An Immuno histochemical Study On Tumor Progression

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Abstract **- Oral Squamous Cell Carcinoma (OSCC) is a serious health concern that can lead to a reduced quality of life or even death. It ranks sixth in terms of cancer incidence and is one of the top causes of mortality in India. PMDs (Potentially Malignant Disorders) are precancerous lesions that have a higher chance of developing into oral squamous cell carcinoma. Tumor angiogenesis is a key biomarker that may influence the evolution of a precancerous lesion into a cancerous one. By generating angiogenesis, Vascular Endothelial Growth Factor (VEGF) plays an important role in carcinogenesis. Angiogenesis is the formation of new blood vessels from existing ones, which is essential for tumors growth, progression, and metastasis. This study was to examine and assess the expression of VEGF in Oral Epithelial Dysplasia (OEDs), Verrucous Carcinoma (VCs), and various grades of Oral Squamous Cell Carcinoma (OSCCs). The study included 100 formalin-fixed paraffin-embedded tissue blocks with 20 cases of OED, 20 cases of VC, and 60 cases of OSCC [20 cases of well-differentiated oral squamous cell carcinoma (WD-OSCC), 20 cases of moderately differentiated oral squamous cell carcinoma (MD-OSCC), and 20 cases of poorly differentiated oral squamous cell carcinoma (PD-OSCC). Anti-human VEGF mouse IgG monoclonal antibody was used to stain all of the cases. Analysis of variance (ANOVA), Post hoc Bonferroni test, Independent t-test, Pearson Chi-square test, and Pearson correlation coefficient test were used to investigate the expression of VEGF for staining intensity, distribution, and statistical analysis. VEGF immuno expression were shown to be higher in OEDs, VC, and Oral Squamous Cell Carcinoma OSCC, in that order. PD-OSCC had the highest proportion of positive, followed by MD-OSCC and WD-OSCC. Angiogenesis plays a significant role in tumors growth and metastasis, according to our findings. The expression of VEGF, as well as increases in vascularity during the transition from OEDs to VCs and OSCCs, were found to be significantly linked.**

Keywords: angiogenesis, Growth factor, Oral cancer and dysplasia.

1. INTRODUCTION

The term dysplasia comes from the Greek word dysplasia, which meaning "abnormal growth." The phrase "exfoliated cells from the cervix" was originally used in pathology by Reagon in 1958 to describe exfoliated cells from the cervix. The presence of OED is thought to be the initial alteration associated with malignant transformation. [1] Oral Potentially Malignant Disorder (OPMD) is a clinical name for precursor lesions in which epithelial dysplasia is the histological diagnosis. Leukoplakia, erythroplakia, actinic keratosis, reverse smoker's palate, oral submucous fibrosis, erosive lichen planus, and lupus erythematosus are examples of OPMDs. It has a global occurrence rate ranging from 1% to 5%, with 36% of dysplastic lesions progressing to cancer. According to an early epidemiological study from India, OPMDs were shown to be the cause of 80 percent of oral malignancies. [2] The progression of oral epithelial dysplasia into invasive carcinoma is marked by an increase in angiogenic switch, which is marked by an increase in vascularization and can be used as a marker of malignant transformation. [3] Angiogenesis is thought to be a critical process in epithelial dysplasia for the dysplastic cells' nourishment and development. Dysplastic cells, tumors cells, stromal cells like fibroblasts, and inflammatory cells like mast cells and macrophages all produce angiogenic factors. Many studies have demonstrated that these angiogenic agents are responsible for tumors growth, invasion, and metastasis, as well as increasing significant alterations in vascularity along the transition from normal oral mucosa to dysplasia to various degrees of carcinoma. (4), (5) Angiogenesis is a crucial phase in tumors growth, and metastasis refers to the formation of new blood vessels from existing ones. Capillary sprouting will be used to accomplish this. The tumors can only grow up to 1-

2mm with the current blood supply, and without any further blood supply, the tumors cells will get exhausted, resulting in hypoxia-induced death. [6-8] The growth factor vascular endothelial growth factor (VEGF) belongs to the Platelet-Derived Growth Factor (PDGF) superfamily. VEGF was thought to be a powerful angiogenic stimulator when combined with its associated receptor. It stimulates the motility and maturation of endothelial cells (ECs), allowing blood and oxygen to flow into the hypoxic environment. ECs in tumorss multiply 20-2000 times faster than ECs in healthy tissue. [9, ten] VEGF expression is a sensitive angiogenesis marker that may be used to assess tumors angiogenesis cells and has been found to be effective in tumors promotion, tumors development, and even metastasis prediction. The goal of this study is to look at VEGF immuno expression in OEDs, VCs, and different grades of OSCCs.

