Role of Immunohistochemical Markers in Assessing the Recurrence in Oral Squamous Cell Carcinoma: A systematic Review

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Abstract:

Detection of recurrence of oral carcinoma at an early stage is the most effective means of improving survival and reducing morbidity from this disease, yet a significant proportion of patients succumb to death as a result of recurrence. The literature on immunohistochemical markers associated with recurrence of oral squamous cell carcinoma (OSCC) was searched systematically to access relevant data published till 2022. Twenty studies met the inclusion criteria for the review. In all these studies, the cases of OSCC were analysed histopathologically and then immunohistochemistry was done. Six studies included immunohistochemistry which was correlated with PCR. Immunohistochemical markers play an important role in recurrence but the research in this area is sparse, poorly understood and under-researched. Systematic, high-quality and theory-driven research in this area is essentially required.

Introduction:

Oral squamous cell carcinoma (OSCC) is one of the most common type of cancer in the world. It ranks as the first in India among the male and is a major cause of cancer morbidity and mortality. In India, high incidence of oral cancer is associated with tobacco consumption.[1,2] Its etiology is multifactorial and both internal and external factors affect the development of cancer.[3] Invasion into adjoining tissues and regional lymph node involvement are two major problems leading to 90% of treatment failures. Clinical staging of the disease based on TNM classification (tumor size, lymph node spread and distant metastasis) and the histopathological grading are the most relevant OSCC prognostic factor, however, the behavior of some OSCC is uncertain. Morphological changes with loss of adhesion, extracellular matrix (ECM) degradation and increase in cell migration is exhibited by metastatic and invasive tumour cells, which is the result of rearrangements of the cytoskeletal microfilaments[2]. Another important aspect in progression of oral squamous cell carcinoma is the role of intercellular signaling proteins called as cytokines[4]. Local recurrence is influenced by tumour biology related to cell morphology and tumour microenvironment. Thus, it is necessary to identify the factors and molecular events which contribute to local recurrence and invasion of OSCC, so that a novel therapeutic approach can be developed. Summing it up, recurrence has a major influence on five years survival in oral squamous cell carcinoma, so it is essential to identify the molecular elements responsible for recurrence in it. To assess the role of immunohistochemical markers in the recurrence of OSCC, till date no study has been systematically evaluated. We have followed the preferred reporting for systematic reviews in this research.

Method:

Eligibility criteria

The following criteria was used for chosen studies:

Study Design:

- Included: Original research studies which were mainly retrospective from the date of inception to 2022
- Excluded: All the case series, animal studies, review papers, conference papers, abstracts and unpublished data.

Intervention:

- Included: Immunohistochemistry [IHC] studies.
- Excluded: RT-PCR, DNA methylation, gene analysis, serum used in studies.

Participants:

- **Included:** Patients with primary disease as oral squamous cell carcinoma

- **Excluded:** Patients with past history of malignancy of other site or recurrent oral squamous cell carcinoma and patients with metabolic or immune disorders were excluded.

Outcome:

This study might help in identifying the role of immunohistochemical markersinrecurrence of oral squamous cell carcinoma so that these patients could be treated appropriately for better prognosis.

Search strategy: Scientific literature published up to December 2022 was search including the Medline, Embase, and Web of Science databases by three independent reviewers (MT, LS, and RDB) to identify papers, and relevant data were extracted. Disagreements among reviewers were resolved by discussion. The search strategy was consistent across the databases, and it was performedusing the following keywords: Oral squamous cell carcinoma [MeSH Major Topic] AND [recurrence] AND [immunohistochemical markers]. To ensure that all studies assessing anyoutcome of interest were captured, no selective keywords referring to the outcome were introduced in the search strategy. Cross-referencing from relevant studies was performed to confirm the retrieval of all possible studies.There were no restrictions in the search in terms of the year of publication or language.

Data extraction: Two authors created a systematic data extraction sheet to exclude irrelevant articles. Based on the inclusion and exclusion criteria, the included articles were evaluated. The third author helped to resolve the discreepencies arising in these two steps. This data sheet was used to extract data from relevent studies under the headings: author, year, country, sample size, recruitment period, detection method and antibody source.

Results:

Search results and outcome: A total of 98 articles were found in the initial search strategy. After screening of titles and duplicates , 57 articles were screened. After excluding the abstracts, a total of 34 articles were found eligible. Out of which 14 articles were excluded in which among the four articles method other than immunohistochemistry was used. Out of the other ten articles, two studies included lymph node metastasis, two were serum based studies, one study included prognosis in anatomic subsets, one study included digital scoring of markers, two studies were based on survival of patients and one study included role of markers in OSCC arising from OSMF. Finally twenty articles met the criteria of the present review and were included in it.

