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REVIEW ARTICLE

MARKERS OF ANGIOGENSIS IN POTENTIALLY MALIGNANT LESIONS AND ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT:
Angiogenesis, the growth of new blood vessels from pre-existing ones, is one of hallmark of tumor formation and is also important in a number of normal physiologic processes including growth and development, wound healing. Folkman and colleagues demonstrated that solid tumors cannot grow any larger than 2-3 mm in diameter without being able to induce their own blood supply.Tumor growth can be stunted via a number of anti-angiogenic agents which are under clinical trial, VEGF inhibitors being the most important one. But ongoing researches have shown that monotherapy with VEGF was not as effective as needed with various other side effects. Therefore there is an arising need for discovering alternative strategies to target angiogenesis in tumors.Various biomarkes for angiogenesis has been tried over the years, in both potentially malignant lesions as well as head and neck squamous cell carcinoma and their co-relation with lymph lode metastasis, grading, site, size, association with inflammation, TNM staging, bone invasion, perineural invasion has been studied by various authors.A thorough understanding of angiogenesis and its role in cancer prevention is needed to increase the overall survival rate and prognosis of cancer.
Key Words: Angiogenesis, potentially malignant lesions, oral squamous cell carcinoma, tumor markers.

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INTRODUCTION

Overall, head and neck cancer accounts for more than 550,000 cases annually worldwide [1] Several factors contribute to poor outcome of oral cancers like oral cancer is often diagnosed in an advanced stage.[2,3] Development of second primary tumors are most common in patients with head and neck squamous cell carcinoma and is the most common cause of treatment failure and death.[4,5] HNSCC is also believed to arise via a multistep process that involves the activation of oncogenes as well as the inactivation of tumor suppressor genes.[6-10]

For the metastatic spread of cancer tissue, growth of the vascular network is important. The processes whereby new blood and lymphatic vessels form are called angiogenesis and lymphangiogenesis, respectively. Both have an essential role in the formation of a new vascular network to supply nutrients, oxygen and immune cells, and also to remove waste products.[11]

Angiogenesis, the growth of new blood vessels from pre-existing ones, is one of the essential phenotypes of tumor formation and is also important in a number of normal physiologic processes including growth and development, [12] wound healing, [13] and reproduction.[14-17]. Folkman and colleagues demonstrated that solid tumors cannot grow any larger than 2-3 mm in diameter without being able to induce their own blood supply

Neovascularization, including tumor angiogenesis, is basically a four-step process. First, the basement membrane in tissues is injured locally. There is immediate destruction and hypoxia. Second, endothelial cells activated by angiogenic factors migrate. Third, endothelial cells proliferate and stabilize. Fourth, angiogenic factors continue to influence the angiogenic process [1]

Blood vessels supply oxygen and nutrients and provide gateways for immune surveillance. Endothelial cells (ECs) line the inner surface of vessels to support tissue growth and repair. As this network nourishes all tissues, it is not surprising that structural or functional vessel abnormalities contribute to many diseases. Inadequate vessel maintenance or growth causes ischemia in diseases such as myocardial infarction, stroke, and neurodegenerative or obesity-associated disorders, whereas excessive vascular growth or abnormal remodeling promote

many ailments including cancer, inflammatory disorders, and eye disease.[17-19] Vessels are also used as routes for tumor cells to metastasize.

DEVELOPMENT OF BLOOD VESSELS-

In the embryo, new vessels form de novo via the assembly of mesoderm-derived endothelial precursors (angioblasts) that differentiate into a primitive vascular labyrinth (vasculogenesis).[20] Subsequent vessel sprouting (angiogenesis) creates a network that remodels into arteries and veins.[21] Recruitment of pericytes and vascular smooth muscle cells that enwrap nascent EC tubules provides stability and regulates perfusion (arteriogenesis).[22] In the adult, vessels are quiescent and rarely form new branches. However, ECs retain high plasticity to sense and respond to angiogenic signals. The term ‘‘angiogenesis’’ is commonly used to reference the process of vessel growth but in the strictest sense denotes vessel sprouting from pre-existing ones.

