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Original Article

Immunohistochemical comparison of CD44 and Hypoxia Inducible Factor-1 Alpha (HIF-1α) in Oral Squamous Cell Carcinoma and Oral Epithelial Dysplasia: A comparative study

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ABSTRACT

This study aimed to evaluate the expression of hypoxia inducible factor-1 alpha (HIF-1α) and CD44 markers to detect their potential role in the progression of oral epithelial dysplasia (OED) to oral squamous cell carcinoma (OSCC). The study involved 50 patients with different grades of OED and OSCC taken from different anatomical sites by the head and neck surgeons. The pathologists used ordinary stained slides for histological evaluation and immunostaining of HIF-1α and CD44 antibodies using conventional and remote examination tools. The results showed that the mean area percentage of HIF-1α and CD44 immunostaining was significantly higher in severe dysplasia, followed by moderate dysplasia and mild dysplasia. Similar results were observed in OSCC, with poorly differentiated OSCC showing the highest mean expression and the mean area percentages in all cases were higher in OSCC (44.24%) and (47.09%) than in OED (14.50%) and (20.29%). The study concluded that HIF-1α and CD44 markers could be helpful in accurate classification of oral epithelial lesions with potential and therapeutic value, however further clinical studies are recommended.

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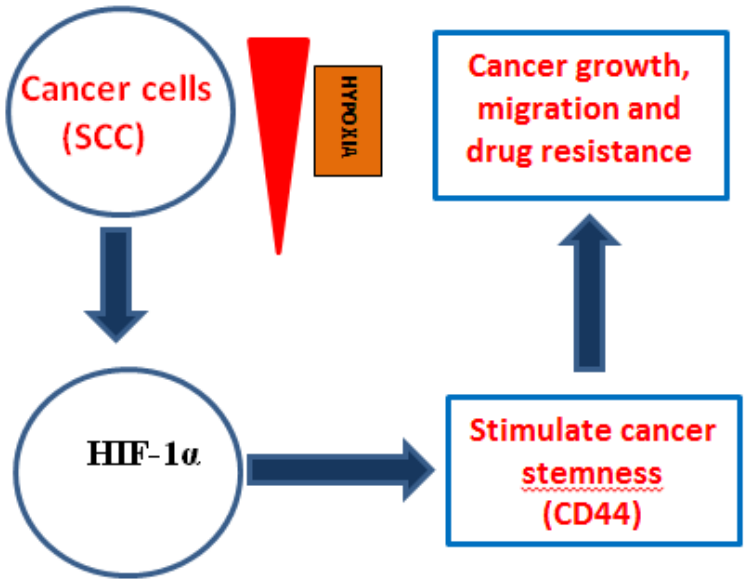
GRAPHICAL ABSTRACT



Introduction

Oral cancer is the 16th most prevalent malignancy and the 15th most common cause of death worldwide. About 350,000 new cases of oral cancer are detected each year according to the International Agency for Research on Cancer (IARC). Oral squamous cell carcinoma is the most widespread type of oral cancers, which has substantial morbidity and mortality (5-year survival is around 60%) despite treatment. Oral epithelial dysplasia (OED) is a common precursor or associated with many OSCCs, if increasing the risk of developing cancer when the dysplasia progresses [1-4]. Tumorigenesis is a multi-step and complex process that is partly correlated with the interaction of many cancer-promoting pathways and the emergence of the tumor microenvironment. The tumor lacks oxygen due to the cancer cells' quick proliferative cycle and the ineffective blood supply. Cancer cells employ various metabolic pathways, including activation of the HIF1 factor, the most important regulator of oxygen homeostasis, to make up for oxygen deficit and adaptability within the tumor microenvironment [5]. In response to hypoxia, cancer cells in solid tumors, like head and neck squamous cell carcinoma (HNSCC), express the hypoxia-inducible factor-1 Alpha (HIF-1α), which is important for tumor development, cell migration, angiogenesis, metastasis, cancer stem cells (CSCs) maintenance, and resistance to therapy [6-9].

HIF-1α overexpression has been associated with unfavorable outcomes for several malignancies, including OSCC, according to numerous studies [10-12]. Cluster of differentiation 44 (CD44) is a type I transmembrane glycoprotein that serves as a key adhesion molecule and hyaluronic acid receptor. It has been linked to CSC-like characteristics such as enhanced cell motility, migration, and invasion. Furthermore, epithelial to mesenchymal transition (EMT) induction by CD44 has been detected in various cancer types including HNSCC [13-16]. Numerous studies have shown that CD44 and hypoxia-inducible signaling are related. The CD44 expression and other markers conferring stem cell capability is elevated by hypoxic stimulation of different cancer cell types. These imply that CD44 is increased in hypoxic conditions via HIF-1α, which aids in HIF-1-mediated cancer growth [17, 18]. However, there has not been much research done on HIF-1α and CD44 correlation in OSCC yet. In addition, little research has been done on the connection between these two proteins and the development of oral dysplasia into OSCC.

Concerning these insightful foundations, we designed our research to investigate the immunohistochemical expression of HIF-1α and CD44 in OED and OSCC to comprehend their roles in progression of tumor and to predict their potential prognostic and probable therapeutic value in cases of OSCC. We used both conventional microscopy (CM) and telepathology system (TP) for evaluation of cases in agreement with independent examiners.

Materials and Methods

Patients

This retrospective study evaluated 60 specimens of paraffin-embedded tissue blocks including 25 cases of OED and 25 cases of OSCC in addition to 10 cases of normal oral mucosa after exclusion of abnormalities that were collected from archives of Al-Azhar University Hospitals (Al-zahraa), Faculty of Medicine, pathology and oral pathology departments between 2019 and 2022. The available pathological data were retrieved from the pathology reports and medical files of the patients with communication of the clinicians in concern taking consultation from outside experts when indicated. The clinical examination performed by the clinicians concerned in maxillofacial and ENT departments and biopsies were obtained from the anatomical sites as follows: The 25 specimens of OED were taken from the buccal mucosa (16 cases) and floor of the mouth (9 cases) while 25 specimens of OSCC were taken, six from the tongue, three from the gingiva, three from the floor of the mouth, nine from the buccal mucosa, and four from the hard palate. Routine histological examinations were carried out by pathologists to validate their diagnosis and assess their histopathologic grade in accordance with the World Health Organization (WHO) categorization [19]. The final diagnoses of all the studied patients were decided with aid of telehealth technology to support communication at a distance with the investigators, clinicians, and patients when indicated for this study.

Exclusion criteria include cases lacking agreement with at least four independent pathologists on the diagnosis of the ordinary stained slides or the scoring of the immunohistochemical staining. Furthermore, cases lacking archived tissue blocks or relevant clinic-histological data were also excluded.