2. MATERIALS AND METHODS

With the archived samples that were subjected to histological inspection, this immunohistochemistry (IHC) investigation was undertaken in the Department of Oral Pathology & Oral Microbiology, Vinayaka Mission's Sankarachariyar Dental College, India. Three groups of 100 formalin-fixed paraffin-embedded tissue samples were used in the investigation. Group 1 includes 20 OED instances, Group 2 includes 20 VC cases, and Group 3 includes 60 OSCC cases [20 WD-OSCC (Group 3A), 20 MD-OSCC (Group 3B), and 20 PD-OSCC (Group 3C)]. The study used normal oral epithelium obtained for marginal clearing as a positive control. The Institutional Ethical Committee, Vinayaka Mission Sankarachariyar Dental College, and Salem all gave their approval to the project. Mouse monoclonal anti-human antibody VEGF (Pathnsitu, USA-RTU) and secondary conjugated polymer were used to treat the samples (Pathnsitu, USA).

Interpretation of staining: Under 10x/40x magnifications, the immuno stained slides were examined for positive and a high-quality photomicrograph was taken. Brown precipitate in the cytoplasm of tumors cells suggested a positive reaction. Each section's entire area was evaluated and analysed, with the primary portion that demonstrated intensity being taken into account.

***Evaluation of VEGF Immuno reactivity*:** A semi-quantitative scoring system was used to assess VEGF immunostaining: A. Percentage of VEGF immuno reactivity B. Intensity of staining C. VEGF Final Score The percentage of VEGF positive in each field was documented by two observers, and the staining region was graded. The scoring criteria for IHC-labeled cells developed by Klein et al. were followed [11, 12].

***Statistical Analysis*:** The mean, standard deviation, Analysis of variance (ANOVA), Post hoc Bonferroni test, Independent t-test, Pearson Chi-square test, and Pearson correlation coefficient test were used to statistically analyse the data on immunohistochemical expression of VEGF in all tissue sections using the statistical Package for Social Sciences (SPSS) software version 19.0. Statistical significance was defined as a probability value of less than 0.05.

Results: VEGF immunostaining was diffuse, brownish, and indicated granular cytoplasm of epithelial cells, as well as a few stromal components such as fibroblasts and endothelial cells, as well as inflammatory cells. In the positive control tissue, VEGF expression was determined to be low to moderate (Fig 1).

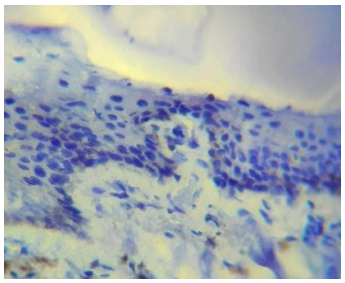

Fig 1: The photomicrograph shows normal oral mucosa with few cells VEGF positive in basal layer

In OEDs, VEGF expression was found in the basal and suprabasal epithelial layers. Negative expression was seen in 30% of dysplasias, mild expression in 70%, and moderate expression in 10%. (Fig 2).

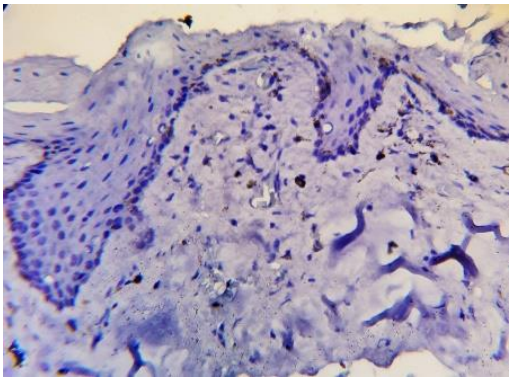

Fig 2: Epithelial Dysplasia shows VEGF positive cells in basal and para basal layer

In the peripheral area, VC showed mild to moderate expression, with 15% being negative, 50% mild, and 35% showing moderate expression (Fig 3).

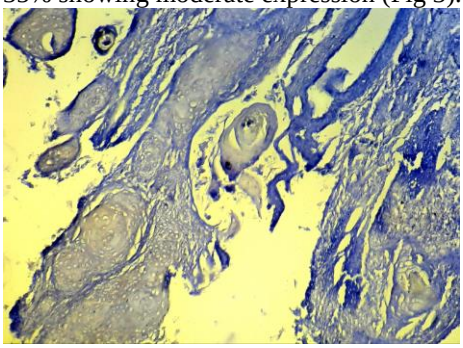

Fig: 3: Immuno histochemical mild expression of VEGF in Verrucous Carcinoma

WD-OSCC was shown to have mild to moderate expression in the keratin pearl area among OSCCs. WD-OSCC expression was found to be 15% negative, 30% mild, and 55% moderate (Fig 4).

