ORAL SQUAMOUS CELL CARCINOMA:
DIAGNOSTIC MARKERS AND PROGNOSTIC INDICATORS

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OSCC is the most frequent malignant tumour of the oral cavity, accounting for more than 90% of
malignant tumours of this anatomic region and it often arises from precursor lesions. Aside from tobacco
and alcohol consumption, further determinants have been considered to increase the risk of OSCC
development, such as micronutrient deficiencies, chronic traumatism, poor oral hygiene and viruses.
Recurrence, survival and conversely, mortality depends on numerous and different biological, histological,
macroscopic and microscopic factors that have been investigated in order to define causes, to help diagnosis
and to refine appropriate treatments that perfectly fit with the different features of OSCCs. For this purpose,
during the last decades, the improvement of scientific technologies and molecular analyses have allowed to
investigate markers and genetic and epigenetic factors, in order to clarify their responsibilities related to
early diagnosis and OSCC progression and prognosis in order to address them as targets in future selective
and individually-shaped therapies. This review will focus on the etiology, advances in diagnostic markers
and prognostic indicators for oral cancers.

Oral Squamous Cell Carcinoma (OSCC) belongs
to the group of the “Head and neck cancers” (H&N
cancers), which define a heterogeneous group of
epithelial malignant tumours affecting the lining
mucosa of nasal cavity and paranasal sinuses,
nasopharynx, hypopharynx, larynx and trachea,
oropharynx, oral cavity and salivary glands (1).
Johnson N. et al. defined OSCC as “an invasive
epithelial neoplasm with varying degrees of squamous
differentiation and a propensity to early and extensive
lymph node metastases, occurring predominantly
in alcohol and tobacco-using adults in the 5th and
6th decades of life” (2). OSCC is the most frequent
malignant tumour of the oral cavity, accounting for
more than 90% of malignant tumours of this anatomic
region and it often arises from precursor lesions
(3). Aside from tobacco and alcohol consumption
(4), further determinants have been considered to
increase the risk of OSCC development, such as
micronutrient deficiencies (5), chronic traumatism,
poor oral hygiene (6) and viruses (7). Among
viruses, the role of human papillomavirus (HPV)
infection is the most investigated but still debated.
It is universally accepted that HPV is responsible
for all cases of cervical cancer (8), where more than
120 different HPV genotypes have been identified

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and almost 45 subtypes have been grouped into high- and low-risk HPV types according to their risk potential in inducing invasive cervical cancer (9). Some studies report that HPV could also play a role in some cancers of the oral cavity (mainly the base of the tongue and tonsils) and oropharynx (10). Herrero et al. found HPV infection prevalence is higher in oropharyngeal SCC than in OSCC and HPV could play a definite etiologic role in a small subgroup of cancers of the oral cavity, where HPV E6 and E7 proteins were isolated (11). Scapoli et al. confirmed these results (12), performing a similar study limited to SCCs of the proper oral cavity and testing for the presence of four high-risk human papillomavirus (HPV16, HPV45, HPV18 and HPV31). They reported a clinically significant amount of HPV16 DNA in only 7 samples out of 569, with a prevalence as low as 2% and no significant difference with respect to peritumoral normal tissue. Two years later, the same authors investigated the presence of multiple high-risk (HPV16) and low-risk (HPV6 and HPV11) type human papillomavirus in a large sample of SCC limited to the oral cavity, with a prevalence rate of 1.8% for HPV11, confirming the prevalence of HPV16 (13). Recently, Pannone et al. showed that HPV-E7 protein inactivating pRb is expressed in oral cancer cells infected not only by classical high risk HPV16 and HPV18, but also by HPV70, usually considered a low risk virus and HPV53, classified as a possible high risk virus. They also identified a subgroup characterized by HPV infection (10.5%) among OSCCs (14, 15).

OSCC easily tends to spread to the regional lymph nodes of the neck and then metastasize. In order to summarize OSCC features related to the primary tumour size (T), the occurring nodal involvement (N) and distant metastases (M), TNM-classification systems have been refined during the years (16), according to histological differences existing among primary sites (17, 18) and to the consequent different behaviour, in terms of severity, tendency to metastasize and prognostic implications (19-21). The latest worldwide accepted classification, was revised in 2009 (22) with the aim to correlate with prognosis, extent of surgery and need for further non-surgical treatment, such as chemo- and radio-therapy.