Attracted by proangiogenic signals, ECs become motile and invasive and protrude filopodia. These so-called tip cells spearhead new sprouts and probe the environment for guidance cues. Following tip cells, stalk cells extend fewer filopodia but establish a lumen and proliferate to support sprout elongation. Tip cells anastomose with cells from neighboring sprouts to build vessel loops.

The initiation of blood flow, the establishment of a basement membrane, and the recruitment of mural cells stabilize new connections. The sprouting process iterates until proangiogenic signals abate, and quiescence is re-established. Although vessels can grow via other mechanisms, such as the splitting of pre-existing vessels through intussusception or the stimulation of vessel expansion by circulating precursor cells.[23,24]

Tumor Vessels Are Abnormal

Tumor vessels display abnormal structure and function [25,26] with seemingly chaotic organization. Highly dense regions neighbor vessel-poor areas, and vessels vary from abnormally wide, irregular, and tortuous serpentine-like shape to thin channels with small or compressed lumens. Every layer of the tumor vessel wall is abnormal. ECs lack a cobblestone appearance, are poorly interconnected, and are occasionally multilayered. The basement membrane is irregular in thickness and composition, and fewer; more loosely attached hypocontractile mural cells cover tumor vessels, though tumor-type-specific differences exist.

The resulting irregular perfusion impairs oxygen, nutrient, and drug delivery.[25,26] Vessel leakiness together with growing tumor mass increases the interstitial pressure and thereby impedes nutrient and drug distribution. The loosely assembled vessel wall also facilitates tumor cell intravasation and dissemination. As a consequence of poor oxygen, nutrient, and growth factor supply, tumor cells further stimulate angiogenesis in an effort to compensate for the poor functioning of the existing ones. However, this excess of proangiogenic molecules only leads to additional disorganization as the angiogenic burst is nonproductive, further aggravating tumor hypoperfusion in a vicious cycle. The hypoxic and acidic tumor milieu constitutes a hostile microenvironment that is believed to drive selection of more malignant tumor cell clones and further promotes tumor cell dissemination. The uneven delivery of chemotherapeutics together with a reduced efficacy of radiotherapy, owing to the lower intratumoral oxygen levels, limits the success of conventional anticancer treatment.

Modes of Tumor Vascularization

Besides sprouting, tumors utilize other modes of vessel growth. For example, tumor cells can co-opt pre-existing vasculature without a need to stimulate vessel branching initially. Once the tumor outgrows this supply, hypoxia evokes a secondary angiogenic

response. Bone marrow-derived progenitors can also promote tumor vascularization or control the angiogenic switch
during metastasis, but their importance is debated and context dependent.[27] If tumors would be able to switch mechanisms of vascular growth and some of these mechanisms rely less on VEGF, they would possess the means to escape from treatment with VEGF (receptor) inhibitors and therefore there was an urge to discover more antiangiogenic mechamnisms.

Angiogenic Factors and Regulation of Angiogenesis-

A number of molecules are involved in the complex angiogenic cascades. Angiogenic factors can be categorized as follows: [28]

(1) Soluble growth factors such as acidic and basic fibroblast growth factor (aFGF and bFGF) and vascular endothelial growth factor (VEGF), which are associated with EC growth and differentiation;

(2) Inhibiting factors that inhibit the proliferation and enhance the differentiation of ECs, such as transforming growth factor β (TGF-β), angiogenin, and several low molecular weight substances ; and

(3) Extracellularmatrix-bound cytokines that are released by proteolysis, which may contribute to the regulation of angiogenesis and include angiostatin, thrombospondin, and endostatin.

In addition, a number of microphages secreting bFGF, tumor necrosis factor (TNF), and VEGF were shown to be associated with tumor angiogenesis. Angiogenesis is governed by a balance between inducers and inhibitors. It also can be regulated by EC proliferation, which is regulated or restrained by pericytes through the sequestration of potent mitogens in the extracellular matrix, changes in EC shape that reduce the sensitivity of the cells to growth factors, and certain endothelial factors.

