SINAI International Scientific Journal (SISJ)

Vol.1 Issue 1, July 2024

Do Stromal Cells and Micro-Vessel Densities Have a Role in Malignant Transformation of Potentially Malignant and Metastasis of Oral Squamous Cell Carcinoma?

Asmaa S Algharyani[1], Doaa AM Esmaeil[2], Marwa M Shakweer[3], Mohamed S Negm[4], Nadia M Lotfy[5]*

[1]*Oral Pathology Department, Faculty of Dentistry, University of Tripoli, Libya*

[2]*Oral Pathology Department, Faculty of Dentistry, Sinai University, Kantra, Egypt*

[3]*Department of Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt*

[4]*Department of Pathology, Faculty of Medicine Cairo University, Giza, Egypt*

[5]*Department of Oral Pathology, Faculty of Dentistry, Mansoura University, Mansoura, Egypt.*

**Corresponding author*

Correspondence:

Doaa AM Esmaeil
doaa.ismail@su.edu.eg

Citation:

Algharyani A. S, Esmaeil D. AM Shakweer M.M, Negm M.S, Lotfy N. M. "Do Stromal Cells and Micro-Vessel Densities Have a Role in Malignant Transformation of Potentially Malignant and Metastasis of Oral Squamous Cell Carcinoma?", SINAI International Scientific Journal (SISJ), vol. 1, pp. 17-32, 2024

Received: 25 March 2024

Accepted: 30 June 2024

ABSTRACT

Tumour microenvironments are crucial for cancer progression and metastasis. There are two major routes of spread, one by blood vessels and the other through the lymphatic system. There are limited data regarding the immune cell potential, the role of both lymphatic and blood vessels in oral epithelial dysplasia (OED), and metastatic & non-metastatic oral cancer. This study is designed to determine the role that eosinophil cell and mast cell densities play, along with the role of podoplanin (D2-40) (lymphatic marker), CD105 (vascular marker) and PCNA (proliferative marker), in the different grades of oral squamous cell carcinoma and OED. In this cross-sectional study, paraffin blocks were selected, including 60 cases of OSCC and 10 cases of OED. From each block, 6 sections were prepared for hematoxylin and eosin, special stains (Toluidine blue and congo red) for mast cells and eosinophils, and immunohistochemistry with CD105, D2-40, PCNA. The immunoreactivity of this marker was analyzed by an image analysis program. The study revealed a significant increase in lymphovascular density (LVD), microvascular density (MVD) and PCNA in OSCC when compared to OED. Metastatic OSCC demonstrated an increase in LVD, and MVD when compared to non-metastatic OSCC. On the other hand, the degree of differentiation of the infiltrated immune cells revealed that eosinophils and mast cells reduce when OSCC's grade is advanced. In addition, non-metastatic cases showed higher counts of immunological cells than metastatic cases. Tumour cell proliferation, lymphangiogenesis and angiogenesis are important phenomena of cancer cell spreading and are correlated with poor prognosis. The large number of immunological cells in OSCC when compared to dysplastic groups confirms that they have a critical role in stromal invasion. Additionally, the non-metastatic group demonstrated a significantly higher mean count of tumour-associated tissue eosinophils and mast cells than the metastatic group. Thus, we concluded that increased infiltration of eosinophil and mast cells in OSCC is directly related to favourable prognosis and indicates their potential protective role against tumour metastasis.

KEYWORDS: Oral Cancer, tumour microenvironment, podoplanin, CD105, lymphangiogenesis.

1. INTRODUCTION

Oral cancer is a worldwide health burden, with 443,000 new cases and 241,450 deaths globally [1]. Oral squamous cell carcinoma (OSCC) has a high incidence rate of occurrence and mortality worldwide despite advanced treatment modalities [2]. Tumour microenvironment (TME) consists of extracellular matrix (ECM) and diverse cell types, such as cancer-associated fibroblasts and various immune cells. TME plays as positive and negative regulator on tumour growth and development [3]. Thus, the role of mast cells and tumour-associated tissue eosinophils (TATE) in the biological behaviour of tumours has recently been a major point of concern in literature, including their value in angiogenesis and tumourigenesis [4]. Metastasis comprises sequential steps, including proliferation, stimulation of angiogenesis, cell detachment from the primary tumour, invasion through the lymphatic (LVs) or blood vessels (BVs) and interaction with components of the new TME [5]. Several vascular markers have been tested in many studies for the evaluation of micro-vessels in tumours, including CD105, which was associated with increased microvascular density (MVD) and related to advanced stage and poor prognosis [6]. OSCC is characterised by early lymphatic spread to regional lymph nodes (LNs), which proceeds to systemic spread. Thus, cervical LN metastasis was determined to be one of the prognostic factors in OSCC [7]. Several lymphatic endothelial markers have been employed to estimate the density of lymph vessels in cancer, including podoplanin, which is detected using the monoclonal D2-40 antibody [8].

This work was conducted with the aim of evaluating the presence of immunological cells in dysplastic epithelium, metastatic and non-metastatic OSCC. Additionally, we aimed to measure microvascular and lymphovascular density in the three groups to evaluate their roles in tumour growth and metastasis.

2. MATERIAL AND METHODS

2.1. Tissue Specimens

Seventy formalin-fixed paraffin-embedded (FFPE) specimens of OSCC and oral epithelial dysplasia (OED) were divided into 5 cases exhibiting mild dysplasia and 5 cases exhibiting severe dysplasia, while OSCC were classified into two groups, group I (32 negative LN) and group II (28 positive LN).

The paraffin blocks were collected from the archives of the oral and maxillofacial pathology department, Faculty of Dentistry, Mansoura University, from files of the pathology laboratory at Ain Shams University Hospital (El-Demerdash) and form the pathology department, Faculty of Medicine, Cairo University.

2.2. Special Stains Procedure

FFPE sections were deparaffinized and rehydrated before staining. Sections were stained with 1% toluidine blue solution for about 30 minutes, then by washing with filtered water. Other tissue sections were stained with 1% Congo red for about 30 minutes with frequent agitation, then rinsed quickly in filtered water. Sections were counterstain in hematoxylin for 15-30 seconds and then washed in tap water. These sections were processed and mounted in synthetic resin. Slides were examined under a microscope.

2.3. Immunohistochemical (IHC) Stains Procedure

At room temperature, endogenous peroxidase was eliminated from hydrated tissue sections by treating them with 3% hydrogen peroxide for 15 minutes. A pressure cooker was utilized for retrieving antigens by incubating the tissue section for 15 minutes in 10% serum. Tissue sections were treated with protein block serum at room temperature, and then they were incubated in a prediluted primary antibody solution. Both CD105 and PCNA were used as primary antibodies. Other antibodies used are Dako mouse monoclonal anti-CD105 antibody, QBEnd-10 (M7165) (dilution 1:25) and pre-diluted PCNA antibody (clone MIB-1; PC: N1633; DD). Also, mouse monoclonal antibody (ab77854; Abcam, Cambridge, MA, USA) was used to detect podoplanin [2].