General characteristics of eligible studies: The total number of cases were 1870, maximum articles were from Asian ethinicity. In all articles, there were cases of OSCC. All were retrospective studies except one which was prospective study. All were analysed histopathologically and then immunohistochemistry was done. Six studies included immunohistochemistry which was correlated with PCR.

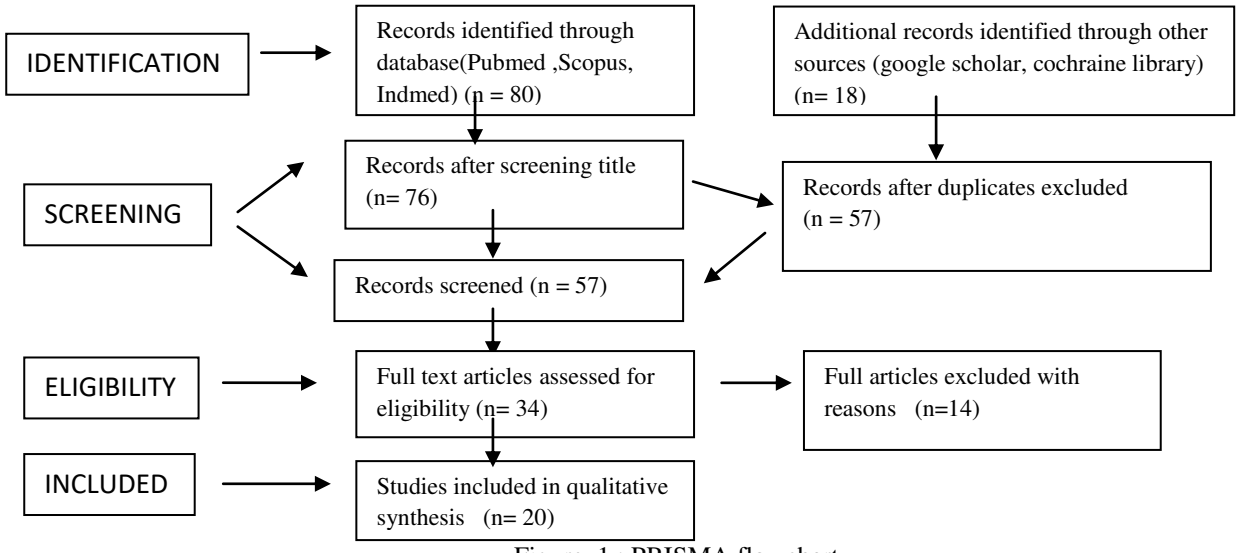

Figure .1 : PRISMA flowchart

Parameters related to immunohistochemical markers of related studies: There are diverse variety of "IHC markers" that can be used either alone or in combination to detect recurrence potential in patients of OSCC. The studies included here used, , , 'NNMT[5]','NBS1[6]', 'β-Catenin[7,9]', 'CCL18[8]', 'E-Cadherin[9]', 'Cyclin-D1[9,11]',',','HIF-1α[10]', 'VEGF[10]', 'EGFR[11]', 'NRP-1[12]', 'HER-2[12]','eIF4E[13]', 'L1EBP1[13]', 'p16[15,16]', 'Derlin-1[17]', 'ELMO3[18]','CD56[19]', 'HSP70[19]', 'ZNF703[20]', AKR1B10[21], 'Talin[22]' 'TRF-2[23]', LC3[24] ,'DPD[25]',

'Thymilidate synthetase[25]'. Nuclear staining is seen of NNMT, NBS1 and TRF2 at a dilution of 1:10000 and 1:500 respectively[5,6,23]. β-Catenin is used in one study at a dilution of 1:500 where it showed brown staining in cytoplasm[7]. In one more study, a combination of E-cadherin, Cyclin-D1 and β-Catenin' was used. In that, the dilution of E-cadherin is 1:50, and 1:150 for Cyclin-D1 and β-Catenin. E-cadherin showed membranous, Cyclin-D1 showed nuclear and β-Catenin showed cytoplasmic and membranous staining[9]. In one study, HIF-1α from Neomaker is used with VEGF from Biogenex showing brown staining in cytoplasm[10]. NRP-1 and HER-2 from Santacruz Biotechnology revealed brown staining in cytoplasm[12]. Both elF4E (Abcam) and 4EBP1 (Abcam) used in one study exhibited nuclear staining at a dilution of 1:500[13]. A combination of markers is used in one study which included CD4, CD8,(ThermoFisher scientific) CD204(transgenic),TGFβ1(R and D systems),CK(Nicheria Bio),PD-L1(Abcam) and PAX7 (Novus biological)[14]. Derlin-1 (Sigma), CCL18, CD56 (Novocastra) and HSP70 (DAKO) exhibits cytoplasmic staining at a dilution of 1:1000, 1:300, 1:500 and 1:500 respectively[8,17,19]. In one study,ELMO3 showed nuclear staining at a dilution of 1: [18]. ZNF703 (Abcam) was used in one study at a dilution of 1:400. Positive cells were stained as brown in nucleus[20]. The expression of AKR1B10 was analyzed by immunohistochemistry in one of the study. In one study, expression of the autophagy-related markers LC3A/B and p62 was evaluated by immunohistochemistry[21,24]. Another study used Dihydropyrimidine dehydrogenase (DPD) and thymidylate synthase which showed cytoplasmic immunoreactivity[25].