Recurrence, survival and conversely mortality depends on numerous and different biological, histological, macroscopic and microscopic factors that have been investigated in order to define causes, help diagnosis and refine appropriate treatments that perfectly fit with the different features of OSCCs.

The most investigated prognostic indicators for survival may be considered site, size and thickness/depth of invasion of the primary tumour (23-26). Survival decreases the closer the tumour origin is to the inner sites of the mouth and in relation to the progressive involvement of regional lymph nodes (27).

Another main predictor for survival of patients affected by OSCC is the nodal status (28). More than 60% of OSCC are diagnosed in locally advanced stages with a 5-year survival <50-60% (29-31).

As in many carcinomas, the first step for OSCC dissemination to other distant tissues (metastases) is the involvement of the regional lymph nodes (32). The progressive involvement usually starts from the most proximal lymph node draining the anatomic area of the primitive tumour and from here it orderly progresses to other lymph nodes of the chain. This first lymph node colonized by the tumour is called “sentinel lymph node” (SNL) and it is detected in conjunction with radiotracer injection and lymphoscintigraphy (33) during the intraoperative session of the primary tumour surgery. This is when SNL biopsy allows the real-time fresh histologic analysis to find metastases occurring at these levels and established more extended therapeutic procedures such as neck dissection (34-36) and postoperative radiotherapy or chemo-radiotherapy, depending on the presence of intermediate- or high-risk features (37, 38). Association of neck dissection plus chemo-radiotherapy can be useful in the event of unresectable advanced carcinomas (39).

However, overall and disease-free survivals depend on multiple factors, influencing the type of surgical and non-surgical therapeutic procedures (39, 40).

The value of the SNL positivity has been discussed and recent works are aimed to change dissection protocols in the neck (41). Accordingly, if the SLN is free of tumour, it is assumed that the remaining cervical LNs are free from cancer as well.
(42). It was confirmed by Contaldo et al. that SNL resulted the sole positive node affected by metastasis in small cT1- cT2/cN0 OSCC, thus considering the neck dissections subsequent to its positivity during intraoperative assessment an overtreatment, associated with worse quality of life and higher morbidity. They also demonstrated different overall survival according to pre-surgical staging, number of lymph nodes harvested and intent to surgery and it was statistically significant (43).

A common feature of many cancers is the presence of inflammatory cells in the peri-tumoral microenvironment but its role in respect to tumour development, progression and spreading still remains controversy (44). In fact, although peri-tumoral inflammation has been traditionally considered a defence mechanism against cancer progression and invasion (45), further studies report evidence that peri-tumoral stromal inflammation plays a supporting and aggravating role in some carcinomas (46,47). Other studies investigated the role of the tumour-associated macrophages, that seem to induce epithelial to mesenchymal transition and has been associated with poor prognosis (48).

Recently, morphometry of the single tumoral cells has been used as a diagnostic tool in OSCC diagnosis (49).

Classical histopathological parameters are not sufficient to accurately predict the clinical behaviour of oral SCC, hence the identification of molecular markers that can accurately define those lesions that will manifest an aggressive behaviour and worse prognosis, is of pivotal importance (50).

For this purpose, during the last decades, the improvement of scientific technologies and molecular analyses have allowed to investigate markers and genetic and epigenetic factors, in order to clarify their responsibilities related to early diagnosis and OSCC progression and prognosis, thus addressing them as targets in future selective and individual customized therapies (51, 52).

In regards to epigenetic changes occurring in H&N SCC, Tosi et al. detected important changes in the phosphorylation program during cancer progression (53). In detail, they measured the phosphorylation levels of the serine-threonine kinase Akt, mitogen-activated protein kinase (MAPK) and protein kinase C (PKC) in a group of specimens with SCC and a paired control group, demonstrating Akt and MAPK significant under phosphorylation in tumours, whereas PKCs showed no differences from control samples. This evidence has been recently confirmed by Chaisuparat et al, who discovered Akt/mTOR pathway activation in oral verrucous carcinoma a subtype of OSCC (54).