Immunohistochemistry

4 μm sections of formalin-fixed, paraffin-embedded tissue were submitted to immunohistochemistry. An automated immunohistochemical stainer was used (Bond, Leica Biosystems) with Bond Polymer Refine Detection System. The protocol in immunostainer was: Antigen retrieval with citrate buffer pH 6.0 for 20 minutes, and then the slides were incubated with anti-HIF-1α (clone [H1alpha67], cat. ab179483, Abcam, USA, dilution 1: 200) and anti-CD44 (CLONE, [EPR18668], Cat. Ab189525, Abcam, USA, dilution 1: 500) both of them are mouse monoclonal antibodies, for 30 min at room temperature. ImmPRESS kit (Vector Laboratories, Burlingame, USA) was used for polymeric detection. Thereafter, chromogen 3.30-diaminobenzidine (DAB, Vector Laboratories, Burlingame, USA) was used to detect the antigen-antibody reaction. The sections were counterstained with Hematoxylin (Mayer's, Modified, [ab220365]). Positive immunohistochemical control slides for HIF1α and CD44 were tonsillar tissue and breast carcinoma, respectively. Negative control sections were prepared with the exclusion of the primary antibody.

Histomorphometric analysis

Using a computerized Leica image analyzer (Leica Qwin 500 software, Germany), immunoreactivity for both HIF1 and CD44 was assessed by determining the area percentage of +ve immunostained cells. The measurement units in pixels automatically changed into micrometer units. A standard measuring frame of 11434.9 μm^2 was used as a reference with a magnification (200) used to calculate the area % of both HIF-1 and CD44 expression. A blue binary color was used to mask the reactive areas of positive immunostaining. Ten fields/slide sections of each case histomorphometrically evaluated. After that,

mean values for each specimen were calculated and recorded as prepared for comparison.

Whole slide imaging and telepathology

All cases were scanned at magnification of x40 by a slide scanner machine (Aperio XT image Scope, Leica Biosystem, Buffalo Grove, IL) and were loaded onto their accompanying proprietary server (Spectrum E_Slide Manager, v.11.2.0.780, Leica Biosystem). Images were accessed via local server through an individual login used by staff in concern. Examiners were asked to give their description, diagnosis, and evaluation regarding type of staining and staining pattern throughout the WSI evaluation. After evaluation of the slides by the pathologists in laboratory, slides were shared with remote pathology experts using the technology of telepathology to give (blindly) their description and evaluation of the studied cases. The pathology departments had no telepathology platform, but electronic approach software was used for this study to provide accurate diagnosis and evaluation of the studied cases depending on the telecommunication links enabling electronic transmission of the digital pathology images and immunohistochemical stained slides' images when indicated to get a sufficient agreement on the final reporting. A simple secured sharing technology was used to share high quality images.

Statistical analysis

The SPSS software version 23.0, (SPSS Inc., Chicago, Illinois, USA) was used to evaluate the recorded data. The numerical information was displayed as mean, standard deviation, and ranges. The One-way ANOVA was used to compare several means and Tukey's test was applied for multiple comparisons between several variables. When comparing two means, an independent-sample t-test was used. The correlation between two sets of variables was evaluated using Pearson's correlation coefficient (r) test. Concordance was calculated for case subtype and the methodology as the number of histopathology or immunostaining cases in agreement with the primary examining pathologists divided by the total number of evaluated cases. To compare the interpretation methodologies, 95% confidence intervals (CI) were considered using incorporating continuity correction score method. Differences with a P-value<0.05 is regarded as statistically significant.

Results and Discussion

This study assessed cases with 100% diagnostic agreement between pathologists. Of the 25 cases of OED, 9 cases were diagnosed as mild dysplasia (18%), 8 cases were moderate dysplasia (16%), and 8 cases were severe dysplasia (16%), and of the 25 cases OSCC, 10 cases were diagnosed as well-differentiated OSCC (WDOSCC) (20%), 8 cases were moderately differentiated OSCC (MDOSCC) (16%), and 7 cases were poorly differentiated OSCC (PDOSCC) (14%). Patients' age ranged from 25-72 years old with male predominance (35 males versus 15 females). A concordance of 87% was achieved for immunohistochemical expression whereas histological diagnosis of oral lesions revealed 96% concordance between telepathology and conventional microscopy. Nine cases were excluded due to lack of concordance.

Histopathological findings

In mildly dysplastic tissue, changes appeared in basal and parabasal cells, the changes included basal cell loss of polarity, hyperchromatism, pleomorphism, and abnormal mitosis. In moderate dysplastic tissue, changes appeared showing loss of polarity of basal cells; pleomorphism and hyperchromatism not extend beyond the mid portion of the epithelial thickness. In severe dysplastic tissue, the full thickness of epithelium showed cytological and architectural changes involved the full thickness of epithelium with intact basement membrane. The underlying connective tissue showed dilated blood vessels and chronic inflammatory cells (Figure 1a, b and c). WDSCC specimens showed invasive islands of malignant epithelial cells within the underlying connective tissue, exhibited pleomorphic neoplastic cells with hyperchromatic nuclei and abnormal mitotic figures with formation of multiple keratin pearls and individual cell keratinization, while in

MDSCC, the invasive nests of neoplastic cells showed increased criteria of malignancy with decrease keratin formation. In PDSCC, the tumor cells displayed loosely adherent pleomorphic cells invading deep connective tissues without keratin formation (Figure 2a, b, and c).

Immunohistochemical findings

HIF1α immunostaining was detected in cytoplasm and nuclei of the basal cell layer in mild dysplasia, basal and suprabasal layers in moderate dysplasia, while in severe dysplasia, it was present in the full thickness of epithelium (Figure 1d, e and f). HIF1α immunostaining was detected in the cytoplasm and or nuclei of neoplastic epithelial cells invading connective tissue of the three grades of OSCC (Figure 2d, e and f). Regarding HIF-1α expression in different grades of OED, the mean HIF-1α area % was found to be the highest in severe dysplasia (23.79%) followed by moderate dysplasia (11.95%), and then mild dysplasia (7.76%) with statistically significant difference between different grades of OED cases (P<0.05).

While the mean HIF-1α area % in different grades of OSCC was found to be the highest in PDSCC (56.16%), followed by MDSCC (46.01%) and the lowest mean value was in WDSCC (30.56%) with a highly significant difference between different grades of OSCC cases (P<0.001) (Table 1).