Table 1: Overview of the different angiogenic factors [29]

Category	Names	Major functions
Proteolytic enzymes	(i) Matrix metalloproteinases (MMPs): matrilysin (MMP-7), interstitial collagenase (MMP-1), neutrophil collagenase (MMP-8), collagenase-3 (MMP-13), stromelysin-1 (MMP-3), stromelysin-2 (MMP-10), stromelysin-3 (MMP-11), metalloelastase (MMP-12), MMP-19, enamelysin (MMP-20), gelatinase A (MMP-2), gelatinase B (MMP-9), MT1-MMP (MMP-14), MT2-MMP (MMP-15), MT3-MMP (MMP-16), MT4-MMP (MMP-17) (ii) Plasminogen activators (PAs)	MMPs; taking different substrates according to MMPs; substrates can be collagen, gelatin, laminin, fibronectin, proteoglycans, and proMMPs
Angiogenesis inducers	Vascular endothelial growth factor family (VEGF-A or VEGF, PlGF, VGGF-B, VEGF-C, VEGF-D, orf virus VEGF or VEGF-E), fibroblast growth factor family (aFGF, bFGF, etc.), angiopoietin 1 (Ang-1), transforming growth factor-alpha/beta (TGF α/β), platelet-derived growth factor (PDGF), hepatocyte growth factor/scatter factor (HGF/SF), tumor necrosis factor-alpha (TNFα), interleukin-1/8, angiogenin, ephrins, integrins $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_5\beta_1$, cyclooxygenase-2 (COX-2)	(i) Induction of EC proliferation, migration, and differentiation (ii) TGF-β shows opposite effect in some contexts
Angiogenesis inhibitors	Thrombospondin-1/2 (TSP-1/2), angiostatin (plasminogen fragment), endostatin (collagen XVIII fragment), vasostatin (calreticulin fragment), tumstatin, platelet factor-4 (PF4), antiangiogenic antithrombin III, kringle 5 (plasminogen fragment), prolactin 16-kD fragment, fragment of SPARC, 2-methoxyestradiol, metalloproteinase inhibitors (TIMPs), interferon-alpha/beta/gamma (IFN $\alpha/\beta/\gamma$), interleukin-12 (IL-12), IP-10, Ang-2	(i) Inhibit EC proliferation/migration (ii) Induce EC apoptosis (iii) TIMPs: inhibit MMP or uPA activity (iv) Ang-2: inhibit blood vessels maturation, antagonist of Ang-1

ANGIOGENESIS MARKERS -

MICROVESSEL DENSITY-

The microvessel density is usually considered a promisisng prognostic marker in several human tumors. However, its possible clinical role in tumors of head and neck region is not completely understood, its association with clinic-pathological parameters and prognosis being still controversial. As tumors grow, MVD increases parallel to tumor volume, as assessed by T stage and stage of invasion. MVD is maintained during carcinogenesis and increases with tumor growth, these characteristics suggest that some angiogenic factors released from OSCCs promote neovascularization.

VEGF –

VEGF is a potent angiogenic cytokine involved in the development of blood supply and is located on chromosome 6p12. It has been known to induce both physiologic and pathologic angiogenesis. The mechanism by which VEGF induces angiogenesis includes increased vascular permeability, leakage of proteins cell proliferation, promotion of endothelial cell growth, migration and differentiation. [30]

The family of vascular endothelial growth factors (VEGFs), including VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, placental growth factor and VEGF-F, play a key role in angiogenesis and lymphangiogenesis. Among them, VEGF-A is known to be a key angiogenic factor, and is the most frequently used by a tumor to switch on its angiogenic phenotype. In fact, VEGF-A overexpression has been reported in most types of cancer, including oral cancer, and it is thought to be a prognostic factor for survival. VEGF-C stimulates the proliferation of both vascular and lymphatic endothelial cells *via* the VEGF receptor (VEGFR)-2 and VEGFR-3 in many physiological and pathological processes. VEGF-C has been detected in several different types of cancer, and its level in some studies seems to correlate with nodal metastasis and patient survival.