The DAKO Cytomation Envision+System- HRP kit (AEC) was employed as a secondary antibody following the manufacturer's instructions. To demonstrate how the antigen-antibodies react, they were stained with 3,3′-Diaminobenzidine (DAB) in the supplied solution and then counterstained with Mayer's hematoxylin. Sections were treated with phosphate buffer solution (PBS) as a negative control rather than primary antibodies. The slides were then examined under a light microscope by two individuals in a semi-quantitative manner. The technique used the Avidin-Biotin Complex (ABC) Method.

2.4. Evaluation of stained slides

2.4.1. Evaluation of PCNA-Positive Tumour Cells

The modified score of the samples was based on the intensity and the percentage of positive nuclei as follows. In terms of extent, score (0) is less than 5%, score (1) is 6% to 25%, score (2) is 26% to 50%, score (3) is 51% to 75% and score (4) is 75% to 100%. In terms of intensity, the scale moves between negative (0), mild (1), moderate (2) and strong or intense (3). The final score was computed by multiplying the immunostaining intensity by the proportion of positive cells. This finally gives four groups: negative expression (-, 0), mild positive expression (+, 1 - 3), moderate positive expression (++, 4 - 7) and substantially positive expression (+++, 8 - 12) [9].

2.4.2. Evaluation of Podoplanin-Positive Tumour Cells in Oral Epithelial Dysplasia

We assessed podoplanin expression following the guidelines put forth by Kawaguchi et al. [1] as follows. Score (0): no expression was detected in any area of the epithelium. Score (1): expression is limited to the epithelial basal layer. Score (2): expression is detected in the basal and suprabasal layers at one area. Score (3) suprabasal layer expression is detected at two or three sites. Score (4): suprabasal layer expression is detected at more than three focal areas. On the other hand, we scored immunostaining using the scoring system of Kawaguchi et al. [10] and Vicente et al. [11], which is:

- Score 0-1: negative or low risk.
- Score 2 or more: positive or high risk.

In OSCC, podoplanin immunoreactivity was graded as reported by Rodrigo et al [12] by the positive tumour cell numbers on a scale of 0 to 5, as shown below. (0): Negative: (1) <10% (2): >10% but < 30%. (3) >30%, but <50%. (4) >50% but <80%, and (5) >80% positive staining. The immunoreactivity score (IRS) was determined by multiplying the quantity and staining intensity scores. Scores can vary from 0 to 15 (7 or greater indicates excellent responsiveness; 0 to 6 indicates weak reactivity).

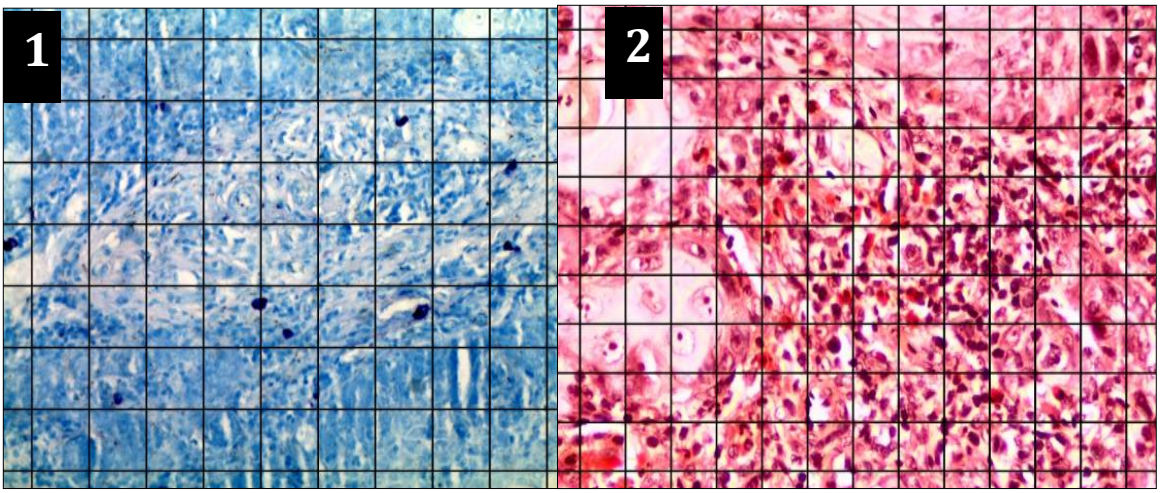
2.4.3. Quantification of LVD and BVD

The densities of lymphatic (D2-40-positive cells) and BVs (CD105-positive cells) in the intra-tumoural zone were measured using the approach published by Weidner et al. [13]. To determine locations with more stained vessels, the slides were inspected at low power (10×). The total number of vessels was assessed by counting five hot spot fields at a magnitude of 40×.

Images were acquired on a computer with (×200) objectives for determining the stained vessel number using Image J software. The lymphatics and blood vessel numbers were converted to several vessels per area (mm2) to yield the LVD and BVD, respectively. The procedure was done for all sections [14].

2.4.4. Evaluation of histochemical staining

Mast cell granules were stained purple-violet with toluidine blue, while eosinophilic granules were brilliant red with Congo red. These cells were further investigated using a microscope. The measurement was performed at random by selecting 10 high-power fields from each slide that revealed a high density of these cells. Each field was inspected under ×40 objective lens using a "software grid" approach to evaluate TATE and mast cells. The entire number of cells was calculated and divided by the overall amount of fields to get the average cell number [15] (Figures 1 and 2).



Figures 1 and 2. Presenting a grid field for eosinophils and mast cells counting in Congo red and toluidine blue, respectively (×40).

2.4.5. Statistical Analysis

Data was analyzed statistically with IIBM SPSS, version 24.0 (IBM Corporation, Armonk, New York). A one-way analysis of variance (ANOVA) test was applied to compare the mean values among more than two groups. A student t-test was conducted to compare the mean values between the two groups. A p-value < 0.05 is considered significant.

3. RESULTS

3.1. Special Staining

In our study, we compared mean TATE and mast cells among mild and severe dysplasia, the variation was substantial (Table 1). On the other hand, we detected a statistically significant rise in the mean mast cells and eosinophil density from dysplasia to OSCC (Table 2).

Table 1: The mean density of eosinophils, mast cells, D2-40, CD 105 and the PCNA in OED.

Lesion	density of eosinophils Mean ± SD	the density of mast cells Mean ± SD	LVD; D2-40 Mean ± SD	MVD; CD105 Mean ± SD	PCNA Mean ± SD
Mild OED	6.14±1.5	3.94±0.54	5.3±2.22	7.9±2.9	1.2±0.4
Severe OED	6.62±0.5	4.26± 0.75	12.4±2.15	14.6±1.5	4.2±2.04
SD indicates standard deviation; LVD, lymphatic vessel density; MVD, microvessel density.					