Colella et al., in 2011, reported a low expression of Androgen Receptors (AR) mRNAs and a high expression of Estrogen Receptors (ER) mRNAs in malignant tissues of oral mucosa, thus suggesting an involvement of these two sexual hormones in oral cancer (55). This evidence has been recently strengthened by the work of Doll et al. (56) who asserted the de-differentiating role of ER sub-type, that they found predominantly and significantly higher expressed in poorly-differentiated OSCC compared to healthy peritumoral mucosa.

The down-regulation of cell adhesion molecules is another investigated step responsible for OSCC progression: low E-Cadherin and P-cadherin expression and their delocalization from membrane to cytoplasm have been considered as negative prognostic factors of OSCC, due to its aggressive biological behaviour with tendency to infiltrate and metastasize (14, 57).

Similarly to cadherins, several authors hypothesized the prognostic role of CD44 in oral and oropharyngeal SCC (58), since low expression of CD44 correlates with a decreased survival and, conversely, increased expression of CD44 in primary tumours was consistent with a longer survival.

Overexpression of oncogenes inhibiting apoptosis is another event occurring in OSCC. In terms of prognostic significance, Lo Muzio et al. considered bcl-2 and survivin expression as early markers of prognosis (50). They found bcl-2 expression was determined in the early diagnostic phase, whereas in the relapse phase its presence was not found. This evidence was confirmed by further studies reporting the frequent expression of bcl-2 in oral leukoplakia (OL) with malignant transformation, associated with the reduction in number of apoptotic cells (59). With regards to survivin, Lo Muzio et al. suggested that
its expression may identify cases of oral squamous cell carcinoma with more aggressive and invasive phenotype, due to the evidence they found significant negative association among survival and survivin expression.

Loss of Heterozygosity (LOH) -indicating the absence of a functional tumour suppressor gene in the lost regions- (60), DNA ploidy –DNA content per cell higher or lower than normal - (61, 62) and chromosome instability (62) are common occurrences in OSCC. Scapoli et al, in 2011 observed LOH in 53% of the 51 squamous cell carcinoma they analysed, with a significant association between UICC stage grouping and LOH for 3 gene loci: PDCD4 (programmed cell death 4), CTNNB1 (catenin beta 1), and CASP4 (caspase 4) (63). LOH is significantly associated with tumour severity, demonstrating that LOH contributes to tumour progression of OSCC (64) and it is selectively present in preneoplastic and neoplastic lesions, while it lacks at the perilesional healthy tissue in the same patient (65).

These data correlate with lack of fluorescence at the so called “region of interest” marked during clinical autofluorescence assessment (66). Direct visualization of oral cavity tissue fluorescence affirmed its value during last decades in orienting diagnosis of suspicious lesions, showing sensitivity but not specificity in OSCC and dysplasia diagnosis, as toluidine blue vital staining (67). Auto-fluorescence is a property of some molecules of the tissues, related to their different biophysical and biochemical composition. Cancerous tissues strongly lost fluorescence and this could orient diagnostic procedures in a time saving and non-invasive way (68). Due to their non-invasiveness, other imaging technique have been studied in order to understand their potential role in diagnosing early tumoral signs of transformation thus reducing the need for biopsy, such as confocal microscopy (69, 70). These devices were found to not replace the histopathology procedure. However, the literature agrees its usefulness for oral tissue examination, especially within an oral medicine secondary care facility, before performing a biopsy and in monitoring oral lesions. Another interesting topic is survival rate of implants rehabilitation after surgical exeresis of OSSC and development of peri-implantitis. In fact, even if the main factor for survival rate of implants is the quality of bone of receiving sites, the bacteria of peri-implantitis may be the main cause of failure of implants (71-80). Further studies are needed to investigate this problem.

In conclusion, the early detection of the asymptomatic early stage of oral cancer is still the first and most important step to obtain a satisfactory clinical outcome and cure in most patients.

REFERENCES


49. Christopher, V, Murthy, S, Sr, A, Singh, S, Cp, A, Shivaram, SK and Neethupriya. Morphometry As a