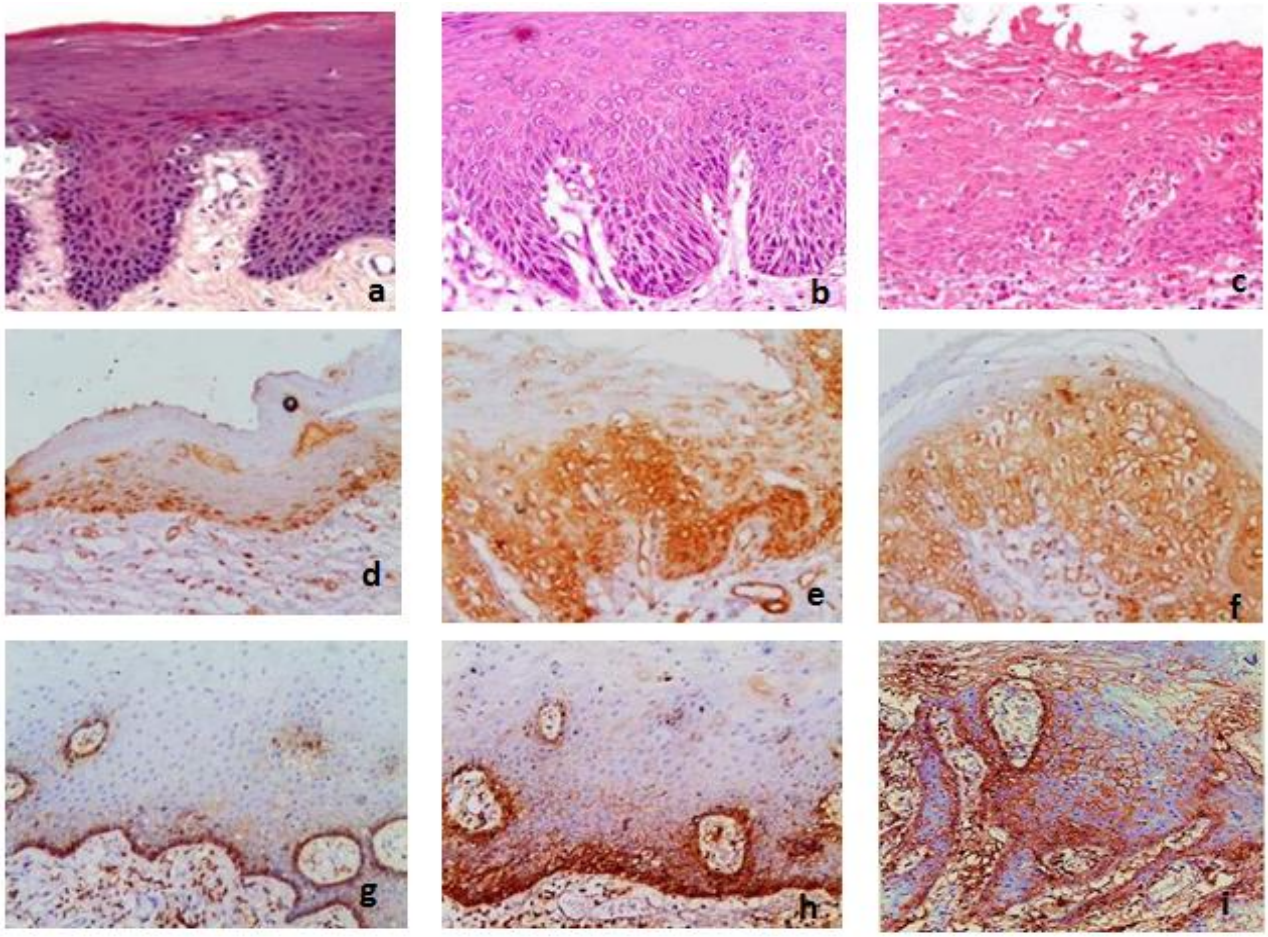


Figure 1: Photomicrographs of different histologic grades of OED. Histopathological features of mild dysplasia (a), moderate dysplasia (b), and severe dysplasia (c) (H&E staining, 200x). Immunohistochemical staining for HIF-1α in mild dysplasia showing cytoplasmic and nuclear expression in the basal cell layer (d), in moderate dysplasia showing cytoplasmic and nuclear expression in basal and suprabasal dysplastic cells (e), and in severe dysplasia showing cytoplasmic and nuclear expression in the whole thickness of dysplastic epithelial cells (f) (HIF-1α IHC staining, 200x). Immunohistochemical staining for CD44 in mild dysplasia showing membranous expression in basal cells (g), in moderate dysplasia showing membranous expression in basal and suprabasal cells (h), and in severe dysplasia showing membranous expression in the full thickness of dysplastic epithelial cells (i) (CD44 IHC staining, 100x).