CD34 –

Is a cell surface sialomucin-like glycoprotein expressed by endothelial cells. CD34 is a highly glycosylated transmembrane cell surface glycoprotein, expressed by hematopoietic stem and progenitor cells and, on the luminal cell membrane of quiescent endothelial cells of small blood vessels and lymphatic. CD34 play a more selective role in chemokine-dependent migration of eosinophils and dendritic cell precursors.[31]

CD31 - platelet-endothelial cell adhesion molecule

CD31, also known as platelet endothelial cell adhesion molecule-1 (PECAM-1), is a 140 kDa type I integral membrane glycoprotein that is expressed at high levels on early and mature endothelial cells, platelets, and most leukocyte subpopulations. The expression on endothelial cells is concentrated at junctions between adjacent cells. CD31 is also expressed on a major population of macrophage/dendritic cell precursors in the bone marrow. PECAM-1 is known to have various roles in vascular biology including angiogenesis, platelet function, and thrombosis. It is a mechanosensor of endothelial cell response to fluid shear stress and it is involved in the regulation of leukocyte migration through venular walls.

CD 105 (ENDOGLIN)-

Is a cell adhesion molecules, first found in a human pre-B cell line. It is a receptor that is strongly up-regulated in proliferating endothelial cells, and - as such - an optimal indicator of proliferation of endothelial cells also in tumour neovasculature. In contrast to pan-endothelial
markers, CD105 is preferentially expressed on endothelial cells of all angiogenic tissues, including tumours, but weakly or not at all with those of normal tissues, giving the superiority oF
CD105 as a marker of tumour angiogenesis. CD105 (endoglin) is a disulfide-linked homodimeric cell membrane glycoprotein of 180 kDa. It is a transmembrane phosphorylated glycoprotein, a component of the receptor complex of transforming growth factor (TGF)-β, which is a pleiotropic cytokine that modulates angiogenesis by the regulation of different cellular functions, including proliferation, differentiation and migration.19 CD105 binds several components of the TGF-β superfamily, in particular TGF-β1 and TGF-β2. [32]

ICAM1

ICAM-1 (Intercellular Adhesion Molecule 1) also known as **CD54** (**C**luster of **D**ifferentiation 54) is a protein that in humans is encoded by the *ICAM1* gene. This gene encodes a cell surface glycoprotein which is typically expressed on endothelial cells and cells of the immune system.

It binds to integrins of type CD11a / CD18, or CD11b / CD18 and is also exploited by rhinovirus as a receptor. Is a member of the immunoglobulin family that binds to integrin 2_, LFA1 and Mac1. LFA1 is expressed by almost all leukocytes in blood circulation and the reaction between ICAM1 and LFA1 has high importance in the adhesion and migration of leukocytes to the sites of inflammation. Stained blood vessels were observed in a normal mucosa exposed to ICAM1 in the study of Walton et al.; there was also an apparent increase in the number and intensity of vascular staining in oral lichen planus.

VCAM1-

The VCAM-1 gene contains six or seven immunoglobulin domains, and is expressed on both large and small blood vessels only after the endothelial cells are stimulated by cytokines. It is alternatively spliced into two known RNA transcripts that encode different isoforms in humans. The gene product is a cell surface sialoglycoprotein, a type I membrane protein that is a member of the Ig superfamily. It is a member of the immunoglobulin family that binds to integrin 1_ and VLA4 and exists on the memory T-cells..Upregulation of VCAM-1 in endothelial cells by cytokines occurs as a result of increased gene transcription(e.g., in response to Tumor necrosis factor-alpha (TNF-α) and Interleukin-1 (IL-1)) and through stabilization of Messenger RNA (mRNA) (e.g., Interleukin-4 (IL-4)). The promoter region of the VCAM-1 gene contains functional tandem NF-κB (nuclear factor-kappa B) sites. The sustained expression of VCAM-1 lasts over 24 hours. Primarily, the VCAM-1 protein is an endothelial ligand for VLA-4 (Very Late Antigen-4 or integrin α4β1) of the β1 subfamily of integrins. VCAM-1 expression has also been observed in other cell types (e.g., smooth muscle cells). It has also been shown to interact with EZR and Moesin.[33]