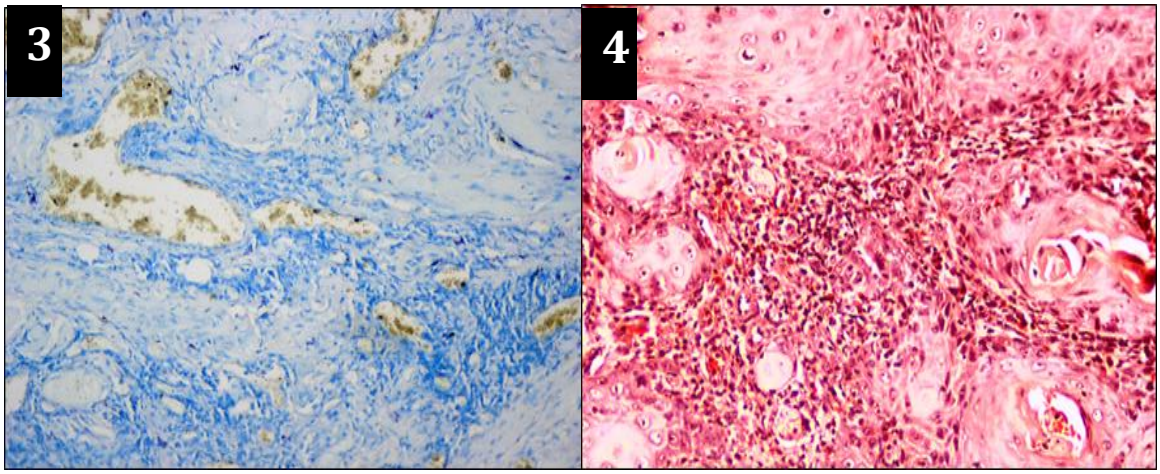
Table 2: The mean density of eosinophils, mast cells, D2-40, CD 34 and PCNA between OED and OSCC.

Lesion	density of eosinophils Mean ± SD	the density of mast cells Mean ± SD	LVD; D2-40 Mean ± SD	MVD; CD105 Mean ± SD	PCNA Mean ± SD
OED	6.38±1.08	4.1±0.6	8.8±4.3	11.2±4.1	2.7±2.11
OSSC	8.3±3.9	5.2±2.4	23.95±14.8	33±15.74	7.1±3.9
SD indicates standard deviation; LVD, lymphatic vessel density; MVD, microvessel density.					

The mean count of eosinophils in the metastatic group was 6.41± 3.15/10 HPF and in the non-metastatic group was 10.36 ± 3.78/10HPF, while the mean count of mast cells in the metastatic group was 4.05± 1.9/10HPF and in the non-metastatic group was 6.2 ± 2.4/10HPF. There was a highly statistically remarkable variance in mean eosinophils and mast cells between the two groups (P< 0.001; Table 3). The mean score of infiltration of both cell types was decreased from well to poorly differentiated SCC in both groups (Figures 3 and 4; Figure 13).

Table 3: The mean density of eosinophils, mast cells, D2-40, CD 105 and the between non-metastatic (Gr.I) and metastatic (Gr. II).

Lesion	density of eosinophils Mean ± SD	the density of mast cells Mean ± SD	LVD; D2-40 Mean ± SD	MVD; CD105 Mean ± SD	PCNA Mean ± SD
Group (I)	10.36±3.8	6.24± 2.4	13.6±8.36	22.6 ± 8.8	4.2±2.9
Group (II)	6.41±3.15	4.05 ± 1.9	35.75±10.9	44.5 ± 13.6	10.2±1.9
SD indicates standard deviation; LVD, lymphatic vessel density; MVD, microvessel density.					



Figures 3 and 4. Photomicrograph showing of mast cells in toluidine blue (a) and (b) eosinophils in Congo red stained section of OSCC (×40).

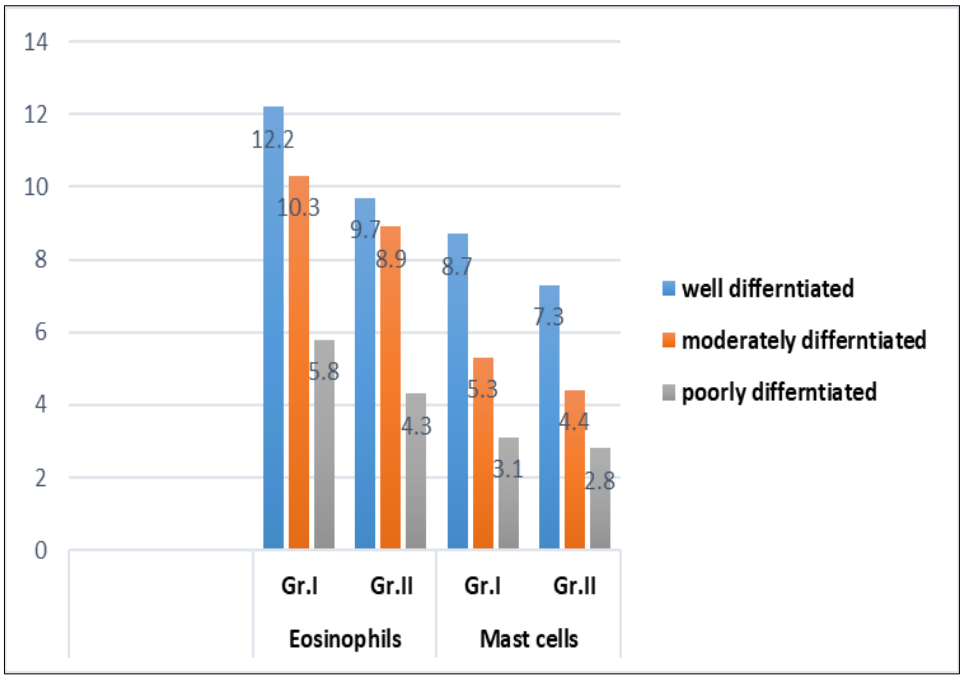


Figure 13. Comparison between mean hot spot of mast cell and eosinophils according to differentiation.

3.2. Immunoreaction for Podoplanin

Immunostaining of podoplanin was found as a cytoplasmic/membranous pattern in tumour cells and in endothelial cells of lymphatic vessels. However, blood endothelial cells expressed a negative reaction.

3.3. Evaluation of Epithelial Podoplanin Score

All studied cases of OED (100%) showed positive reactivity of podoplanin expression, while only 96.7% (58 cases) of OSCC showed such positivity. The staining of podoplanin was confined to the basal layer of the epithelium. No reaction was detected in the upper two-thirds of the epithelium in mild dysplasia. However, severe dysplasia demonstrated a high positive reaction throughout the majority of its thickness (Figure 5). Our observation was based on the mean immunoreactivity scores (MIRS) for podoplanin expression, which showed a highly statistically significant difference between OED and OSCC (P>0.001; Table 4).

Table 4: Distribution of podoplanin immunoreactivity through study cases.