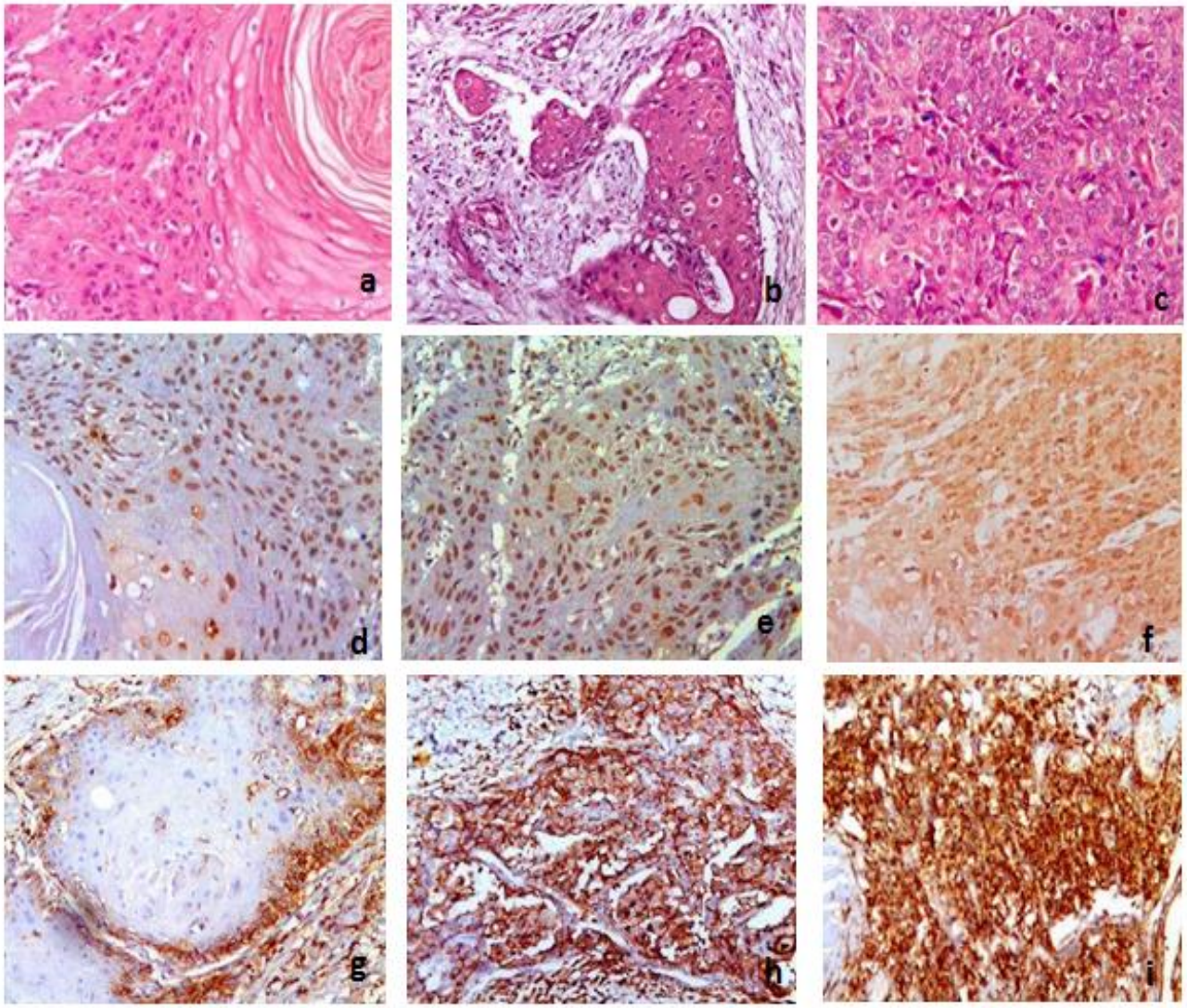


Figure 2: Photomicrographs of different histologic grades of OSCC. Histopathological features of well differentiated SCC (WDSCC), (a) moderately differentiated SCC (MDSCC), (b) poorly differentiated SCC (PDSCC), and (c) (H&E staining, 200x). Immunohistochemical staining for HIF-1α showing cytoplasmic and nuclear expression in WDSCC, (d) MDSCC, and (e) PDSCC (f) (HIF-1α IHC, 200x). Immunohistochemical staining for CD44 in WDSCC showing membranous expression (g), showing membranous and cytoplasmic expression in MDSCC (h), and PDSCC (i) (CD44 IHC staining, 100x)

Table 1: Comparison between different grades of OED and OSCC regarding HIF-1α mean area %

HIF-1α area %				
	Mean±SD	Range	ANOVA test	P-value
Dysplasia				
Mild Moderate Sever	7.76±4.21 11.95±6.87 23.79±8.18	4.57-14.49 5.41-23.06 13.77-32.54	7.862	0.007*
Carcinoma				
WDSCC	30.56±12.32	17.50-47.11	6.065	0.015*
MDSCC	46.01±11.02	36.21-64.69		
PDSCC	56.16±11.76	42.05-70.47		

**ANOVA and Tukey's tests were used; **P-value <0.05 is significant.; OSCC: Oral squamous cell carcinoma, WDSCC: Well differentiated squamous cell carcinoma, MDSCC: Moderately differentiated squamous cell carcinoma, and PDSCC: Poorly differentiated squamous cell carcinoma.*

CD44 immunostaining was detected in cytoplasm and/or nuclei of the basal layer in mild dysplasia, basal and suprabasal layers in moderate dysplasia, while in severe dysplasia, it was detected in the whole dysplastic cell layers (Figure 1g, h and i). Cell membrane and sometime cytoplasmic immunostaining of CD44 was detected in the neoplastic epithelial cells invading connective tissue of the three grades of OSCC (Figure 2g, h and i). Concerning the comparison of CD44 expression between different grades of OED, the mean CD44 area % was found to be the highest in severe dysplasia (30.03%), followed by moderate (17.25%), and then mild dysplasia (13.58%) with a highly significant difference between different grades of OED cases (P<0.001). Regarding CD44 expression in different grades of OSCC, the mean CD44 area % was found to be the highest in PDSCC (56.01%), followed by MDSCC (51.26%) and the lowest mean value was in WDSCC (33.99%) with a highly significant difference between different grades of OSCC cases (P<0.001) (Table 2).

Comparison of HIF-1α expression in the OED group with the OSCC group revealed that the mean HIF-1α area % in OED was (14.5%) while in OSCC was (44.24%) which was significantly higher (P<0.001) in OSCC compared with OED. Regarding the comparison of CD44 expression in the OED group with the OSCC group revealed that the mean CD44 area % in OED was (20.29%), while in OSCC was (47.09%) which was significantly higher (P<0.001) in OSCC as compared with OED (Table 3).

Regarding OED, there was a highly significant positive correlation (P<0.001) between both markers when using Pearson's correlation coefficient (r) test, where (r-value 0.713), (p-value 0.003) (Figure 3). Furthermore, OSCC showed a significant positive correlation (P<0.05) between both markers, where (r-value 0.581), (P-value 0.023) (Figure 4).

Oral cancers are caused by a long-term accumulation of mutations that eventually transform normal mucosa into dysplasia and then invasive carcinoma. Early detection of high-risk oral premalignant condition is essential for reducing mortality and morbidity while maintaining the most effective method of preventing cancer transformation [20, 21].

Table 2: Comparison between different grades of OED and OSCC regarding CD44 mean area %

CD44 area %.				
	Mean±SD	Range	ANOVA test	P-value
Dysplasia				
Mild Moderate Severe	13.58±5.15 17.25±3.19 30.03±5.04	9.31-21.38 12-20.04 23.86-37.02	18.033	<0.001**
Carcinoma				
WDSCC	33.99±5.33	27.13-39.41	18.446	<0.001**
MDSCC	51.26±7.96	39.53-61.50		
PDSCC	56.01±4.18	51.20-60.6		

**ANOVA & Tukey's tests were used.*

***P-value <0.001 is highly significant.*

Table 3: Comparison between OED and OSCC according to HIF-1α and CD44 mean area %

Area% for HIF1	OED	OSCC	t-test	P-value
Mean–SD	14.50±9.33	44.24±15.37	-6.406	<0.001**
Range	4.57-32.54	17.5-70.47		
Area% for CD44				
Mean–SD	20.29±8.43	47.09–11.28		
Range	9.31±37.02	27.13-61.5	-7.373	<0.001**

**Independent Sample t-test was used; **P-value <0.001 is highly significant.*

Despite the accessible histopathologic examination, more than 60% of all OSCCs are diagnosed when metastatic condition is already present [22]. The most common associated and well-known risk habits for this type of malignancy include tobacco and alcohol consumption [23]. However, molecular studies have revolutionized cancer research in recent times, leading to the discovery of hundreds to thousands of genes involved in the development of various types of cancers including OSCC. These studies are trying to find more useful markers as the indicators for cancer risk and disease progression [24]. The search for the early stage OSCC biomarkers has been the goal for a number of researches; however, none of them yielded with clinically useful exploitable biomarkers for OSCC early diagnosis [25].

