. The results showed that severe

dysplasia, cancer in situ and SCC had significantly higher vascular density compared to mild to moderate dysplasia, but the difference in vascularization between severe dysplasia, cancer in situ and SCC was not statistically significant. Michailidou et al reported that the number of micro-vessels (stained SC34) in SCC increased significantly compared to leukoplakia with mild to moderate dysplasia. However, no significant increase in the number of microvessels between severe dysplasia and leukoplakia and SCC was found. The results are also Gandolfo et al it was found that CD34 was stained with blood vessels, and in leukoplakia dysplasia, the weed vasculature was higher than normal mucosa, and the highest vascular density was observed in SCC[12]. They therefore suggested that when the amount of vascularization is sufficient, this can be assessed in H and E stained sections to assess the severity of the lesion. In addition, according to the findings of the present study and previous studies angiogenesis is mainly induced in the early stages of malignant transformation[13].

The relationship between inflammation and cancer has long been anticipated. It is clear that the interaction between transformed cells and surrounding environments, such as immune cells, can lead to the progression and progression of many epithelial cancers. In this study, it was observed that the intensity of infiltration of inflammatory cells increased with the increase in the severity of epithelial dysplasia on sections stained H and E up to mild dysplasia and cancer[14]. This finding is based on Gannot et al found that the total number of immune cell infiltrates (CD4, CD8 and B cells) were significantly higher in patients with moderate to severe dysplasia compared to hyperkeratosis and mild dysplasia. In addition, this study showed that B cells are very pronounced in moderate to severe dysplasia, and even more pronounced in SCC. As a result, although the study attempted to count the vessels in non-inflammatory areas, a relationship was expected between the increase in inflammatory cell infiltration and the mean number of vessels. Assessment of the correlation between inflammation cell infiltration density and vascular density in the underlying connective tissue of the examined groups showed that the average number of vessels appeared in samples with mild inflammatory cell infiltration density[15]. In fact, in this study, a correlation was observed between blood vessels and inflammation at an early stage of dysplastic lesions instead of at the invasive stage. As far as we know, no study has yet examined this relationship.

CONCLUSION:

This study result showed that observation of inflammatory infiltration and submucosal infiltration of leukoplakia in routinely stained H&E could be used to evaluate the lesion severity. In particular, the observation of inflammation and high vascular density in H and E staining of oral leukoplasia prophesies more destructive epithelial dysplasia.

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