DECORIN

Decorin is a member of the small leucine-rich repeat proteoglycan (SLRP) family, which was first discovered 'decorating' collagen I fibrils and was subsequently shown to regulate fibrillogenesis.Both the protein core and the single, covalently attached glycosaminoglycan (GAG) moieties of decorin are involved in this function, the relevance of which is demonstrated by the phenotype of the decorin null mouse, which exhibits loose, fragile skin due to dysregulated fibrillogenesis. Interestingly, a role for decorin in postnatal angiogenesis was also revealed by studies in the decorin null background. Corneal neoangiogenesis was reduced. Conversely, neo-angiogenesis was enhanced during dermal wound healing, although surprisingly
this led to delayed wound closure. In this case, skin fragility due to the absence of decorin may have hindered wound closure, despite an increased blood supply. It is apparent however, that decorin plays a role in inflammation-associated angiogenesis. Indeed, endothelial cells undergoing angiogenic morphogenesis in this environment express decorin, while quiescent endothelial cells do not indicating that decorin modulates endothelial cell behaviour specifically during inflammatory-associated remodelling of the vascular system.[34]

ANGIOGENESIS IN ORAL SQUAMOUS CELL CARCINOMA AND POTENTIALLY MALIGNANT LESIONS-

G.Ascani etal in 2006 evaluated microvessel density in 64 cases of squamous cell carcinoma of oral cavity using immunohistochemical analysis with CD34 monoclonal antibody and correlated it with different clinicopathological parameters. Microvessel density differed in three histological groups (grade 1, grade 2, grade 3) (p=0.0331) and between node positive and node negative patients (p< 0.0001). no statistical correlation was observed between microvessel density and other clinical parameters such as age , sex , tumor site and size. [35]

Giuseppe-Alessandro Scardina et al in 2009 compared the vascular endothelial growth factor (VEGF) and adhesion of molecules in the biopsy samples of 30 patients affected by OLP, in order to research the presence of the angiogenetic phenomenon and to understand its pathogenetic mechanism. Immunohistochemical analysis of the VEGF and vascular endothelial
adhesion molecules was carried out by means of primary antibodies and anti-CD34, anti-VEGF, anti-CD106 antigen (VCAM-1) and anti-CD54 antigen (ICAM-1). Presence of a significant
angiogenesis in OLP patients for the VEGF, CD34, CD106 and CD54 (P < 0.001) was revealed. The number of vessel in the biopsies of the patients with OLP (mean±SD: 21.27±4.85), compared with the healthy subjects (mean±SD: 4.74±0.97) was

significantly more (Mann-Whitney test, $P < 0.001$). The positive expression rate of VEGF, CD34, VCAM-1 and ICAM-1 in oral lichen samples was 64.2%, 54.3%, 32.5% and 29.7%, respectively showing that
significant neoangiogenesis occurs in oral lichen planus.[36]

Shu-Hui Li et al in 2009 investigated the sensitivity and specificity of different endothelial markers for evaluating microvessel density (MVD) in OSCCs. 84 OSCC specimens were immunohistochemicaly stained for three common endothelial markers: van Willebrand factor (vWF), CD31 and CD34. There was no significant association between peritumoral MVD and clinicopathological parameters. However, the intratumoral MVDs determined using CD31 and CD34 were significantly associated with tumor size ($P = 0.003$ and $P < 0.0001$, respectively), with histological differentiation ($P = 0.0025$ and $P = 0.018$, respectively) and with tumor stage ($P = 0.001$ and $P < 0.0001$, respectively). Also, the intratumoral MVD counted using CD34 immunostaining was significantly associated with lymph node metastasis of OSCC ($P = 0.005$). Showing endothelial marker CD34 better in the assessment of tumor vascularization of OSCCs. Furthermore, they concluded that hotspot selection, especially intratumoral MVD, is important in examining OSCC progression.[37]

