ORAL SQUAMOUS CELL CARCINOMA: DIAGNOSTIC MARKERS AND PROGNOSTIC INDICATORS

D. LAURITANO[1], A. LUCCHESE[2], M. CONTALDO[2], R. SERPICO[2], L. LO MUZIO[3], F. BIOLCATI[4] and F. CARINCI[4]

[1]Department of Medicine and Surgery, University of Milan-Bicocca, Milan, Italy; [2]Multidisciplinary Department of Medical-Surgical and Odontostomatological Specialties - Second University of Napoli (SUN), Naples, Italy; [3]Department of Clinical and Experimental Medicine, University of Foggia, Italy; [4]Department of Morphology, Surgery and Experimental Medicine, University of Ferrara, Ferrara, Italy

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, oroparhynx, 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

Key words: carcinoma, head, neck, oral, markers

Mailing address:
Alberta Lucchese,
Multidisciplinary Department of Medical-Surgical and Odontostomatological Specialties,
Second University of Napoli (SUN),
Via Luigi De Crecchio 6, 80138 Naples, Italy
Tel.: +39 081 5665476 - Fax: +39 081 5665477

0393-974X (2016)
Copyright © by BIOLIFE, s.a.s.
This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.
Unauthorized reproduction may result in financial and other penalties
DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

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 strengthen 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

1. Barnes, L, Eveson, JW, Reichart, P and Sidransky, D. World Health Organization classification of tumours: pathology and genetics of head and neck tumours. Place: IARC Press, 2005.
2. Johnson, NW, Franceschi, S, Ferlay, J, Ramadas, K, Schmid, S, MacDonald, DG, Bouquot, JE and Slootweg, PJ. Squamous cell carcinoma. In: (ed.^(eds. Barnes, L, Eveson, JW, Reichart, P and Sidransky, D). World Health Organization classification of tumours: pathology and genetics of head and neck tumours. Lyon IARC Press, 2005; 168-75.
3. Carinci, F, Lo Muzio, L, Piattelli, A, Rubini, C, Palmieri, A, Stabellini, G, Maiorano, E, Pastore, A, Laino, G, Scapoli, L, Martinelli, M and Pezzetti, F. Genetic portrait of mild and severe lingual dysplasia. Oral Oncol 2005; 41(4):365-74.
4. Castellsague, X, Quintana, MJ, Martinez, MC, Nieto, A, Sanchez, MJ, Juan, A, Monner, A, Carrera, M, Agudo, A, Quer, M, Munoz, N, Herrero, R, Franceschi, S and Bosch, FX. The role of type of tobacco and type of alcoholic beverage in oral carcinogenesis. Int J Cancer 2004; 108(5):741-9.
5. Sanchez, MJ, Martinez, C, Nieto, A, Castellsague, X, Quintana, MJ, Bosch, FX, Munoz, N, Herrero, R and Franceschi, S. Oral and oropharyngeal cancer in Spain: influence of dietary patterns. Eur J Cancer Prev 2003; 12(1):49-56.
6. Talamini, R, Vaccarella, S, Barbone, F, Tavani, A, La Vecchia, C, Herrero, R, Munoz, N and Franceschi, S. Oral hygiene, dentition, sexual habits and risk of oral cancer. Br J Cancer 2000; 83(9):1238-42.
7. Lucchese, A. Viruses and Oral Cancer: Crossreactivity as a Potential Link. Anticancer Agents Med Chem