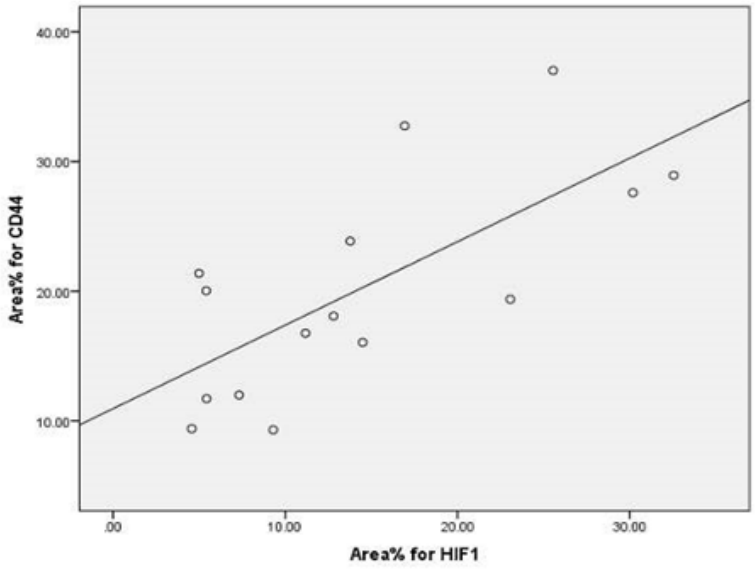


Figure 3: Scatter plot between area % for CD44 and HIF-1α among OED

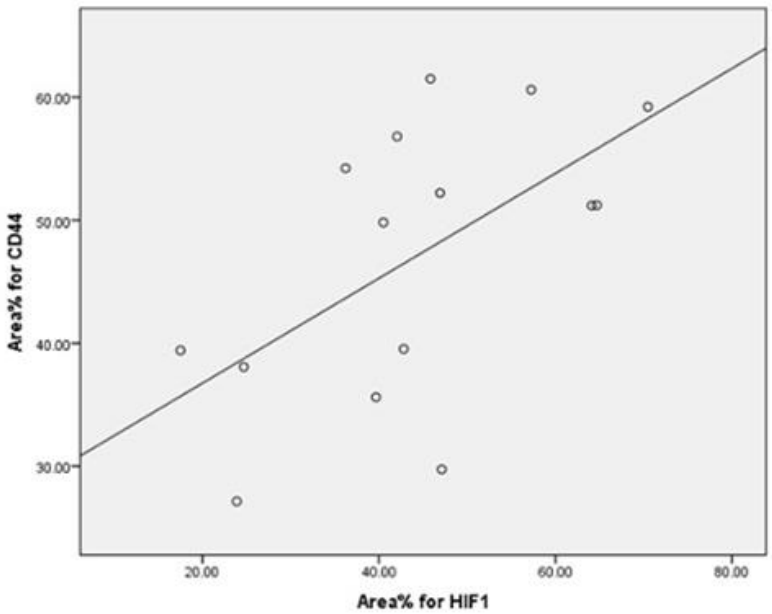


Figure 4: Scatter plot between area % for CD44 and HIF-1α among OSCC

Overexpression of HIF-1α marker has been shown to be associated with tumor cell growth in patients of gastric, colorectal, pancreatic, cervical, endometrial, ovarian, breast, and head and neck carcinomas [26-30]. However, a consensus on this association with head/neck cancer is yet to be attained [31].

Our study showed expression of HIF-1α marker in various grades of OED cases in keeping with Patel *et al.* (2019) and Tilakaratne WM *et al.* (2008) who found that staining of HIF-1α was present up to half of the epithelium for cases of mild and moderate dysplasia and the full thickness in severe dysplasia [32, 33].

HIF-1α degradation is inhibited due to hypoxic conditions in addition to some other conditions including normoxic conditions [34]. HIF-1α activates the downstream target genes transcription which regulate many biological significant processes, including angiogenesis, glucose metabolism, cell proliferation, pH regulation, and migration [35, 36].

Thus, oral premalignant disorders like oral submucous fibrosis (OSMF) were also found to have a significant HIF-1α expression in the basal and suprabasal layers of the epithelium, according to Pereira TV *et al.* (2020) which demonstrates the role of hypoxia in OSMF malignant transformation and that the HIF-1α expression is an early event in oral carcinogenesis [36].

Regarding HIF-1 expression in different grades of OSCC, nuclear HIF-1 expression was more pronounced and intense in advanced grades in this study. Our findings concurred with those of Ogane *et al.* (2010), who examined HIF-1 expression in OSCC by emphasizing the nuclear distribution rather than the cytoplasmic one. They found that the activation of HIF-1α, which is translocated to the nucleus to dimerize with the HIF-1β subunit to form the HIF-1 molecule, highly correlates to its nuclear pattern predominance, which may control various signaling pathways involved in tumor aggressiveness [37].

In the current study, the mean HIF-1α area % in various grades of OED was significantly higher in severe dysplasia (23.79%) followed by moderate (11.95%), and then mild dysplasia (7.76%). This was consistent with the findings of Patel et al. (2019) [32].

When comparing the various OSCC grades according to the HIF-1α mean area% in our study, PDSCC (56.16 %) had a significantly higher mean than MDSCC (46.01%) and WDSCC (30.56%). This finding is concordant with Zheng *et al.* (2013) who discovered that HIF-1α mRNA levels were significantly elevated in tongue cancer and correlated positively with the pathological grade [38]. Similalry, Hu X. *et al.* (2020) reported that HIF-1α expression was strongly associated with the aggressiveness of esophageal squamous cell carcinoma (ESCC) and tumor metastasis [39].

Furthermore, HIF-1α area % showed a significantly higher mean in OSCC (44.24%) compared to OED (14.50%). Our findings concurred with those of Patel *et al.* (2019) who found there was a significantly higher mean HIF-1α labeling index in OSCC compared to OED. HIF-1α expression may have been elevated in oral malignancies because of activation of growth factors such as epithelial growth factor, cytokines (like PGE 2), and Ras protein, which increased HIF-1α expression in human cancer cells. Hence, HIF-1α expression increases from OED to OSCC indicating that HIF-1α upregulation occurs in the early stages of carcinogenesis [32].

In the current study, CD44 immunopositivity in various grades of OED cases was similar to the results of Gupta P *et al.* (2021) who found that there is an increase in the intensity and the percentage of immunostaining of CD44 with increasing degree of dysplasia [40]. On the evaluation of the OSCC cases in our result, the staining pattern varied from membranous in well-differentiated grades to membranous and cytoplasmic immunostaining in moderately and poorly differentiated grades. These results are concordant with another study that found membranous and cytoplasmic staining of CD44 in OSCC cases. The cytoplasmic pattern of CD44 might be interpreted by the connection of intracellular domain of CD44 with cytoskeletal linker proteins like ezrin and ankyrin via which it can facilitate cell migration on hyaluronan [41, 42]. In the current study, the mean CD44 area % in various grades of OED was significantly higher

in severe dysplasia (30.03%), followed by moderate (17.25%), and then mild dysplasia (13.58%). These findings concurred with those of Surendran S. *et al.* (2017) who found that the CD44 expression positively correlated with the progression of oral potentially malignant disorders to more aggressive histologic type [43].

Concerning CD44 expression in different grades of OSCC in the current study, CD44 mean area % was significantly higher in PDSCC (56.01%) than in MDSCC (51.26%) and WDSCC (33. 99%). This finding agreed with another study which found a significant increase in CD44 expression from well to poorly differentiated OSCC and has been associated with poor prognosis after surgical resection following neoadjuvant therapy [44].

Similar to our study, the results of Adnan Y. *et al.* (2022) confirmed that increased expression of CD44 would increase the risk of malignancy and was associated with advanced T stages. It is hypothesized that CD44 stimulates pathways that initiate and promote the growth of tumor cells [44, 45].

In contrast, Hema K. *et al.* (2014) reported that the CD44 expression decreased with increasing grade in OSCC and found that CD44's low expression may be correlated to lymph node metastasis and tumor metastasis. This was explained by reduced cell-cell or cell-matrix adhesion in OSCC, which causes cells to separate. Therefore, the reduced expression of CD44 could be an indication of invasion and metastasis of the tumor [46].