Groups	Mean IRS±SD	P-value
OED	3.8±3.4	<0.001
OSCC (Gr. I +Gr.II)	6.6±4.9	

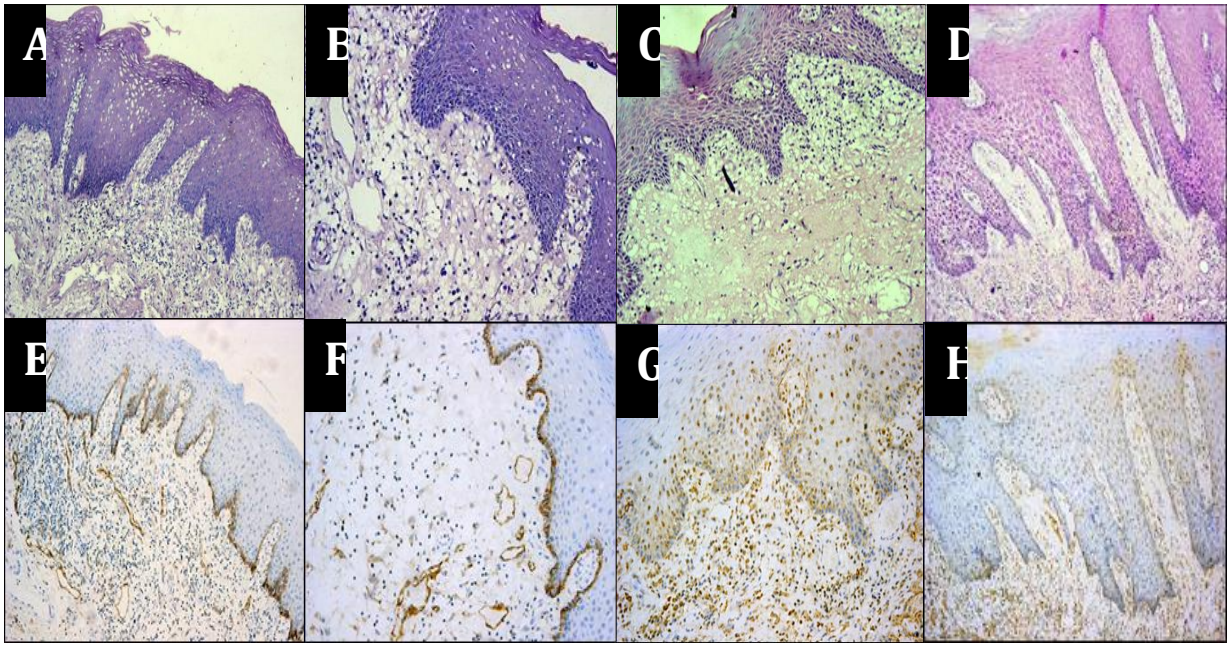


Figure 5. (A and B) mild dysplasia and (C and D) severe dysplasia (A-D H&E ×200). (E and F); podoplanin expression is limited to the basal cell layer in mild dysplasia with score 1, whereas, (G and H) exhibit podoplanin expression in the basal cell layer and suprabasal score 2 (E and F: Anti-podoplanin × 200).

Regarding the correlation of MISR to OSCC grades, it was observed that most of the examined cases of well-differentiated OSCC demonstrated positive membranous and cytoplasmic podoplanin immunoreactivity, including fifteen cases (88%) with high reactivity (12.5±3.1 and 7.4±1.9) in Gr. I and Gr.II, versus only two cases in Gr. II with low

immunoreactivity. The staining was concentrated in the periphery of tumour islands, while the majority of cells at the centre were devoid of the staining reaction. However, the cases of poorly differentiated OSCC in both groups exhibited low membranous immunoreactivity (1.6±1.7 and 2.3±1.07) in Gr. I and Gr.II, (Figure 14) and (Figure 6).

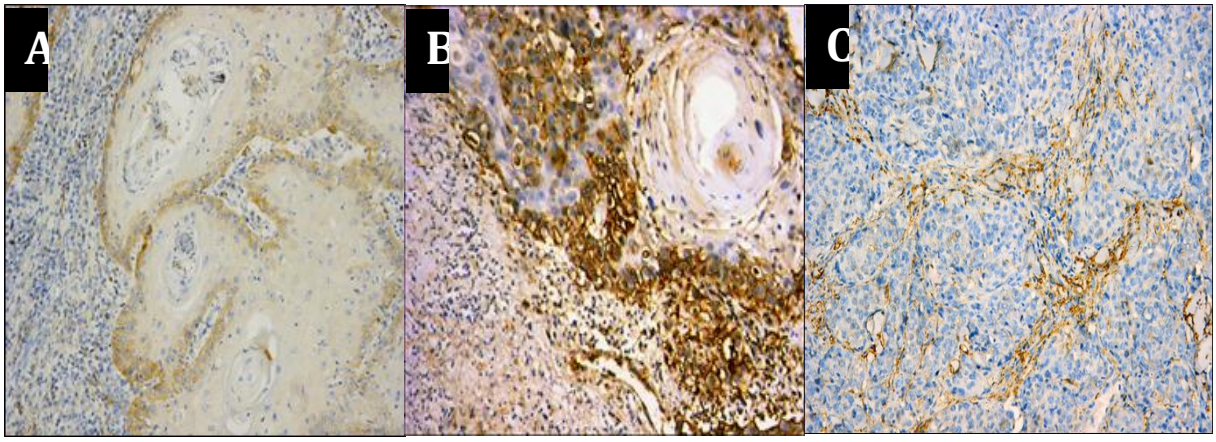


Figure 6. (A and B); Well differentiated OSCC demonstrating immunoexpression of D2-40 at the periphery of tumour islands, whereas, the central cells expressed negative reaction). (C) Poorly differentiated illustrated weak expression of podoplanin in tumour cells (Anti-podoplanin ×200).

3.4. Evaluation of Lymphatic Microvascular Density (LVD)

When LVD was compared between OED and OSCC, the LVD was highest in OSCC (23.95±14.64), whereas OED showed the least M±SD (8.8±4.3; Table 2). Concerning the mean number of stained lymph vessels among the groups, the highest mean value of lymphatic density was recorded in Gr. II with M±SD (35.75±10.89), whereas, the lowest value was recorded in Gr. I with M±SD (13.6±8.36; Table 3). In addition, when comparing LVD based on the histological differentiation, the minimum score of LVD was well-differentiated and increased in moderately differentiated to reach the maximum value in poorly differentiated SCC in Gr. II (42.8) and Gr. I (29). The difference in LVD among the three grades of OSCC when compared with each other was statistically significant. (Figure 7; Figure 14). Cancers of the tongue have much greater LVD than malignancies in other oral locations ($P < 0.001$) Nevertheless, no association was detected between LVD and age, gender, or tumour site. In our study, tumour emboli were seen in 25% (15 cases) of lymph vessels, ten of them were in Gr. II (Figure 8).