Claudiu Margaritescu et al in 2010 investigated by immunohistochemistry vascular endothelial growth factor (VEGF) expression in tumor cells and correlated VEGF expression to microvessel area, evaluated by using CD105 as a marker of endothelial cells, in biopsy specimens of 54 human OSCC and demonstrated that VEGF is highly expressed in OSCC tumor specimens when compared to pre-neoplastic and normal tissues, without differences between the edge and inside the tumor. Moreover, VEGF expression is reduced in poor differentiated OSCC tumors when compared to moderate and good differentiated forms, and tumor microvessel area is higher in tumors when compared to pre-neoplastic lesions and normal tissues.

Tomofumi Naruse et al in 2011 aimed to clarify the relationship between vascular endothelial growth factor (VEGF) expression and clinicopathological factors in oral squamous cell carcinoma (OSCC) and also examined the correlation between the VEGF expression and the mammalian target of rapamycin (mTOR)–hypoxia inducible factor-1α (HIF-1α) pathway. 120 smaples of OSCC were stained immunohistochemically for VEGF-A, VEGF-C, p-mTOR and HIF-1α proteins. VEGF-A and VEGF-C protein expression was detected in 76 out of 120 (63%) and 81 of 120 (67.5%) OSCCs, respectively, and their expression was significantly higher in primary OSCC than in normal oral mucosa. VEGF-A expression was significantly associated with the tumor stage and age. VEGF-C expression was significantly associated with the cancer cell invasion. The cases with combined p-mTOR+/ HIF-1α+/VEGF-A+ expression had a significantly higher tumor stage and invasion grade, and combined p-mTOR+/HIF-1α+/ VEGF-C+ expression was significantly associated with tumor stage, regional lymph node metastasis and invasion grade, indicating that the mTOR–HIF-1α–VEGF pathway affects the progression of OSCC, and inhibition of this pathway may be useful for the treatment of OSCC.[38]

Sabarinath et al in 2011 aimed at comparing and correlating mast cell density and microvascular density in different grades of oral squamous cell carcinoma. MCD was assessed imunohistochemically using anti mast cell tryptase and MVD using anti factor VIII related von willebrand factor, a statistically significant increase in MCD and MVD among osmf cases was observed and also a positive correlation between MCD and MVD i.e. as MCD increases there is an exponential increase in MVD was observed, both playing a role in pathogenesis of OSMF.[39]

Madhusudan Astekar et al in 2012, evaluated microvessel density (MVD) and vascular endothelial growth factor (VEGF) expression in oral tumorigenesis and correlated it with the clinicopathological characteristics. VEGF expression and MVD were quantified immunohistochemically using anti-VEGF and anti-CD34 antibody, in 10 normal oral mucosa (NOM), 7 mild epithelial dysplasia (Mild ED), 8 moderate epithelial dysplasia (Mod ED), 5 severe epithelial dysplasia (SED), 14 well-differentiated SCC, 11 moderately-differentiated SCC, and 5 poorly-differentiated SCC. VEGF and MVD appeared to increase with disease progression and were statistically higher in oral SCC than in epithelial dysplasia and normal buccal mucosa. There was

significant correlation between VEGF expression and MVD.[40]

Prakhar Kapoor et al in 2012 analyzed the expression of VEGF in OSCC tissues of different histological grades, clinical sizes and lymph node status and used this as an indicator for disease progression by helping in delineating a risk population, that may benefit from an attractive adjuvant therapeutic strategy for OSCC. This was the first study that takes into account the clinical status of the lymph nodes and VEGF expressivity in a sample size of 30 cases which were immunohistochemically analysed using VEGF antibody. VEGF positivity was seen in approximately. 90% of cases which was independent of histological grade of OSCC. However the intensity increased with the clinical size of cancer and from palpable lymph node to a tender and hard lymph node.[41]

Ipsita Kukreja et al in 2013 evaluated the correlation between expression of VEGF and CD 34, the role of MVD in progression of OSCC and compared the degree of angiogenesis in different grades of OSCC and observed the relation between angiogenesis and MVD and the overall effect of this on 33 cases of oral cancer using immunohistochemically VEGF and CD 34 antibody**.** VEGF positivity as well as MVD was found to be independent of the grade of the tumor. Tumor MVD was found to be independent of expression of VEGF.[42]