2015; 15(10):1224-9.
8. Bosch, FX, Lorincz, A, Mun ˜oz, N, Meijer, CJ and Shah, KV. The causal relation between HPV and cervical cancer. J Clin Pathol 2002; 55:244–65.
9. Campisi, G and Giovannelli, L. Controversies surrounding human papilloma virus infection, head and neck vs oral cancer, implications for prophylaxis and treatment. Head Neck Oncol 2009; 1:8.
10. Mork, J, Lie, AK, Glattre, E, Hallmans, G, Jellum, E, Koskela, P, Moller, B, Pukkala, E, Schiller, JT, Youngman, L, Lehtinen, M and Dillner, J. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. N Engl J Med 2001; 344(15):1125-31.
11. Herrero, R, Castellsague, X, Pawlita, M, et al. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. J Natl Cancer Inst 2003; 95(23):1772-83.
12. Scapoli, L, Palmieri, A, Rubini, C, Martinelli, M, Spinelli, G, Ionna, F and Carinci, F. Low prevalence of human papillomavirus in squamous-cell carcinoma limited to oral cavity proper. Mod Pathol 2009; 22(3):366-72.
13. Palmieri, A, Scapoli, L, Martinelli, M, Pezzetti, F, Girardi, A, Spinelli, G, Lucchese, A and Carinci, F. Incidence of low risk human papillomavirus in oral cancer: a real time PCR study on 278 patients. Int J Immunopathol Pharmacol 2011; 24(2S):83-7.
14. Pannone, G, Santoro, A, Carinci, F, et al. Double demonstration of oncogenic high risk human papilloma virus DNA and HPV-E7 protein in oral cancers. Int J Immunopathol Pharmacol 2011; 24(2S):95-101.
15. Santoro, A, Pannone, G, Ninivaggi, R, et al. Relationship between CK19 expression, deregulation of normal keratinocyte differentiation pattern and high risk-human papilloma virus infection in oral and oropharyngeal squamous cell carcinoma. Infect Agent Cancer 2015; 10:46.
16. Carinci, F, Pelucchi, S, Farina, A and Calearo, C. Changing the staging of oral cancer. J Oral Maxillofac Surg 1999; 57(3):356.
17. Carinci, F, Curioni, C, Padula, E and Calearo, C. Cancer of the nasal cavity and paranasal sinuses: a new staging system. Int J Oral Maxillofac Surg 1996; 25(1):34-9.
18. Carinci, F, Farina, A, Padula, E and Calearo, C. Primary malignancies of the nasal fossa and paranasal sinuses: comparison between UICC classification and a new staging system. J Craniofac Surg 1997; 8(5):405-12.
19. Carinci, F, Pelucchi, S, Farina, A and Calearo, C. A comparison between TNM and TANIS stage grouping for predicting prognosis of oral and oropharyngeal cancer. J Oral Maxillofac Surg 1998; 56(7):832-6; discussion 36-7.
20. Carinci, F, Farina, A, Pelucchi, S, Brunelli, G, Pastore, A and Calearo, C. Stage grouping of oropharyngeal cancer: evaluation of three systems by means of survival analysis. J Craniofac Surg 1999; 10(1):73-8.
21. Carinci, F, Farina, A, Bovicelli, A, Pelucchi, S and Calearo, C. Disease-specific survival for a new t-stage of oropharyngeal cancer. Br J Oral Maxillofac Surg 2000; 38(4):402-4.
22. Edge, SB, Byrd, DR, Compton, CC, Fritz, AG, Greene, FL and Trotti, A. AJCC Cancer Staging Manual (7th Ed.)Place: Springer 2009.
23. Carinci, F, Pelucchi, S, Farina, A, De Franciscis, G and Calearo, C. Extension as a prognostic factor in oropharyngeal cancer: largest mucosal dimension compared with number of (sub)sites involved. Br J Oral Maxillofac Surg 1998; 36(6):440-5.
24. Kumar, T and Patel, MD. Pattern of lymphatic metastasis in relation to the depth of tumor in oral tongue cancers: a clinico pathological correlation. Indian J Otolaryngol Head Neck Surg 2013; 65(1):59-63.
25. Ota, Y, Aoki, T, Karakida, K, Otsuru, M, Kurabayashi, H, Sasaki, M, Nakamura, N and Kajiwara, H. Determination of deep surgical margin based on anatomical architecture for local control of squamous cell carcinoma of the buccal mucosa. Oral Oncol 2009; 45(7):605-9.
26. Suzuki, H, Fukuyama, R, Hasegawa, Y, Tamaki, T, Nishio, M, Nakashima, T and Tatematsu, M. Tumor thickness, depth of invasion, and Bcl-2 expression are correlated with FDG-uptake in oral squamous cell carcinomas. Oral Oncol 2009; 45(10):891-7.
27. Carinci, F, Pelucchi, S, Farina, A, Bonsetti, G, Mastrandrea, M and Calearo, C. Site-dependent survival in cancer of the oral cavity. J Craniofac Surg 1997; 8(5):399-403; discussion 04.
28. Kapoor, C, Vaidya, S, Wadhwan, V and Malik, S.