Sr.no.	Year	Sample size	Method	Markers	Author	Country	Dilution
1.	2006	23	IHC + PCR	Thymidylate synthase , DPD	Sakakura et al	Japan	NA
2.	2010	125	IHC	NBS-1	Hsu et al	Taiwan	NA
3.	2012	19	IHC	NRP-1,HER-2	Mehta et al	USA	NA
4.	2015	210	IHC	ZNF703	Hang Yang et al	China	1:400
5.	2016	125	IHC	ELMO3	Lorenz K. et al	Austria	NA
6.	2016	290	IHC	Cyclin D1, p53, EGFR	Gupta et al	India	NA
7.	2017	77	IHC	CD56,HSP 70	Stangle et al	Germany	1:500
8.	2017	114	IHC	Derlin 1	Lieming et al	China	1:1000
9.	2018	40	IHC+ PCR	p16	Babji et al	India	NA

	8						
10.	2018	38	IHC	HIF1 α, VEGF	Lee et al	Taiwan	NA
11.	2018	71	IHC	LC3A, LC3B	Terabe et al		
12.	2019	54	IHC	elF4E, L1EBPI	Huang CT et al	Taiwan	1: 500
13.	2019	147	IHC	p16	Ni et al	China	NA
14.	2019	107	IHC	AKR1B10	Yun Ho et al	Taiwan	
15.	2020	144	IHC	p16	Hashmi et al	Pakistan	NA
16.	2020	102	IHC	CCL18	Mao et al	China	1:300
17.	2021	80	IHC	β - catenin	Kar et al	India	1:500
18.	2021	80	IHC	TRF-2	Kar et al	India	1:500
19.	2021	65	IHC	E–cad, cyclin D1, β - catenin	Al Rawi et al	UAE	1:50,1:150,1:150
20.	2022	40	IHC+ PCR	NNMT	Zhang et al	China	1:10,000

Immunohistochemical studies: Potential Markers: Formalin fixed paraffin embedded and archived blocks of OSCC were included in the studies. NNMT was used in 90 cases out of which 36 cases showed significant increase[5].NBS1 was used in 125 OSCC cases, 35 samples(28%) showed IHC level 0, 38 cases (30.4%) were IHC level + ,whereas 52 samples (41.6%) showed IHC ++ defined as increased expression. All NBS1 IHC ++ tumor samples demonstrated a greater than 2-fold increase in NBS1 mRNA expression[6]. β-catenin was used in one study in tumor and surgical cut margin. Among 80 patients, β-catenin marker was positive in tumorin 33 cases (41.25%), whereas in 47 cases (58.75%), it was negative, it was just reverse at cut margin, respectively. This outcome showed that when β-catenin is positive in tumor, the recurrence was low (22.2%, 6 out of 27 patients while the recurrence was high (21 out of 27 patients) showing negative β-catenin in tumor. The recurrence was more when β-catenin was positive in cut margin, that is, 21 out of 27 recurrences (77.8%)