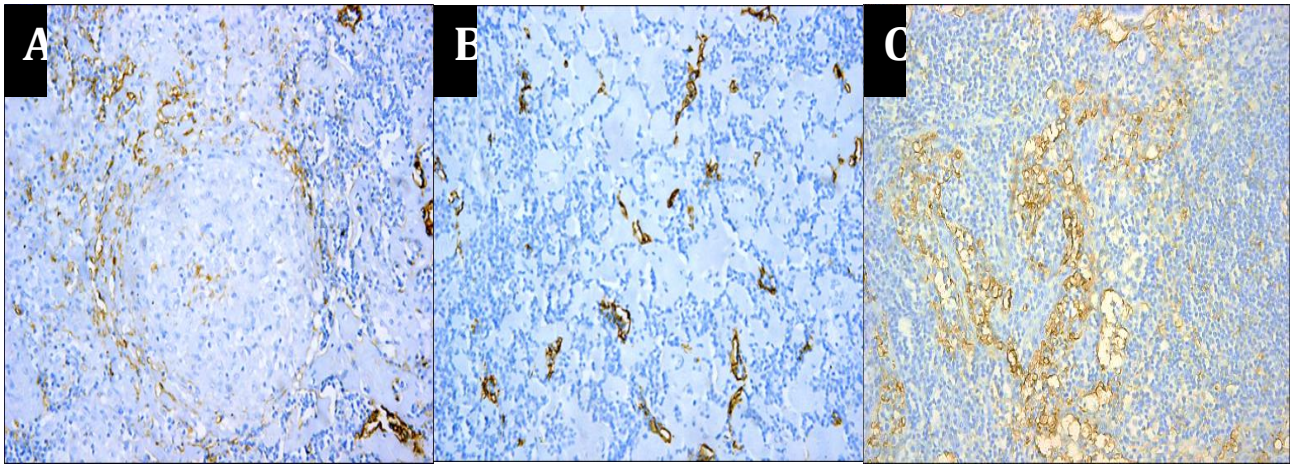


Figure 7. (A); Well differentiated OSCC showing a scanty number of lymphatic vessels, (B); Moderately differentiated OSCC showing an obvious increase in the number of lymphatic vessels (C); Poorly differentiated OSCC illustrated numerous lymphatic vessels, (anti-podoplanin × 200).

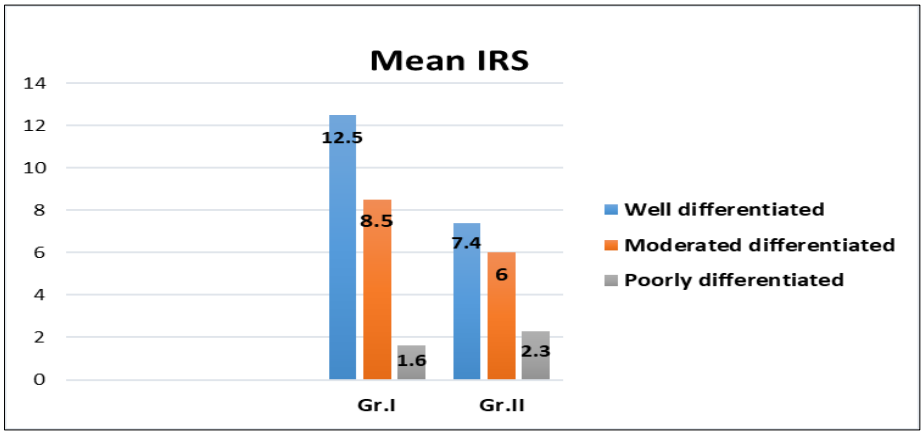


Figure 14. Distribution of podoplanin immunoexpression score through selected groups.

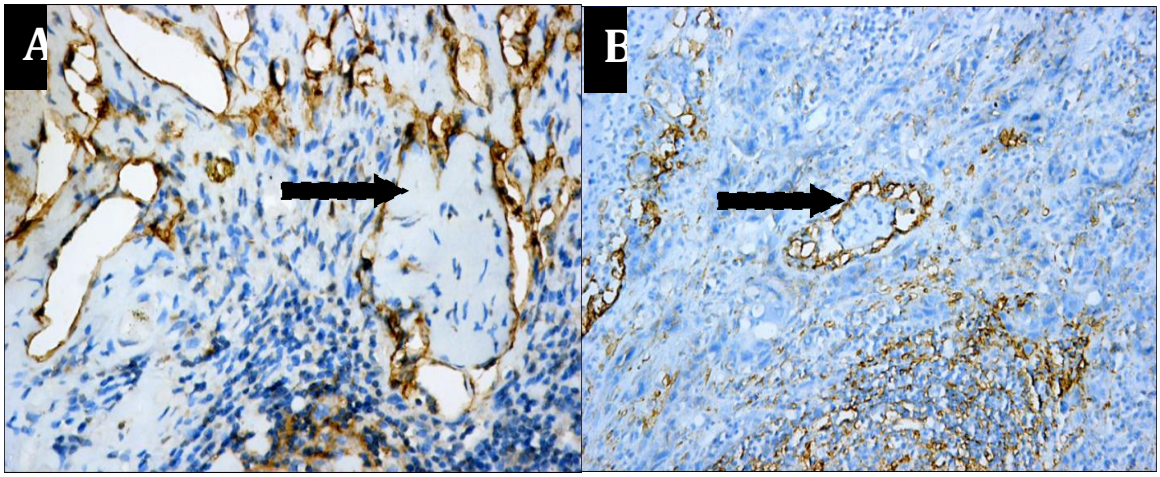


Figure 8 (A and b): Photomicrograph of poorly differentiated OSCC illustrating tumour emboli present within lymphatic vessels (black arrow refers to tumour emboli).

3.5. Immunoreaction for CD105

CD105 positive reactivity was observed in the cytoplasm of the endothelium lining of the blood vessels, whether individually, in collections, or in newly created BVs. Native BVs with thick muscle walls and big lumina displayed weak to negative CD105 immunoreactions. The mean value of the assessed hot spot of MVD was 11.2±4.1 in OED, whereas it was 33±15.74 in OSCC. The findings revealed a substantial elevation in CD105 expression from OED dysplasia to OSCC. (P<0.001*; Table 2). In terms of histopathological grading, the BVD in well-differentiated OSCC specimens was lower than rhat in moderately differentiated instances. In contrast, the weakly differentiated in Gr. I (29.75) and Gr. II (36.9) had the highest mean micro-vessel density (Figure 15; Figures 9 and 10).

When MVD in Gr. I and Gr. II were compared with each other, the average number of the blood vessels (MVD) for the LN negative cases (Gr. I) was 22.6 ± 8.8, while it was 44.5 ± 13.6 for the LN positive OSCC cases (Gr. II). These differences in MVD between the studied groups were found to be statistically significant. Greater MVD values were positively associated with greater scores of involvement in LNs (P<0.001; Table 3).