Likewise, the CD44 area % showed a significantly higher mean in OSCC (47.09%) compared to OED (23.64%). That was consistent with another study that reported that CD44 expression was high in oral potentially malignant disorders and upraise subsequently into OSCC, suggesting that the CSCs could be involved in the progression of OED to OSCC [47]. Regarding the correlation between HIF-1α and CD44 in the current study, there was highly significant ($P<0.001$) and significant ($P<0.05$) positive correlation between HIF-1α and CD44 mean area % among OED, and OSCC cases, respectively. Furthermore, it was found that HIF-1α increases CD44 expression in several malignancies, including breast cancer cells and gastric cancer cells suggesting that a hypoxic microenvironment provides stem cells with an ideal environment to maintain their precursor status [48, 49]. To provide accurate diagnosis for the studied cases, we used telepathology technique in sharing cases with experts. Studies showed that any of the five main categories of telepathology (static, dynamic, robotic, whole slide imaging, or hybrid methods) can offer diagnostic accuracy rate similar to the traditional or conventional microscopy examination [50, 51]. We also compared the results of using such technique and high concordance was achieved.

The limitations of this study were related to the lack of molecular features of related genes and also the correlation of both studied immunohistochemical markers with some clinical features and anatomical sites in addition to the lack of follow up data of the OED cases due to several factors related to the management centers specific for head and neck tumors in our institution. The number of the studies cases was not large number and the cases were taken from single institution. These should be considered in the future studies.

Conclusion

This study concluded that the expression of CD 44 and Hypoxia Inducible Factor-1 Alpha markers are significantly increased in oral squamous cell carcinoma compared to oral epithelial dysplastic lesions and normal mucosa which can provide diagnostic accuracy as well as the potential prognostic role of both markers as the role of hypoxic microenvironment in oral squamous cell carcinoma development can be demonstrated by the gradual increase of immunostaining in different grades of OED and OSCC. More studies on both markers in larger number of cases with clinicopathological evaluation are recommended for testing also the targeting hypoxia as a promising treatment for oral squamous dysplastic to prevent invasiveness. A concordance of 87% was achieved for immunohistochemical expression evaluation whereas histological diagnosis of oral lesions revealed 96% concordance between telepathology and conventional microscopy.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the article and agreed to be responsible for all the aspects of this work.

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References

[1]. a) Alotaibi O.N., Oral and maxillofacial cancer: A 35-year retrospective analysis at a referral dental hospital in Saudi Arabia, *The Saudi Dental Journal*, 2022, **34**:56 [Crossref], [Google Scholar], [Publisher]; b) Hiawi F.A., Ali I.H. Study the Adsorption Behavior of Food Colorant Dye Indigo Carmine and Loratadine Drug in Aqueous Solution. *Chemical Methodologies*, 2022, **6**:720 [Crossref], [Publisher]; c) Hosseini S.M.H., Naimi-Jamal M.R., Hassani M. Preparation and Characterization of Mebeverine Hydrochloride Niosomes as Controlled Release Drug Delivery System. *Chemical Methodologies*, 2022, **6**:591 [Crossref], [Publisher]; d) Monjezi Amin., Karimian P., Yousofvand V., Therapeutic Applications of Salvadora Persica Plant in Medical Sciences: A Review Article, *Asian Journal of Green Chemistry*, 2023, **7**:180 [Crossref], [Google Scholar], [Publisher]; e) Al-Ameer A.H.A., Quinolone antibiotics and their applications in metal complexes: An update, *Asian Journal of Green Chemistry*, 2022, **6**:223 [Crossref], [Google Scholar], [Publisher]

[2]. Ferlay J., Colombet M., Soerjomataram I., Mathers C., Parkin D.M., Piñeros M., Znaor A., Bray F., Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods, *International journal of cancer*, 2019, **144**:1941 [Crossref], [Google Scholar], [Publisher]

[3]. Lenouvel D., González-Moles M.Á., Ruiz-Ávila I., Gonzalez-Ruiz L., Gonzalez-Ruiz I., Ramos-García P., Prognostic and clinicopathological significance of PD-L1 overexpression in oral squamous cell carcinoma: A systematic review and comprehensive meta-analysis, *Oral oncology*, 2020, **106**:104722 [Crossref], [Google Scholar], [Publisher]

[4]. Mahmood H., Bradburn M., Rajpoot N., Islam N.M., Kujan O., Khurram S.A., Prediction of malignant transformation and recurrence of oral epithelial dysplasia using architectural and cytological feature specific prognostic models,