cases[7].CCL18 expression was found to be increased in all the OSCC tissues when compared to their corresponding non-cancerous tissues in the paired IHC staining of 102 OSCC cases[8]. Cyclin D1 was used in two separate studies, in one study 65 cases were taken. It was significantly higher in subjects over 60 years old (86.00±18.3) and significantly higher in subjects with recurrent tumors (90.92±14.84). In this study, E-Cadherin and β-catenin were also used. E-cadherin expression differed greatly between tumor grades, with the highest in grade 1 and the lowest in grade 3. β-catenin expression was slightly lower in patients who smoked or had recurrent tumors[9]. In a study, HIF-1α and VEGF were evaluated in 38 patients (8 hyperkeratosis and 30 oral squamous cell carcinoma). Increased HIF-1α expression was found in OSCC, compared to hyperkeratosis. On the contrary, mild decrease in VEGF expression was found in OSCC, compared to hyperkeratosis. Higher HIF-1α expression in OSCC stages III and IV thanin stages I and II. Highest HIF-1α and VEGF expression were seen in cases of metastasis and recurrence[10].The Cyclin D1, EGFR and p53 protein expressions of 290 OSCC patients were evaluated using IHC.According to Cyclin D1 expression, 31 (10.7%) patients were negative, 197 (67.9%) were positive and 62 (21.4%) were strongly positive. The EGFR expression showed 13 (4.5%), 135 (46.6%) and 142 (49.0%) respectively and p53 expression showed 35 (12.1%), 46 (15.9%) and 209 (72.1%) respectively. The significant association of marker expression (Cyclin D1,EGFR, p53) was found with recurrence. The higher expression of Cyclin D1 and EGFR showed significant association with more probability to recur at distant site along with early time of recurrence[11]. IHC for NRP-1 and HER-2 was performed on 14 samples. Expression of NRP-1 was seen in 43% (6/14) of patients and that of HER-2 was seen in 25% (4/16). Negative HER-2 status was associated with longer survival[12]. 4EBP1 and eIF4E were used in 54 patients in this study; they were divided into two groups, one of 28 patients with non-recurrence and 26 with recurrence. Higher expression levels of eIF4E and p-4EBP-1 were associated with the risk for tumor recurrence[13]. In a study, 28 patients were included where the number of CD8+ T cells and granzyme B+ cells significantly decreased in patients who subsequently developed recurrence. CD204+ macrophage infiltration tended to be higher in patients who developed recurrence than in those who did not[14].In one study, p16 over expression was noted in 22.9% (33 cases), while 21.5% (31 cases) were focal positive and 55.6% (80 cases) were negative. Significant association of p16 expression was noted with nodal metastasis and extranodal spread[15,16]. IHC analysis of Derlin-1 protein in 114SCC and 6 adjacent normal mucosa. There was no stain of Derlin-1 in normal mucosa tissues but positively stained in 94.7% (108/114) of cancer slides. Among them 45.4% (49/108) of patients were detected with high expression of Derlin-1[17]. 125 patients were included in a study where ELMO3 expression was assessed using immunohistochemistry (IHC). ELMO3 expression was detected in 71.2% of the HNSCC cases tested. We found significantly increased overall and disease-free survival rates and decreased recurrence rates in patients with no detectable ELMO3 expression[18]. In a retrospective study, the expression of Hsp70 was determined in relation to tumor infiltrating $CD56^+$ NK cells in formalin-fixed paraffin embedded tumor specimens of 145 patients with SCC. Patients with high Hsp70 expressing tumors (scores 3–4) showed significantly decreased overall survival, local progression-free survival and distant metastases-free survival compared to those with low Hsp70 expression. Low numbers of tumour-infiltrating NK cells correlate with unfavourable outcome[19].210 patients were included in a study where ZNF703 expression was assessed using immunohistochemistry. The overexpression rate of ZNF703 protein was 48.6% in HNSCC tumor cells, it was significantly higher than that of the adjacent noncancerous squamous epithelium cells (48.6% versus 11.6%). The overexpression of ZNF703 was found to be related to recurrence[20].

Significance of Potential Markers: Nicotinamide N-methyltransferase (NNMT), as a cytoplasmic enzyme with a molecular weight of 29 kDa belonging to the N-methyltransferases family, was originally regarded as providing a straightforward mechanism for regulating nicotinamide (NAM)levels. NNMT catalyzes the methylation of NAM and structurally related compounds by using the universal methyl donor S-Adenosyl methionine (SAM) to produce S-adenosyl-L-homocysteine (SAH) and 1-methylnicotinamide.NNMT is overexpressed in a variety of tumors and has been shown to promote progression and poor prognosis of several malignant tumors, such as gastric cancer, esophageal cancer and colorectal cancer. NNMT was highly expressed in OSCC patients and was negatively associated with lymph node metastasis[5].

Nijmegen breakage syndrome (NBS) is a chromosomal-instability syndrome associated with microcephaly, immunodeficiency, cancer predisposition, radiosensitivity, and growth retardation. The product of the defective gene in NBS (the NBS gene), NBS1 (p95, nibrin), is a member of the DNA double- strand break (DSB) repair complex (hMre11 complex). NBS1 carries out its checkpoint functions when it is phosphorylated by the ATM (ataxia-telangiectasia mutated) protein following ionizing radiationhowever, rare or no mutationsof NBS1 have been identified in certain types of human cancer. Overexpression of NBS1 contributes to transformation through the activation of PI3-kinase／Akt. Increased NBS1 expression is present in 45% of advancedHNSCC patients receiving non-surgical treatment and is associated with a worse prognosis[6]. β-catenin is a multifunctional protein that plays a role in two seemingly unrelated processes: cell-cell adhesion and signal transduction. In addition to its role in regulating E-cadherin-mediated cell adhesion, β catenin is a