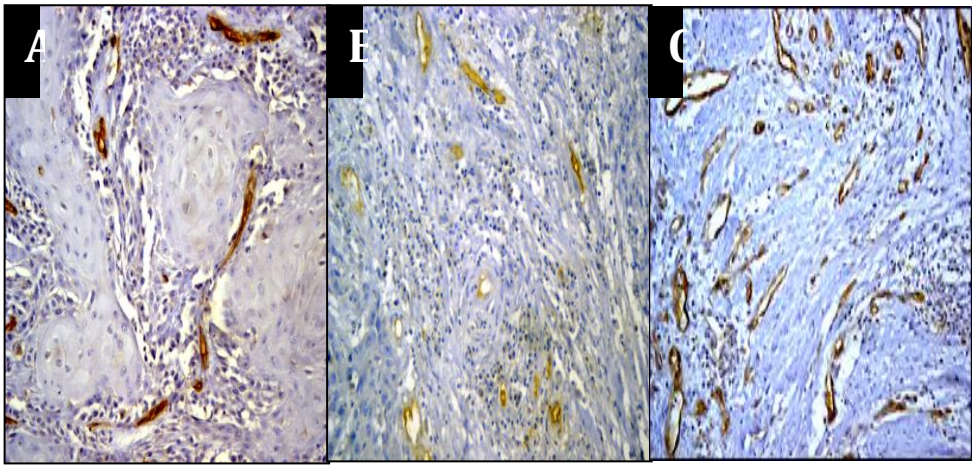


Figure 9. Photomicrograph showing (A) Well-differentiated OSCC with a negative LN and scant BVs. (B); moderately differentiated with a negative LN exhibiting enlarged and tiny irregularly stained BVs. (C); poorly differentiated OSCC with negative LN displaying a substantial amount of stained BVs distributed throughout the tumour stroma.

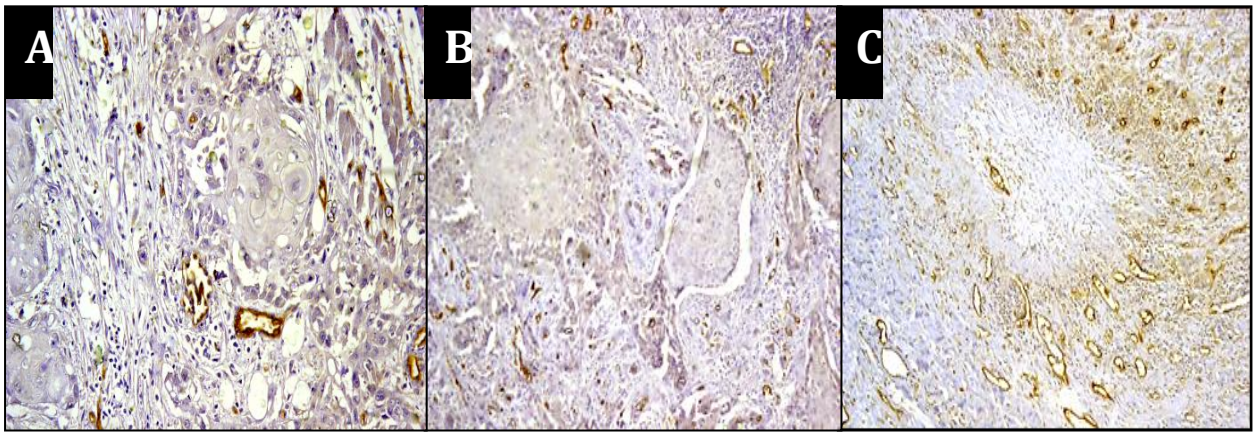


Figure 10. Photomicrograph showing (a) Well-differentiated OSCC with positive LN and a few tiny stained BVs between the tumour cells. (b) Moderately differentiated OSCC with a positive LN showing little collapsed convoluted BVs within neoplastic epithelial cells. (c): Poorly differentiated OSCC with positive LN displaying a large number of stained BVs of various sizes and shapes scattered in tumour stroma (immunostain of CD105 × 200).

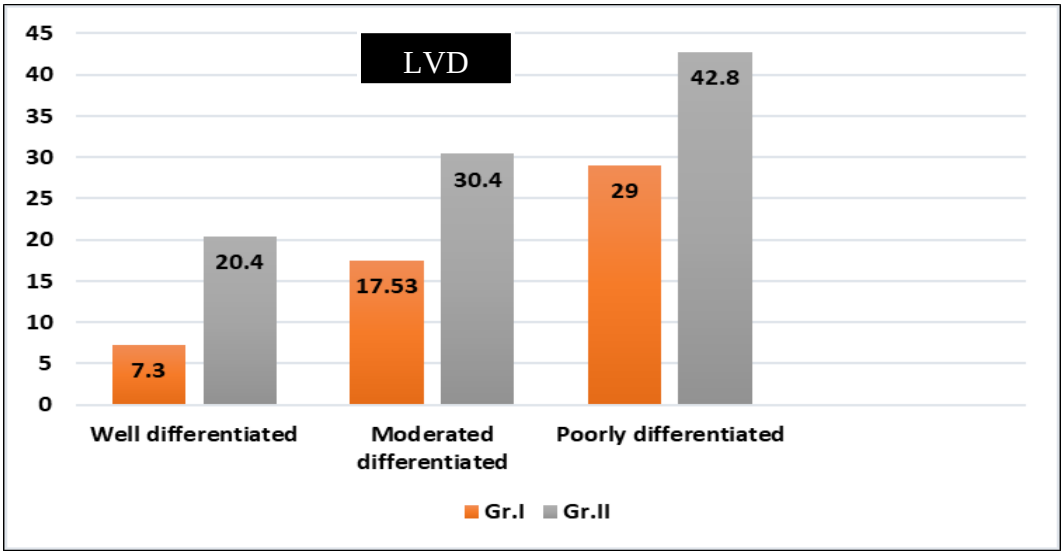


Figure 15. The difference in mean LVD of D2-40 between different grades of OSCC.

3.6. Immunoreaction for PCNA

A remarkable notice in the current work was that the majority of cases in the metastatic group were recorded as high mean numbers (10.2±1.9; P<0.001) in contrast to the non-metastatic group, which was recorded as low mean numbers (4.2±2.9; Table 3; Figure 16). In addition, the average of colored cells in the samples of OSCC had a higher mean value (7.1) than the mean value in OED (2.7) (Table 2).