Modern Pathology, 2022, **35**:1151 [Crossref], [Google Scholar], [Publisher]
[5]. Hajizadeh F., Okoye I., Esmaily M., Ghasemi Chaleshtari M., Masjedi A., Azizi G., Irandoust M., Ghalamfarsa G., Jadidi-Niaragh F., Hypoxia inducible factors in the tumor microenvironment as therapeutic targets of cancer stem cells, *Life sciences*, 2019, **237**:116952 [Crossref], [Google Scholar], [Publisher]
[6]. a) Utispan K., Koontongkaew S., Mucin 1 regulates the hypoxia response in head and neck cancer cells, *Journal of Pharmacological Sciences*, 2021, **147**:331 [Crossref], [Google Scholar], [Publisher]; b) Kalvanagh P.A., Kalvanagh Y.A. A New Therapeutic Approach Based on Silymarin in the Treatment of Breast Cancer. *Adv. J. Chem. Sect. B. Nat. Prod. Med. Che*m., 2023, **5**:75 [Crossref], [Publisher]; c) Kalvanagh P.A., Kalvanagh Y.A. Evaluation of the effects of siRNA on Snail1 and miR-143 gene expression levels in metastatic female breast cancer cells during mastectomy. *Int. J. Adv. Biol. Biomed. Res*., 2023, **11**:56 [Crossref], [Publisher]; d) Adyani Kalvanagh P., Adyani Kalvanagh Y. An Overview of Mastectomy and Its Types in the Treatment of Breast Cancer. *Int. J. Adv. Biol. Biomed. Res*., 2023, **11**:25 [Crossref], [Publisher]; e) Hatami A. Preparation, Description and Evaluation of the Lethality Acid Loaded Liposomal Nanoparticles Against in Vitro Colon and Liver Cancer. *Journal of Chemical Reviews*, 2021, **3**:121 [Crossref], [Publisher]
[7]. Luo W., Wang Y., Hypoxia Mediates Tumor Malignancy and Therapy Resistance, *Hypoxia and Cancer Metastasis*, 2019, **1136**:1 [Crossref], [Google Scholar], [Publisher]
[8]. Han H., Lee S.O., Xu Y., Kim J.E., Lee H.J., SPHK/HIF-1α Signaling Pathway Has a Critical Role in Chrysin-Induced Anticancer Activity in Hypoxia-Induced PC-3 Cells, *Cells*, 2022, **11**:2787 [Crossref], [Google Scholar], [Publisher]
[9]. You L., Wu W., Wang X., Fang L., Adam V., Nepovimova E., Wu Q., Kuca K., The role of hypoxia-inducible factor 1 in tumor immune evasion, *Medicinal research reviews*, 2021, **41**:1622 [Crossref], [Google Scholar], [Publisher]
[10]. Chen B., Li L., Li M., Wang X., HIF1A expression correlates with increased tumor immune and stromal signatures and aggressive phenotypes in human cancers, *Cellular Oncology*, 2020, **43**:877 [Crossref], [Google Scholar], [Publisher]
[11]. Ucaryilmaz Metin C., Ozcan G., The HIF-1α as a Potent Inducer of the Hallmarks in Gastric Cancer, *Cancers (Basel)*, 2022, **14**:2711 [Crossref], [Google Scholar], [Publisher]
[12]. Zoa A., Yang Y., Huang W., Yang J., Wang J., Wang H., Dong M., Tian Y., High expression of hypoxia-inducible factor 1-alpha predicts poor prognosis in pancreatic ductal adenocarcinoma: a meta-analysis and database validation protocol, *Translational Cancer Research*, 2022, **11**:3080 [Crossref], [Google Scholar], [Publisher]
[13]. Young M.J., Chen Y.C., Wang S.A., Chang H.P., Yang W.B., Lee C.C., Liu C.Y., Tseng Y.L., Wang Y.C., Sun H.S., Chang W.C., Estradiol-mediated inhibition of Sp1 decreases miR-3194-5p expression to enhance CD44 expression during lung cancer progression, *Journal of biomedical science*, 2022, **29**:3 [Crossref], [Google Scholar], [Publisher]
[14]. Medrano-González P.A., Rivera-Ramírez O., Montaño L.F., Rendón-Huerta E.P., Proteolytic Processing of CD44 and Its Implications in Cancer, *Stem Cells International*, 2021, **2021**:6667735 [Crossref], [Google Scholar], [Publisher]
[15]. Kar K., Ghosh S., Roy A.K., A Study of CD44 Positive Cancer Cells in Epithelial Ovarian Cancer and their Correlation with P53 And Ki67, *Journal of Laboratory Physicians*, 2021, **13**:050 [Crossref], [Google Scholar], [Publisher]
[16]. Ryu D., Ryoo I.G., Kwak M.K., Overexpression of CD44 Standard Isoform Upregulates HIF-1α Signaling in Hypoxic Breast Cancer Cells, Biomol Ther (Seoul), 2018, **26**:487 [Crossref], [Google Scholar], [Publisher]
[17]. Dubey P., Gupta R., Mishra A., Kumar V., Bhadauria S., Bhatt M.LB., Evaluation of correlation between CD44, radiotherapy response, and survival rate in patients with advanced stage of head and neck squamous cell carcinoma (HNSCC), *Cancer Medicine*, 2022, **11**:1937 [Crossref], [Google Scholar], [Publisher]
[18]. Ghazi N., Saghravanian N., Taghi Shakeri M., Jamali M., Evaluation of CD44 and TGF-B Expression in Oral Carcinogenesis, *Journal of Dentistry*, 2021, **22**:33 [Crossref], [Google Scholar], [Publisher]

[19]. Muller S., Tilakaratne W.M., Update from the 5th Edition of the World Health Organization Classification of Head and Neck Tumors: Tumours of the Oral Cavity and Mobile Tongue, *Head and neck pathology*, 2022, **16**:54 [Crossref], [Google Scholar], [Publisher]
[20]. Liu T.Y., Lee C.C., Chen Y.C., Chang Y.S., Huang H.Y., Lee Y.T., Yen J.C., Chao D., Chang J.G., Mutation Analysis of Second Primary Tumors in Oral Cancer in Taiwanese Patients through Next-Generation Sequencing, *Diagnostics (Basel)*, 2022, **12**:951 [Crossref], [Google Scholar], [Publisher]
[21]. Lorini L., Bescós Atín C., Thavaraj S., Müller-Richter U., Alberola Ferranti M., Pamias Romero J., Sáez Barba M., de Pablo García-Cuenca A., Braña García I., Bossi P., Nuciforo P., Overview of Oral Potentially Malignant Disorders: From Risk Factors to Specific Therapies, *Cancers (Basel)*, 2021, **13**:3696 [Crossref], [Google Scholar], [Publisher]
[22]. Radman M., Glavina A., Sabol I., Mravak-Stipetić M., Knowledge of Oral Cancer among the Fourth and Fifth Year Dental Students, *Acta Stomatologica Croatica*, 2018, **52**:340 [Crossref], [Google Scholar], [Publisher]
[23]. Shao Y., Song Y., Xu S., Li S., Zhou H., Expression Profile of Circular RNAs in Oral Squamous Cell Carcinoma, *Frontiers in Oncology*, 2020, **10**:1 [Crossref], [Google Scholar], [Publisher]
[24]. Holbrook W.P., Ögmundsdottir H.M., Oral Lichen Planus and Mutated TP53—A Road to Cancer? , *Dentistry Journal,* 2022, **10**:176 [Crossref], [Google Scholar], [Publisher]
[25]. Karmelić I., Salarić I., Baždarić K., Rožman M., Zajc I., Mravak-Stipetić M., Bago I., Brajdić D., Lovrić J., Macan D., Salivary Scca1, Scca2 and Trop2 in Oral Cancer Patients—A Cross-Sectional Pilot Study, *Dentistry Journal,* 2022, **10**:70 [Crossref], [Google Scholar], [Publisher]
[26]. Burri P., Djonov V., Aebersold D.M., Lindel K., Studer U., Altermatt H.J., Mazzucchelli L., Greiner R.H., ünther Gruber G., Significant correlation of hypoxia-inducible factor-1alpha with treatment outcome in cervical cancer treated with radical radiotherapy, *International Journal of Radiation Oncology* Biology* Physics*, 2003, **56**:494 [Crossref], [Google Scholar], [Publisher]
[27]. Schindl M., Schoppmann S.F., Samonigg H., Hausmaninger H., Kwasny W., Gnant M., Jakesz R., Kubista E., Birner P., Oberhuber G., Austrian Breast Colorectal Cancer Study Group, Overexpression of hypoxia-inducible factor 1alpha is associated with an unfavorable prognosis in lymph node-positive breast cancer, *Clinical Cancer Research*, 2002, **8**:1831 [Google Scholar], [Publisher]
[28]. Birner P., Schindl M., Obermair A., Breitenecker G., Oberhuber G., Expression of hypoxia-inducible factor 1alpha in epithelial ovarian tumors: its impact on prognosis and on response to chemotherapy, *Clinical Cancer Research*, 2001, **7**:1661 [Crossref], [Google Scholar], [Publisher]
[29]. Chen W.T., Huang C.J., Wu M.T., Yang S.F., Su Y.C., Chai C.Y., Hypoxia inducible factor-1alpha is associated with risk of aggressive behavior and tumor angiogenesis in gastrointestinal stromal tumor, *Japanese Journal of Clinical Oncology*, 2005, **35**:207 [Crossref], [Google Scholar], [Publisher]
[30]. Pérez-Sayáns M., Suárez-Peñaranda J.M., Pilar G.D., Barros-Angueira F., Gándara-Rey J.M., García-García A., Hypoxia-inducible factors in OSCC, *Cancer letters*, 2011, **313**:1 [Crossref], [Google Scholar], [Publisher]
[31]. Zhou J., Huang S., Wang L. Yuan X., Dong Q., Zhang D., Wang X., Clinical and prognostic significance of HIF-1α overexpression in oral squamous cell carcinoma: a meta-analysis, *World Journal of Surgical Oncology*, 2017, **15**:104 [Crossref], [Google Scholar], [Publisher]
[32]. Patel N.R., Jain L., Mahajan A.M., Hiray P.V., Shinde S.S., Patel P.A., An Immunohistochemical Study of HIF-1 Alpha in Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma, *Indian Journal of Otolaryngology and Head & Neck Surgery*, 2019, **71**:435 [Crossref], [Google Scholar], [Publisher]
[33]. Tilakaratne W.M., Iqbal Z., Teh M.T., Ariyawardana A., Pitiyage G., Cruchley A., Stewart J.E., Hagi-Pavli E., Lalli A., Waseem A., Parkinson E.K., Upregulation of HIF-1alpha in malignant transformation of oral submucous fibrosis, *Journal of Oral Pathology & Medicine*, 2008, **37**:372 [Crossref], [Google Scholar], [Publisher]