transcription cofactor in the wingless (Wnt) signaling pathway and a target of the adenomatosus polyposis coil (APC) gene product, which has been linked to the development of various human cancers. In tumors, reduced or no expression of β-catenin causes cell proliferation, migration, and invasion, and is linked to a poor prognosis. The E-cadherin/β-catenin complex can be disturbed during oncogenesis. β-catenin is an anchoring protein present in the cytoplasm and is essential in maintaining normal functions of E-cadherin. β-catenin binds to the E-cadherin molecule's cytoplasmic domain directly. This binding is needed for stable cell-cell adhesion and is controlled in part by β-catenin[7,9]. Chemokine ligand 18 (CCL18) belongs to the small cytokine family, and these are involved in immunoregulatory processes. In tumor tissues, there seems to be a positive feedback loop in order to increase the CCL18 expression. CCL18 was highly expressed in poorly differentiated OSCC patients with early lymphatic metastasis and poor survival rate. This suggests that CCL18 is related to OSCC prognosis[8].E-cadherin helps normal cells adhere together. Cadherins are a wide family of membrane-associated glycoproteins that regulate cell to cell adhesion. Impaired expression of cadherin causes cell adhesion dysfunction, leaving tumor cells less articulate and more invasive. Loss of E cadherin expression has been discovered to increase the likelihood of these cells metastasizing locally or to a distant area. E-cadherin deficiency has been linked to poorly differentiated cancers, lymph node metastases, and a poor prognosis[9]. Cyclin D1 is a central player in oral cancer growth, as it promotes cell proliferation, migration and differentiation. The rate of amplification of the cyclinD1 gene is more than two times greater in other cancers. This amplification of the cyclin D1 gene can lead to overexpression of this protein, which is sometimes linked to prognostic markers such as high T and N stages, advanced level, low differentiation, and decreased survival[9]. Expression of HIF-1α is caused by hypoxia and cumulative genetic alteration. Hypoxia and HIF-1α expression has been shown to promote neovascularization and facilitate tumor spread. It is reported, in dysplastic cervical lesion, that HIF-1α showed an abnormal distribution, especially in the lower third of the epithelium. Not only in hypoxia, it is also expressed in ischemic and inflammatory conditions. VEGF can be activated by HIF-1α which plays a role in promoting neovascularization and facilitate tumor spread. HIF-1α showed an abnormal distribution, especially in the lower third of the epithelium. Not only in hypoxia, HIF-1α can also be expressed in ischemic and inflammatory conditions. VEGF can be activated by HIF-1α.VEGF expression in malignant tumor had been found to increase vascularity, cancer cell growth and lymph node metastasis[10].Activation of p53 and EGFR are known to be inhibitors of apoptosis and play crucial role in initiation of intracellular signaling pathways which regulate the activation of cell proliferation, invasion, angiogenesis and metastasis and thereby influence treatment outcome Expression of these proteins have also been correlated with a more aggressive phenotype and worse prognosis[11]. Neuropilin-1 (NRP-1) is a cell-surface receptor for VEGF and class 3 semaphorins is expressed by neurons and endothelial cells and acts as a mediator of angiogenesis and neuronal guidance. It is overexpressed by many cancers and is associated with increased neoangiogenesis and aggressive tumor behaviour. Neuropilin-1 is expressed in various tumor cells such as breast, prostate, lung, melanoma cells and acute myeloid leukemia. This tumor cell derived NRP-1 is functionally active and may act as a positive modulator of tumor angiogenesis and a negative regulator of tumor cell apoptosis in the presence or absence of VEGF. It has also been reported that NRP-1 is an independent predictor of cancer relapse and poor survival in patients[12]. The PI3K/AKT/mTOR signaling pathway has been indicated to be implicated in the development, progression, and metastasis of numerous cancers. In this pathway, the translation initiators that influence the downstream protein synthesis include eIF4E, 4EBP1, and S6K, and the rate of protein synthesis is dependent on the status of phosphorylation. The expression levels of eIF4E, p-4EBP1, p-eIF4E, p-S6K1, and p-S6R were elevated in both the patients with recurrence and non-recurrence, which may indicate that cell growth and proliferation rates are higher in patients with tumor recurrence than in those without recurrence. Correspondingly, elevated expressions of eIF4E, p-4EBP1, and S6K1 correlated with breast cancer proliferation and survival. Moreover, a strong relationship between MNK-mediated phosphorylation of eIF4E has been demonstrated in prostate and breast cancers. As the phosphorylation of eIF4E, 4EBP1, and S6K is known to affect the rate of translation, it is possible that these proteins play a critical role in the progression of HNSCC[13].The p16 INK4a (p16) is a tumor suppressor gene (TSG) located on the chromosome 9p, locus 21, involved in cell cycle progression blocking process. It is inactive in wide range of human malignancies. A loss of p16 INK4a immunosuppression has been observed in the early stages of oral carcinogenesis and has been considered as first TSGs to be inactivated in OSCC. Microsatellites are extensions of DNA in which a short motif is repeated 5–100 times and is susceptible to inaccurate repetition during DNA replication. These microsatellite alterations are of two types: microsatellite instability (MSI) and loss of heterozygosity (LOH)[15,16]. Derl-like-protein-1 (Derlin-1) is a human homologue of yeast Der1p containing four transmembrane regions within the lipid bilayer of endoplasmic reticulum membrane4. It was initially reported as a partner of the p97 ATPase complex and regulator of misfolded protein degradation from the endoplasmic reticulum (ER) to cytosolic5. The ER is a specialized organelle where cells correct protein folding and modify secretory and membrane proteins. Cancer cellular adaptation to ER stress is mediated by the unfolded protein response (UPR), when cancer cell under intrinsic and external