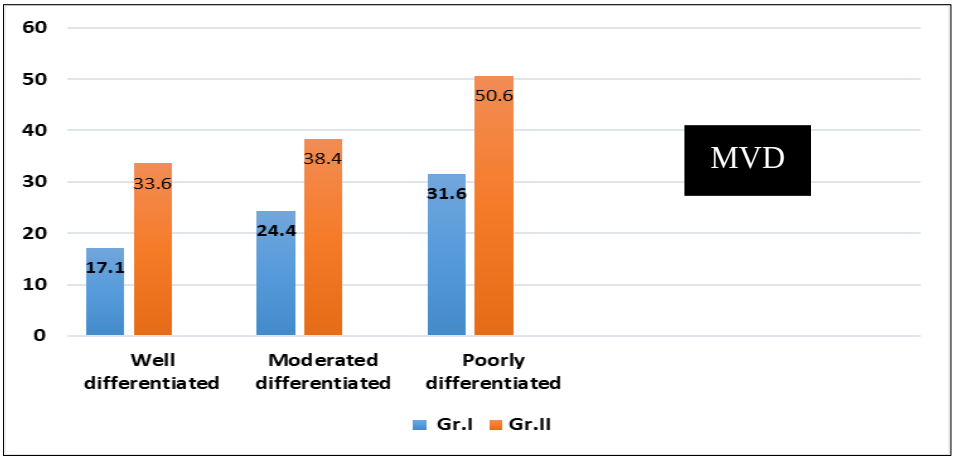


Figure 16. Bar chart illustrating Mean hot spot (CD105) between different groups

4. DISCUSSION

he point of this study was to look into how mast cells and eosinophils work together as important parts of the stromal TME in OED and OSCC. According to this study, the mean TATE count in the negative lymph node (10.36 ± 3.78) was reported to be substantially elevated than the positive lymph node (6.41± 3.15). These observations were fully consistent with Debta et al. [16]. They assumed that TATE is related to a better prognosis, which represented a significant immune response. The evidence may be attributed to the fact that eosinophils have a direct tumouricidal effect and facilitate the penetration of tumour-killing cytokines by increasing their permeability into tumour cells. The cytotoxic proteins that are produced from eosinophils act as anti-tumour factors to restrict cancerous cells. Moreover, TNF-alpha, secreted by eosinophils has a role in the death of tumour cells. Hence, TATE could be related to the suppression of proliferation and TME homeostasis resulting in enhanced survival [17].

In the current work, there was a higher count of mast cells in non-metastatic cases of OSCC (6.2 ± 2.4) than in metastatic cases, which were (4.05± 1.9). This is analogous to the research done by Tomita et al. [18], who explained that mast cells serve an important function in immune cell defense against carcinogens. This could be attributed to mast cells' ability to have anti-tumourigenic properties. There are a variety of cytotoxic molecules released by mast cells, including TNF-α, chymase which assist in the development of inflammation, prevent tumour cell proliferation, induce tumour cell apoptosis and supress metastasis by inducing chondroitin sulfate secretion. Furthermore, upon activation and degranulation of mast cells, there is an active recruitment of cells of the innate and acquired immune system [19].

Still, we showed that eosinophils and mast cells were lower in OSCC with an advanced histologic grade by connecting immune cells to the level of differentiation. A similar result was found by Debta et al. [16], who determined that these cells are promising markers in OSCC. On the other hand, the elevated mean density for mast cells and eosinophils in OSCC compared to dysplasia groups reflects their important role in the transformation of dysplastic lesions into frank invasive carcinoma. This evidence was from the study done by Pereira et al. [20]. It can be explained by the release of IL1 by mast cells in OED, leading to epithelial proliferation, which could have a role in cancer invasion. Furthermore, immune cells can initiate the development of new BVs by promoting the growth of endothelial cells and the breakdown of the ECM before the actual invasion. Several reports have assumed that angiogenesis and lymphangiogenesis are associated with neoplastic growth and metastasis, thus, angiogenesis and lymphangiogenesis in cancer masses can be targeted for cancer management [21]. In the current study, D2-40 and CD105 were utilized to observe the endothelium of lymphatic and BV, respectively.

Our results demonstrated that the MVD showed a significant increase between mild dysplasia, severe dysplasia and oral cancer. As a result, angiogenesis appears to be switched on in the early phases of epithelial transformation into cancer. There was also a significant rise in cases with cervical lymph node metastasis when compared to patients without metastasis. This can be explained by the role of new vessels in the maintenance of tumour growth. These vessels have physiological and structural challenges that include the formation of shunts, vasomotor conditions, circulation instability, and obstruction of micro-vessels, which might enable the passage of cancer cells into the bloodstream. Therefore, OSCCs intensive angiogenesis is crucial for tumour progression and metastasis. In addition, the significantly

higher MVD in high-grade OSCC versus low-grade tumours reflects the strong association between angiogenesis and worse tumour grade. These recent findings were entirely following Elmorsy et al. [22].

We used IHC and image analysis approaches to investigate podoplanin expression in tumour cells, in addition to LVD in OED a and OSCC. Positive podoplanin expression was observed in all patients with oral dysplasia (100%) and OSCC (96.7%). The study found that OSCC had a substantially elevated score of podoplanin (6.6±4.9) compared to OED (3.8±3.4). These results were consistent with those of Parhar et al. [8], who demonstrated that podoplanin is expressed in oral dysplastic and hyperplastic lesions. These are those that are associated with an elevated risk of developing invasive carcinoma. The findings demonstrated that podoplanin expression may be employed as a predictor for a higher likelihood of OED transforming into OSCC.

This investigation of D2-40 expression corresponding to various grades of OSCC demonstrated high reactivity in the majority of well-differentiated patients (88% in both groups), while all poorly differentiated cases (100%) showed low reactivity. These results were consistent with those of Aiswarya et al. (23) and Rodrigo et al. [12], who hypothesized that D2-40 expression is linked to cancer start rather than invasiveness. The study indicated that OSCC had the highest mean number of stained lymph vessels (23.95±14.64), while OED had the lowest (8.8±4.3), with a substantial variance detected. Our results agree with the finding of Parhar et al. [8], which could indicate that early dysplasia is associated with lymphatic vessel proliferation, with a progressive increase in carcinoma in situ, highlighting the role of lymphangiogenesis in the progression from non-invasive to invasive carcinoma.

In contrast with the previously published research by Lee et al. [24], who found no association between LVD and lymph node metastases, the current study found that Gr. I had an increased LVD (35.75±10.89) than Gr. I (13.6±8.36). This finding was consistent with Huber et al. (25) who discovered a positive association between the number of lymphatics and lymph node metastases. So, recently, therapies that stop lymphocytes from growing and therapies that help new blood vessels grow have been used to lower the number of LVs and the rate of systemic metastasis.

In the current investigation, PCNA was higher in OSCC in comparison to OED which indicates an increase in proliferation activity during the transition from dysplasia to carcinoma. Finally, the strong connection between PCNA expression and nodal metastasis shows the connection between a high proliferation index and the aggressive behavior of carcinoma in metastatic groups.

4. CONCLUSION

The current work was conducted to highlight the relation of immunological cells with dysplastic epithelium and invasive OSCC. The study demonstrated that TATE and mast cells were higher in non-metastatic than metastatic OSCC, which are considered favourable histopathological prognostic factors in oral cancer. Also, the fact that there were more immune cells found in OSCC than in dysplastic groups suggests that both types of cells may play a role in stromal invasion. Despite histopathological grading, the formation of new blood and lymphatic vessels is considered a main channel of cancerous cells for loco-regional

dissemination and neovascularization that boosts tumour cell proliferation and, thus, could be valuable in the prognostic assessment of this neoplasm.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

LIMITATIONS OF THE STUDY

The study showed a promising role of markers of lymphovascular density and microvascular density as predictors for distant metastasis. These markers have a role in epithelial malignant transformation from dysplasia and cervical lymph node metastasis. However, patient follow-up and correlation of the presented data to patient overall survival and disease-free survival were not included in the study protocol, which could reveal a prognostic value for the used markers. Further studies on revealing the association between LVD and MVD and overall survival is recommended.