ADINA BUNGET et al in 2013 assessed the tumoral neoformed blood vessels in oral squamous carcinomas, using the CD34 antibody, showed a significant growth of the microvascular density, the average number being 504.66±177.65 vessels/mm2. The diameter of angiogenesis vessels varied between 3.42 and 121.27 μm. The density of lymphogenesis vessels was 508.78±235.93 vessels/mm2, while the diameter varied from 2.82 to 165.28 μm. Both angiogenesis and lymphogenesis vessels were more numerous in the areas where the inflammatory infiltrate was more abundant, suggesting that chronic inflammation plays the part of a promoter factor of neoplastic lesions.

Seema Nayak et al in 2013 aimed to examine molecular and phenotypic expression of two angiogenesis modulators viz. decorin and vascular endothelial growth factor-A (VEGF-A) in human potentially malignant oral lesions (PMOLs) and oral squamous cell carcinomas (OSCC) in relation to clinico-pathological variables and survival outcome. 72 PMOLs, 108 OSCC and 52 healthy controls were tested Immunohistochemically using antibodies against decorin, VEGF-A and CD-31. Messenger-ribonucleic acid (mRNA) expression was analyzed by using real-time polymerase chain reaction. Cytoplasmic staining of decorin was observed in the basal layer of epithelium in 53 (73.61%) cases of PMOLs and in peritumoral stroma in 55 (50.92%) cases of OSCC. None of the cases showed nuclear expression of decorin. Decorin expression both at phenotypic and molecular level was found to be down-regulated from PMOLs to OSCC. Lymph node metastasis and reduced decorin expression independently correlated with overall survival in OSCC. VEGF-A expression had no significant impact on survival outcome.[43]

Maryam Seyedmajidi et al , 2013 aimed to evaluate imunohistochemically VCAM1 and ICAM1 in oral lichen planus and to compare these two markers with normal mucosa for evaluation of angiogenesis. 70 samples of oral lichen planus was evaluated and significant results were obtained. VCAM1 and ICAM1 expression significantly increased compared to normal mucosa in oral lichen planus according to the percentage of stained cells ($p=0.000$& $p=0.000$, Mann-Whitney test). Thirty cases of oral normal mucosa associated with lichen planus showed that the VCAM1 has increased significantly in comparison to normal mucosa ($p<0.001$). Also, ICAM1 expression between lichen planus and normal mucosa, showed a significantly difference ($p<0.001$). A significant difference between VCAM1 and ICAM1 expression and type of lichen planus was not observed ($p>0.05$). Regarding the results, it seems that high expression of VCAM1 and ICAM1 is related to oral lichen planus.[44]

Flavio Monteiro-Amado etal, 2013 aimed to evaluate the immunoexpression of MMP-2, MMP-9 and CD31/microvascular density in squamous cell carcinomas of the floor of the mouth and correlated the results with demographic, survival, clinical (TNM staging) and histopathological variables (tumor grade, perineural invasion, embolization and bone invasion). Data from medical records and diagnoses of 41 patients were reviewed. Histological

sections were subjected to immunostaining using primary antibodies for human MMP-2, MMP-9 and CD31 and streptavidin-biotin-immunoperoxidase system. Histomorphometric analyses quantified positivity for MMPs (20 fields *per* slide, 100 points grade, ×200) and for CD31 (microvessels <50 μm in the area of the highest vascularization, 5 fields per slide, 100 points grade, ×400). There was a statistically significant correlation between immunostaining for MMP-2 and lymph node metastasis. Factors associated negatively with survival were N stage, histopathological grade, perineural invasion and immunostaining for MMP-9. There was no significant association between immunoexpression of CD31 and the other variables. The intensity of immunostaining for MMP-2 can be indicative of metastasis in lymph nodes and for MMP-9 of a lower probability of survival.[45]