adverse conditions such as hypoxia or chemo-radiotherapy. There were evidences showed that the Derlin-1 played crucial roles in cystic fibrosis and cancers such as breast, colon, bladder and lung cancers. Derlin-1 was over-expressed in SCCHN samples comparing to adjacent normal tissues. The expression of Derlin-1 was associated with T-stage, lymph node metastasis and clinical stage. And high expression of Derlin-1 independently predicted a shorter survival time of SCCHN patients. Knock down Derlin-1 expression regulated cell proliferation, apoptosis and migration in two SCCHN cell lines. Furthermore, the classical signal proteins, like p53, Smad2/3, PI3K/Akt, may involve in the regulation of SCCHN by Derlin-1[17] The engulfment and cell motility 3 (ELMO3) protein has been reported to be involved in cell migration and cytoskeletal remodeling. As of yet, nothing is known about the role of ELMO3 in head and neck squamous cell carcinoma[18]. A highly conserved, major stress-inducible Hsp70, also termed HSPA1A, is found in nearly all cellular and subcellular compartments of nucleated cells. Hsp70 fulfils a variety of chaperoning functions, such as maintenance of cellular homeostasis by assisting folding, maturation and transport of unfolded proteins, and preventing of apoptosis under stressed and non-stressed conditions. Elevated Hsp70 levels are associated with poor prognosis in a variety of tumor. Furthermore, tumors in contrast to normal cell have also been shown to present Hsp70 on their cell surface. Membrane localization of Hsp70 on tumor cells is most likely due to a tumor-specific lipid composition that enables anchorage of Hsp70 in the plasma membrane. CD56NK (natural killer) cells are described to secrete pro-inflammatory cytokines rather than exerting cytotoxicity, these NK cells are able to efficiently eliminate Hsp70 membrane-positive tumor cells. Safety and tolerability of these ex vivo stimulated, autologous connected to oropharyngeal cancer [19]. The zinc finger protein 703 (also known as ZPO1,ZEPPO1) gene is located on chromosome arm 8p12. TheZNF703 protein belongs to the NET (Noc/Nlz, Elbow, and Tlp-1) family, which plays an important role in the embryonic development of zebrafish and Drosophila. It has been shown that ZNF703 gene amplification stimulates migration and proliferation while reducing cell to cell adhesion and is speculated to be associated with poor prognosis[20] . Aldo-keto reductase family 1 member B10 (AKR1B10) is an enzyme implicated in physiological xenobiotic detoxification and also in pathological carcinogenesis. Overexpression of AKR1B10 has been reported in oral squamous cell carcinoma. High expression of AKR1B10 was found to be associated with tumor size, perineural invasion ($P = 0.012$), and recurrence in OSCC[21]. Oral squamous cell carcinoma (OSCC) is highly invasive and is associated with frequent tumour recurrences and lymph node metastases. Identification of genes involved in the aggressiveness of OSCC may provide new targets for clinical intervention. Comparative analyses revealed that Talin (TLN1) was the most highly expressed integrin-cytoskeleton cross-linker that can trigger integrin activation. IHC analyses and mouse study also revealed an association between TLN1 overexpression and advanced OSCC with invasion to adjacent tissues. Survival analyses indicated a significant association between TLN-1 genetic overexpression and a reduced overall survival in patients. Functional knockdown by a dominant negative TLN1 fragment reduced cell growth and invasiveness in TLN1 overexpressing cells via inactivation of downstream oncogenic signalling[22]. Telomeric repeat factor-2 (TRF2) is a telomeric shelter in protein which is involved in the maintenance of genome stability by protecting the telomeric ends of chromosomes through t-loop formation and preventing DNA repair mechanism at the telomeres. Apart from being a key telomeric protein, recent reports have detailed an extra-telomeric role of TRF2 in ATM-mediated DNA damage repair at non-telomeric sites. Furthermore, TRF-2 is also reported to be the direct target of β-catenin which in turn is reported to be involved in cancer progression in several solid tumours [23]. Autophagy is involved in tumour suppression through several pathways. The main function of autophagy is to maintain cellular hemostasis through degradation of damaged organelles and protein aggregates. Autophagy can inhibit tumor development by controlling the cellular levels of p62. The main function of p62 is to deliver ubiquinated proteins to autophagosomes for degradation and subsequently p62 is being degraded in this process. p62 tends to be involved in pro-tumorigenic signaling and has been overexpressed in human cancers.Microtubule-associated protein light chains 3 (LC3) is a specific autophagosome marker and has been demonstrated to be an effective prognostic marker in various cancers including oral SCC. LC3 participates in autophagosome membrane elongation, and its activated form binds tightly to the pre-autophagosomal, autophagosomal and autolysosomal membrane. LC3 consists of three main members, which include LC3A, LC3B and LC3C [24].