[34]. Kappler M., Taubert H., Schubert J., Vordermark D., Eckert A.W., The real face of HIF-1 alpha in the tumor process, *Cell Cycle*, 2012, **11**:3932 [Crossref], [Google Scholar], [Publisher]
[35]. Gong L., Zhang W., Zhou J., Lu J., Xiong H., Shi X., Chen J., Prognostic value of HIFs expression in head and neck cancer: a systematic review, *PLoS One*, 2013, **8**:e75094 [Crossref], [Google Scholar], [Publisher]
[36]. Pereira T., Surve R., Shetty S., Gotmare S., Qualitative expression of hypoxia-inducible factor-1α in malignant transformation of oral submucous fibrosis: An immunohistochemical study, *Journal of Oral and Maxillofacial Pathology: JOMFP*, 2020, **24**:106 [Crossref], [Google Scholar], [Publisher]
[37]. Ogane N., Yasuda M., Shimizu M., Miyazawa M., Kamoshida S., Ueda A., Takata K., Sakuma Y., Miyagi Y., Kameda, Y., Clinicopathological implications of expressions of hypoxia-related molecules in esophageal superficial squamous cell carcinoma, *Annals of diagnostic pathology*, 2010, **14**:23 [Crossref], [Google Scholar], [Publisher]
[38]. Zheng Y., Ni Y., Huang X., Wang Z., Han W., Overexpression of HIF-1α indicates a poor prognosis in tongue carcinoma and may be associated with tumour metastasis, *Oncology letters*, 2013, **5**:1285 [Crossref], [Google Scholar], [Publisher]
[39]. Hu X., Lin J., Jiang M., He X., Wang K., Wang W., Hu C., Shen Z., He Z., Lin H., Wu D., HIF-1α Promotes the Metastasis of Esophageal Squamous Cell Carcinoma by Targeting SP1, *Journal of Cancer*, 2020, **11**:229 [Crossref], [Google Scholar], [Publisher]
[40]. Gupta P., Rizvi S.Z., Lal N., Gupta V., Srivastav A.N., Musa O., Expression of CD44 and CD133 stem cell markers in squamous cell carcinoma of esophagus, *Indian Journal of Pathology and Microbiology*, 2021, **64**:472 [Crossref], [Google Scholar], [Publisher]
[41]. Tamatani T., Takamaru N., Ohe G., Akita K., Nakagawa T., Miyamoto Y., Expression of CD44, CD44v9, ABCG2, CD24, Bmi-1 and ALDH1 in stage I and II oral squamous cell carcinoma and their association with clinicopathological factors, *Oncology letters*, 2018, **16**:1133 [Crossref], [Google Scholar], [Publisher]
[42]. Heldin P., Kolliopoulos C., Lin C.Y., Heldin C.H., Involvement of hyaluronan and CD44 in cancer and viral infections, *Cellular signaling*, 2020, **65**:109427 [Crossref], [Google Scholar], [Publisher]
[43]. Surendran S., Siddappa G., Mohan A., Hicks W. J.r., Jayaprakash V., Mimikos C., Mahri M., Almarzouki F., Morrell K., Ravi R., Govindan S., Cancer stem cell and its niche in malignant progression of oral potentially malignant disorders, *Oral oncology*, 2017, **75**:140 [Crossref], [Google Scholar], [Publisher]
[44]. Adnan Y., Ali S.M.A., Farooqui H.A., Kayani H.A., Idrees R., Awan M.S., High CD44 Immunoexpression Correlates with Poor Overall Survival: Assessing the Role of Cancer Stem Cell Markers in Oral Squamous Cell Carcinoma Patients from the High-Risk Population of Pakistan, *International journal of surgical oncology*, 2022, **2022**:9990489 [Crossref], [Google Scholar], [Publisher]
[45]. Xu H., Niu M., Yuan X., Wu K., Liu A., CD44 as a tumor biomarker and therapeutic target, *Experimental Hematology & Oncology*, 2020, **9**:36 [Crossref], [Google Scholar], [Publisher]
[46]. Hema K., Rao K., Devi H.U., Priya N., Smitha T., Sheethal H., Immunohistochemical study of CD44s expression in oral squamous cell carcinoma-its correlation with prognostic parameters, *Journal of Oral and Maxillofacial Pathology: JOMFP*, 2014, **18**:162 [Crossref], [Google Scholar], [Publisher]
[47]. Custódio M., Pelissari C, Santana T., Trierveiler M., Expression of cancer stem cell markers CD44, ALDH1 and p75NTR in actinic cheilitis and lip cancer, *European Archives of Oto-Rhino-Laryngology*, 2018, 275:1877 [Crossref], [Google Scholar], [Publisher]
[48]. Liang G., Li S., Du W., Ke Q., Cai J., Yang J., Hypoxia regulates CD44 expression via hypoxia-inducible factor-1α in human gastric cancer cells, *Oncology Letters*, 2017, **13**:967 [Crossref], [Google Scholar], [Publisher]
[49]. Krishnamachary B., Penet M.F., Nimmagadda S., Mironchik Y., Raman V., Solaiyappan M., Semenza G.L., Pomper M.G., Bhujwalla Z.M., Hypoxia regulates CD44 and its variant isoforms through HIF-1α in triple

negative breast cancer, *PLoS One*, 2012, **7**:e44078 [Crossref], [Google Scholar], [Publisher]

[50]. Dietz R.L., Hartman D.J., Zheng L., Wiley C., Pantanowitz L., Review of the use of telepathology for intraoperative consultation, *Expert review of medical devices*, 2018, **15**:883 [Crossref], [Google Scholar], [Publisher]

[51]. Farahani N., Pantanowitz L., Overview of telepathology, *Surgical pathology clinics*, 2015, **8**:223 [Crossref], [Google Scholar], [Publisher]

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