Vinita V. Murgod etal in 2014 performed to assess the mucosal vasculature in normal oral mucosa(n=10), early (n=30) and advanced (n=30) OSMF, and well differentiated squamous cell carcinoma (WDSCC) (n=30) using morphometry. Morphometric image analysis of blood vessels was performed on H&E-stained sections for evaluation of vascular density, vascular luminal diameter, area and percentage area. A significant increase in all of the parameters was noted in the test groups relative to the controls. The mean vascular density and mean vascular percentage area were significantly increased in early OSMF and WDSCC relative to controls, and also in advanced OSMF and WDSCC in comparison with early OSMF. The vascularity increased progressively from normal to premalignancy and malignancy, emphasizing the importance of angiogenesis in tumor development and progression. The vascularity was increased in early OSMF and reduced in advanced OSMF, suggesting that inflammation may play a role in the early stages while progressive fibrosis may predispose to atrophy of the epithelium and subsequent malignant changes.[46]

Sujatha Varma etal in 2014, investigated the immunohistochemical expression of VEGF in normal oral mucosa (NO)M) (n=5), epithelial dysplasia (n=17) and oral squamous cell carcinoma (OSCC) (n=24) and correlated this expression with histologic features of tumor progression. Statistical analysis indicated an upregulation of VEGF during the transition from NOM through epithelial dysplasia to OSCC.. An increased and intense expression of VEGF was observed in different histologic grades of squamous cell carcinoma with well differentiated OSCC showing the lowest mean VEGF percentage and intensity scores and poorly differentiated OSCC, the highest, suggesting that VEGF is present in elevated levels in dysplasias and OSCCs when compared with normal oral mucosal specimens. Increased levels of this angiogenic factor could enhance tumor growth by promoting neovascularization.[30]

Vijay Wadhwan etal in 2015 morphometrically evaluated the angiogenesis in different grades of oral squamous cell carcinomas (OSCCs) under light microscope by the use of H and E stained sections and assessed that whether the parameters of vascularity like mean vascular density (MVD), mean vascular area (MVA), and total vascular area (TVA) can be used to histologically grade the tumors. A total of 10 cases each of well-, moderately-.and poorly-differentiated SCC cases were retrieved and were morphometrically analyzed for mean vascular density (MVD), MVA, and TVA. They showed significant differences between all the three parameters, that is, MVD, MVA and TVA when poorly differentiated OSCC was compared with the normal mucosa, well-.and moderately-differentiated OSCC. However, when comparison was made between the well-.and moderately-differentiated OSCC, the differences in the three parameters were present but not statistically significant.[48]

CONCLUSION

Cancer has been the second most prevalent disease in India after cardiovascular diseases. Head and neck squamous cell carcinoma and premalignant lesions has always challenged its therapeutic implication due to secondary tumor development.

In today's era the main goal of any clinical and histopathological diagnosis of potentially malignant lesions and HNSCC is to inhibit its growth and spread ; therefore various therapeutic and anticancer agents has come into play like chemopreventives, drugs targeting angiogenesis etc.

Angiogenesis, one of the hall marks of cancer plays a vital role in tumor growth and its spread. Tumor growth can be stunted via a number of anti-angiogenic agents which are under clinical trial, VEGF inhibitors being the most important one. But ongoing researches have shown that monotherapy

with VEGF was not as effective as needed with various other side effects. Therefore there is an arising need for discovering alternative strategies to target angiogenesis in tumors.

Various biomarkes for angiogenesis has been tried over the years, in both potentially malignant lesions as well as HNSCC and their co-relation with lymph lode metastasis, grading, site, size, association with inflammation, TNM staging, bone invasion, perineural invasion has been

studied by various authors as stated above and a positive correlation has been noticed with rumor grade, size and lymph node metastasis.

Therefore a thorough understanding of angiogenesis and its role in cancer prevention is needed to increase the overall survival rate and prognosis of cancer. Studies should be conducted to improve the overall efficacy of antivascular strategies to combat cancer. Also to identify predictive biomarkers tailored for particular tumor stages and treatment.

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