Discussion:

Despite of tremendous search for prognostic markers, no single molecular study till date has been shown to identify and focus at high risk for recurrences which is the most distressing component of treatment failure in the patients of OSCC. Recent advances in molecular biology field and improved conception of the pathogenesis have provided access to many new orientations in the research of OSCC. The molecular markers of concern are those involved in cell cycle regulation and cell signalling pathway. Oncogenes which promote cell and tumor growth includes growth factor receptors (hst-1, int-2, EGFR/erbB,c-erbB-2/Her-2, sis), intracellular signal transducer (ras,raf,stat-3), transcription factors (myc, fos, jun, c-myc), cell-cycle regulators (Cyclin D1), apoptosis regulatory protein (bcl-2, bax) and tumor suppressor gene (p53, Rb) which encodes proteins that typically transduce

negative growth regulatory signals; these have been identified as genetic alterations in each of the pathological stages of OSCC[11]. Despite of significant improvement achieved during the last decades in the detection, prevention and treatment of OSCC the outcome and prognosis related to cure and survival have still been poorer due to tragic event of treatment resistance and tumor recurrence. More than 50% of patients eventually develop local recurrence or metastasis usually within the first two years following completion of treatment[11]. The importance of immunohistochemical markers in evaluating the recurrence of oral squamous cell carcinoma had been focussed by recent studies. The accuracy and the reliability of the markers used constitute the analytical validity of the marker used, which is of prime importance for targeting and formulating a standard treatment regime for OSCC patients in need for better prognosis. The present systematic review was pursued to incorporate the current data on role of immunohistochemical markers in evaluating the recurrence of oral squamous cell carcinoma. Our inclusion criterion was fulfilled by the total of twenty studies.Meta-analysis, case series, animal studies, review papers, conference papers, abstracts and unpublished data were excluded. We have tried to include unequivocal data of the included studies. The presence of slender data due to few studies along with heterogeneity of evidence narrows the inference that can be withdrawn from the present review. So, this substantiates the further need of more studies focussing mainly on combination of markers to indicate cancer recurrence which could be targeted for intensive treatment modalities ultimately leading to better prognosis. These studies included 'p16', 'p53', 'Derlin-1', 'ZNF703', 'CD56', 'HSP70', 'NNMT','NBS1', 'CCL18', 'EGFR', 'β-Catenin', 'E-Cadherin', 'Cyclin-D1','AKR1B10','HIF-1α', 'VEGF','elF4E', 'L1EBP1', 'DPD', 'Thymilidate synthetase', 'TRF-2', 'NRP-1', 'HER-2', 'ELMO3','CD4', 'CD8', 'CD204', 'TGF-β1', 'CK', 'PDL1', 'PAX7' in OSCC. The results varied due to different sample size and other methodological flaws. Immunohistochemistry (IHC) was used to analyze the NNMT expression profile in OSCC in a study. OSCC patients with highly expressed NNMT in tumor cells had higher risk of lymph node metastasis, postoperative recurrence and a worse pattern of invasion. Highly expressed NNMT can independently predict shorter disease-free survival[5]. In another study it was found that the patients who had high levels of β-catenin in cut margin are 3.6 times more likely to have recurrence than those patients who had low levels which highlighted that β-catenin can be included as a prognostic molecular marker, along with routine histopathological study to influence therapeutic decisions[7]. A study demonstrated that CCL18 expression is strongly and negatively related to disease free survival of OSCC patients and could potentially be used as a novel independent prognostic predictor of OSCC[8]. In another study, Cyclin D1 was used along with E-cadherin and β- catenin whereas Cyclin D1 and β- catenin were significantly correlated with depth of invasion and tumor recurrence. E-cadherin expression was significantly correlated with histological grades and metastasis[9]. One of the study showed high HIF-1a and VEGF were associated to tumor metastasis and recurrence[10]. Over expression of Cyclin D1, EGFR and p53 used in one of the study were found to be associated with tumor recurrence and reduced time to recurrence at primary and distant sites. It was also evident that tumors over expressing these markers were resistant to chemoradiation[11]. High levels of NRP-1 and HER-2 in SCCHN samples was found to be associated with decreased survival and earlier progression of disease, respectively, in SCCHN patients and may represent targets for therapy[12]. In one study, the expression level of eIF4E and p-4EBP1 were significantly associated with tumor recurrence and recurrence-free survival. In conclusion, the expression of eIF4E and p-4EBP1 should be considered as predictive biomarkers for the HNSCC patients[13] .In a study, it was found that patients who developed recurrence had a high expression of PAX7 in tumor cells. The number of CD8+ T cells and granzyme B+ cells tended to decrease in patients who subsequently developed recurrence. Specifically, the granzyme B+ cell number was significantly smaller and CD204+ macrophage infiltration tended to be higher in patients who subsequently developed recurrence than in those who did not[14]. p16 was estimated in two studies. In one study, p16 was used in both tumor proper and surgical margins through IHC and PCR. Expression of p16 with IHC was higher in tumor proper as compared to PCR however the results were similar in surgical margins. This study emphasizes that expression of p16 in surgical margins have a high risk of local recurrence and it can be easily performed within 2 weeks of surgery. The limitation of this study was a small sample size and follow up of all patients was not done to check the recurrence. In the other study, significant association of p16 was noted with nodal metastasis and extranodal spread while no significant association was found with recurrence[15, 16]. Increased expression of Derlin-1 was found to be associated with lymph node metastasis, clinical stage and recurrence in SCC patients in another study. High expression of Derlin-1 was significantly associated with shorter overall survival (OS) and disease-free survival (DFS)[17]. ELMO3 was used in a study where increased disease-free survival rate and decreased recurrence rate in patients with its low expression[18]. In reverse, they found that high expression served as an independent marker for a decreased overall and disease-free survival. In another study, a high heat shock protein (Hsp)70 expression and low numbers of tumor-infiltrating NK lymphocytes correlate with unfavourable outcome following surgery in patients with SCCHN, and thus serve as negative prognostic markers[19]. High expression of ZNF703 in HNSCC tumor tissues as compared to noncancerous tissues was found in a study. ZNF703 overexpression was correlated with tumor position