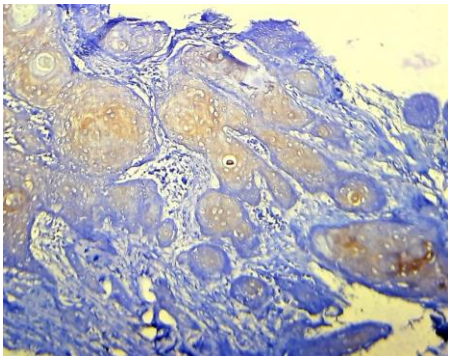

Fig : 4 : Immuno histochemical Moderate expression of VEGF in Well Differentiated OSCC

MD-OSCC shows the complete thickness of the tumors island, there was moderate expression. MD-OSCC expression was found to be 15% negative, 15% mild, 45 percent moderate, and 25% severe (Fig 5).

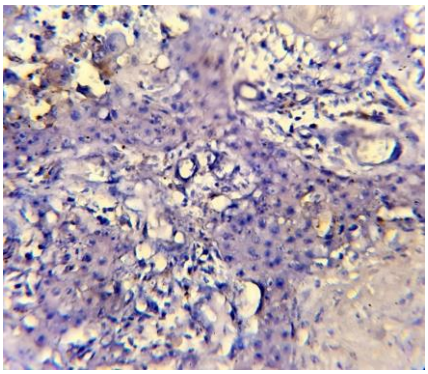

Fig : 5 : Immunohistochemical Mild expression of VEGF in Moderately Differentiated OSCC

The immuno expression of PD-OSCC is moderate to severe in dispersed areas. PD-OSCC expression was found to be 15% negative, 35% moderate, and 50% severe (Fig 6).

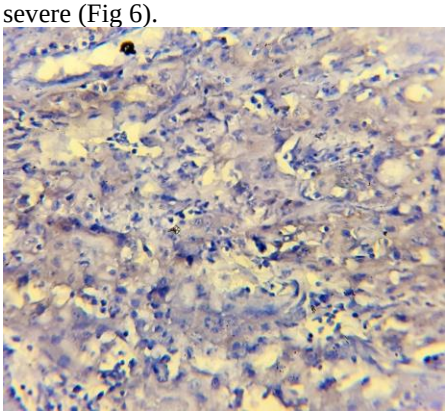

Fig 6: Immuno histochemical Moderate expression of VEGF in Poorly Differentiated OSCC

Percentage of VEGF immuno reactivity: The percentage of VEGF in OED, VC, and different grades of OSCC cases differed significantly (P = 0.001). During the transition from OED to VC and various stages of OSCC, the percentage of immuno reactivity increased.

***Intensity of VEGF immunostaining*:** The intensity of VEGF in OED, VC, and different grades of OSCC cases differs statistically significantly (p=0.001). The intensity of VEGF immunostaining increased as the cases progressed from OED to VC to various stages of OSCC.

***Evaluation of VEGF final score*:** VEGF positive in OED, VC, and different grades of OSCC cases were found to have a statistically significant connection (p=0.001). In OED, VC, and different grades of

OSCC cases, the VEGF immunoreactivity reaction was observed to be greater.

3. DISCUSSION

Carcinogenesis is a cell cycle disruption caused by mutant oncogenes, tumour suppressor genes, and a changed microenvironment. OPMDs displaying epithelial dysplasia were shown to be associated with 80 percent of oral malignancies. [2] Oral carcinogenesis is the progression of cancer from precancerous lesions, and the two-step process is well-documented in the literature. "Altered epithelium with an increased chance of development to squamous cell carcinoma," according to the World Health Organization (WHO). [12] Dysplasia, which is cytological and architectural abnormalities within the epithelium, causes epithelial modification. [13] Angiogenesis is the growth and multiplication of blood vessels from the existing vasculature. Endothelial cells create new vasculature in a series of steps that are mediated by protein molecule mediators. In histological sections of bronchial epithelium, the presence of capillaries next to (juxtaposed) to dysplastic epithelium was identified as Angiogenic Squamous Dysplasia (ASD). The presence of an ASD-like complex in OPMDs could be a biomarker for the upcoming oral carcinogenesis process. Angiogenesis is important in the progression of oral dysplasia to cancer. [3] This entire process is dependent on the formation of new blood vessels from the existing vasculature, which is critical for tumour advancement. Several angiogenic markers have been used in the literature to evaluate blood vessels. The balance between angiogenic stimulators like TGF-, VEGF, and FGF and inhibitors like TSP-1, angiostatin, and PF4 regulates angiogenesis. [14] Through a hypoxic environment, fatigued tumour cells drive angiogenesis. [15] One such powerful angiogenic cytokine involved in tumour angiogenesis is VEGF.

Limitations: A larger sample size and different areas from the same tumors must be analyzed to come to an authenticate conclusion. Mean vascular density (MVD) analysis was not included, neo angiogenesis in the tumors microenvironment could not be located, and larger sample size and different areas from the same tumors must be analyzed to come to an authenticate conclusion.

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