ETHICAL CONSIDERATIONS

The research was conducted under the World Medical Association's Code of Ethics (Helsinki Declaration) for human research. This study was carried out after the approval of the Institutional Review Board (IRB) (number: A08091019), Mansoura University, Faculty of Dentistry.

REFERENCES

[1.] Ahmed S. Doghish, Ahmed A. El-Husseiny, Emad Gamil Khidr, Mahmoud A. Elrebehy, Mohammed S. Elballal, Mustafa Ahmed Abdel-Reheim, et.al, Decoding the role of miRNAs in oral cancer pathogenesis: A focus on signalling pathways, Pathology - Research and Practice,Volume 252,(2023),154949. https://doi.org/10.1016/j.prp.2023.154949

[2.] Tan, Y., Wang, Z., Xu, M. et al. Oral squamous cell carcinomas: state of the field and emerging directions. Int J Oral Sci 15, 44 (2023). https://doi.org/10.1038/s41368-023-00249-w

[3.] de Visser, Karin E., and Johanna A. Joyce. "The evolving tumour microenvironment: From cancer initiation to metastatic outgrowth." Cancer Cell 41, no. 3 (2023): 374-403.

[4.] Prabaharan, Chandra Bose. "Development of targeted radioimmunotherapy for osteosarcoma using a comparative oncology approach." PhD diss., University of Saskatchewan Saskatoon, (2024).

[5.] Juste-Lanas Yago, Silvia Hervas-Raluy, José Manuel García-Aznar, and Alejandra González-Loyola. "Fluid flow to mimic organ function in 3D in vitro models." APL bioengineering 7, no. 3 (2023).

[6.] Kathuriya PT, Bartake AR, Palaskar SJ, Narang BR, Patil SS, Pawar RB. Cd34 and mast cell analysis in normal oral mucosa and different grades of oral squamous cell carcinoma: a comparative study. J Clin Diagn Res. 2015;9(7).

[7.] Xie, Diya, Yunlong Li, Jiahong Shi, Yao Ping Zhu, Yiqing Wang, Christopher J. Butch, and Zhiyong Wang. "A novel near-infrared EGFR targeting probe for metastatic lymph

node imaging in preclinical mouse models." Journal of Nanobiotechnology (2023): 21, no. 1 342.

[8.] Parhar S, Kaur H, Vashist A, Verma S. Role of podoplanin in potentially malignant disorders and oral squamous cell carcinoma and its correlation with lymphangiogenesis. Indian J Cancer. 2015; 52:617–22

[9.] Ma YL, Peng JY, Zhang P, Liu WJ, Huang L, Qin HL. Immunohistochemical analysis revealed CD34 and Ki-67 protein expression as significant prognostic factors in colorectal cancer. Med Oncol. 2010;27(2):304–9.

[10.] Kawaguchi H, El-Naggar AK, Papadimitrakopoulou V, Ren H, Fan YH, Feng L, et al. Podoplanin: A novel marker for oral cancer risk in patients with oral premalignancy. J Clin Oncol. 2008; 26:354–60

[11.] De Vicente J. C., Rodrigo J. P., Rodriguez-Santamarta T., Lequerica-Fernandez P., E. Allonca, and Garc J. M., "Podoplanin expression in oral leukoplakia: tumourigenic role," Oral Oncology, vol. (2013) 49, no. 6, pp. 598–603,

[12.] Rodrigo JP, García-Carracedo D, González MV, Mancebo G, Fresno MF, García-Pedrero J, et al. Podoplanin expression in the development and progression of laryngeal squamous cell carcinomas. Mol Cancer. 2010; 9:48.

[13.] Weidner N, Semple JP, Welch WR, Folkman J. Tumour angiogenesis and metastasis--correlation in invasive breast carcinoma. N Engl J Med 1991; 324:1-8

[14.] Oliveira LR, Ribeiro-Silva A. Prognostic significance of immunohistochemical biomarkers in oral squamous cell carcinoma. Int J Oral Maxillofac Surg 2011; 40:298-307

[15.] Jain S, Phulari RG, Rathore R, Shah AK, Sancheti S. Quantitative assessment of tumour-associated tissue eosinophilia and mast cells in tumour proper and lymph nodes of oral squamous cell carcinoma. J Oral Maxillofac Pathol. 2018;22(2):227- 233

[16.] Debta P, Debta FM, Chaudhary M, Wadhwan V: Evaluation of prognostic significance of immunological cells (Tissue eosinophil and mast cell) infiltration in oral squamous cell carcinoma. J Cancer Sci Ther. 2011; 3:201-204

[17.] Legrand F, Driss V, Delbeke M, Loiseau S, Hermann E, Dombrowicz D, Capron M. Human eosinophils exert TNF-alpha and granzyme A-mediated tumouricidal activity toward colon carcinoma cells. J Immunol. 2010;185(12):7443–51.

[18.] Tomita M, Matsuzaki Y, Edagawa M, Shimizu T, Hara M, Onitsuka T, et al. Distribution of mast cells in mediastinal lymph nodes from lung cancer patients. World J Surg Oncol. 2003; 1:25.

[19.] Hempel HA, Cuka NS, Kulac I, Barber JR, Cornish TC, Platz EA, et al. Low intratumoural mast cells are associated with a higher risk of prostate cancer recurrence. Prostate; (2017);77(4):412–424.

[20.] Pereira T, Tiwari N, Tamgadge A, Tamgadge S, Shetty SJ, Gotmare S. Quantitative analysis of eosinophils and mast cells in potentially malignant disorder and squamous cell carcinoma using special stains. Niger J Exp Clin Biosci; 2018; 6:19-25.

[21.] Kapoor S, Shenoy P S and Bose B: CD34 cells in somatic, regenerative and cancer stem cells: Developmental biology, cell therapy, and omics big data perspective. J Cell Biochem. 2020; 121:3058–3069.

[22.] Elmorsy, A.A., Omar, T.A., Fouad, H.A. and Fata, M.M.,. Blood vessel density as a predictor in oral squamous cell carcinoma using cd34. Alexandria Dental Journal, (2019) 44(2), pp.46-51.

[23.] Aiswarya A, Suresh R, Janardhanan M, Savithri V, Aravind T, Mathew L. An

immunohistochemical evaluation of podoplanin expression in oral leukoplakia and oral squamous cell carcinoma to explore its potential to be used as a predictor for malignant transformation. J Oral Maxillofac Pathol. (2019);23(1):159.

[24.] Lee SW and Park YW: Expression of endoglin and podoplanin in early and advanced oral squamous cell carcinoma: J Korean Assoc Oral Maxillofac Surg (2012) ; 38:145-5.

[25.] Huber GF, Fritzsche FR, Züllig L, Storz M, Graf N, Haerle SK, et al. Podoplanin expression correlates with sentinel lymph node metastasis in early squamous cell carcinomas of the oral cavity and oropharynx. Int J Cancer (2011); 129:1404-9.