(laryngeal carcinoma) and recurrence. ZNF703 overexpression is associated with adverse prognosis in HNSCC, which might be a novel biomarker of HNSCC[20]. High expression of AKR1B10 was found to be associated with tumor size, perineural invasion and recurrence in OSCC. It was also found to be associated with a reduced survival in patients with well and moderately differentiated OSCC and even a high incidence of tumor recurrence in the patients with late-stage disease[21]. One of the study correlated the expression level of TRF2 in cases with increasing stage, grade of tumour and recurrence. Recurrence was more when TRF2 was positive in cut margin which suggested that use of TRF2 as the molecular marker at surgical margins can predict recurrences better than histopathological analysis[23]. In one of the study, the patients with high dihydropyrimidine dehydrogenase (DPD) expression had significantly higher levels of recurrence compared with those with low DPD expression. They concluded that assessment of dihydropyrimidine dehydrogenase (DPD) in oral squamous cell carcinoma (OSCC) may be a useful tool in evaluating clinical outcomes[25]. The comparison between staining intensity between groups in different studies were done by one way ANOVA, Student's t-test, Chi square test, Kruskal Wallis, Pearson's Chi square test, Mann Whitney test. The independent sample t test was used to compare intensity within groups and between groups. The studies using combined biomarkers used logistic regression analyses models. Univariate and multivariate analyses to assess the clinicopathological parameters with recurrence and discriminant analysis to identify combined biomarker model. Kappa analysis was done to evaluate the interobserver variability. The difference between the expression of markers and clinicopathological parameters in the study groups were assessed by Kruskal Wallis, Pearson's Chi square test, Mann Whitneytest, Spearman's rank correlation coefficient test and Fisher's exact tests. Limitations are always part of of systematic review which should be considered. These limitations are mainly due to difference in study groups like many of the studies included in this review has a wide range of difference in sample size. Few had very low sample size and the biomarkers used are also not cost effective. The most important limitation was wide range of immunohistochemical markers used in studies varying from single to combination of markers in different study groups. It becomes very difficult to create a standard biomarker for predicting cancer recurrence and prognosis due to such divergence and variability. More elaborate studies in future should be conducted on large sample size and with more focus on combination of biomarkers.

Conclusion:

The synopsis of this review shows that use of immunohistochemical markers to evaluate the process of recurrence in oral squamous cell carcinoma can be of significant importance in post operative patients. Despite of tremendous search for prognostic markers, no single molecular study till date has been shown to identify and focus at high risk for recurrences which is the most distressing component of treatment failure in the patients of OSCC. Hence, comprehensive and collaborative interpretation of expression of immunohistochemical markers would contribute to the identification of patients at increased risk of tumor recurrence and further these patients might be benefited from more intensive and targeted treatment regimen.

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