Lymph node metastasis: A bearing on prognosis in squamous cell carcinoma. Indian J Cancer 2015; 52(3):417-24.
29. Siegel, R, Naishadham, D and Jemal, A. Cancer statistics, 2012. CA Cancer J Clin 2012; 62(1):10-29.
30. Neville, BW and Day, TA. Oral cancer and precancerous lesions. CA Cancer J Clin 2002; 52(4):195-215.
31. Parkin, DM, Bray, F, Ferlay, J and Pisani, P. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55(2):74-108.
32. Bogenrieder, T and Herlyn, M. Axis of evil: molecular mechanisms of cancer metastasis. Oncogene 2003; 22(42):6524-36.
33. Alkureishi, LW, Burak, Z, Alvarez, JA, et al. Joint practice guidelines for radionuclide lymphoscintigraphy for sentinel node localization in oral/oropharyngeal squamous cell carcinoma. Ann Surg Oncol 2009; 16(11):3190-210.
34. Wenzel, S, Sagowski, C, Kehrl, W and Metternich, FU. The prognostic impact of metastatic pattern of lymph nodes in patients with oral and oropharyngeal squamous cell carcinomas. Eur Arch Otorhinolaryngol 2004; 261(5):270-5.
35. Woolgar, JA, Scott, J, Vaughan, ED, Brown, JS, West, CR and Rogers, S. Survival, metastasis and recurrence of oral cancer in relation to pathological features. Ann R Coll Surg Engl 1995; 77(5):325-31.
36. Amar, A, Chedid, HM, Rapoport, A, Cernea, CR, Dedivitis, RA, Curioni, OA and Brandao, LG. Prognostic significance of the number of lymph nodes in elective neck dissection for tongue and mouth floor cancers. Braz J Otorhinolaryngol 2012; 78(2):22-6.
37. Pathak, KA, Gupta, S, Talole, S, Khanna, V, Chaturvedi, P, Deshpande, MS, Pai, PS, Chaukar, DA and D'Cruz, AK. Advanced squamous cell carcinoma of lower gingivobuccal complex: patterns of spread and failure. Head Neck 2005; 27(7):597-602.
38. Vishak, S, Rangarajan, B and Kekatpure, VD. Neoadjuvant chemotherapy in oral cancers: Selecting the right patients. Indian J Med Paediatr Oncol 2015; 36(3):148-53.
39. Carinci, F, Cassano, L, Farina, A, Pelucchi, S, Calearo, C, Modugno, V, Nielsen, I, Api, P and Pastore, A. Unresectable primary tumor of head and neck: does neck dissection combined with chemoradiotherapy improve survival? J Craniofac Surg 2001; 12(5):438-43.
40. Carinci, F, Arcelli, D, Lo Muzio, L, et al. Molecular classification of nodal metastasis in primary larynx squamous cell carcinoma. Transl Res 2007; 150(4):233-45.
41. Woolgar, JA and Triantafyllou, A. Pitfalls and procedures in the histopathological diagnosis of oral and oropharyngeal squamous cell carcinoma and a review of the role of pathology in prognosis. Oral Oncol 2009; 45(4-5):361-85.
42. Civantos, FJ, Stoeckli, SJ, Takes, RP, Woolgar, JA, de Bree, R, Paleri, V, Devaney, KO, Rinaldo, A, Silver, CE, Mondin, V, Werner, JA and Ferlito, A. What is the role of sentinel lymph node biopsy in the management of oral cancer in 2010? Eur Arch Otorhinolaryngol 2010; 267(6):839-44.
43. Contaldo, M, Di Napoli, A, Pannone, G, et al. Prognostic implications of node metastatic features in OSCC: a retrospective study on 121 neck dissections. Oncol Rep 2013; 30(6):2697-704.
44. Campisi, G, Calvino, F, Carinci, F, et al. Peri-tumoral inflammatory cell infiltration in OSCC: a reliable marker of local recurrence and prognosis? An investigation using artificial neural networks. Int J Immunopathol Pharmacol 2011; 24(2S):113-20.
45. Nespoli, A, Gianotti, L, Totis, M, Bovo, G, Nespoli, L, Chiodini, P and Brivio, F. Correlation between postoperative infections and long-term survival after colorectal resection for cancer. Tumori 2004; 90:485-90.
46. Allavena, P, Sica, A, Solinas, G, Porta, C and Mantovani, A. The inflammatory micro-environment in tumor progression: the role of tumor-associated macrophages. Crit Rev Oncol Hematol 2008; 66(1):1-9.
47. Somma, P, Lo Muzio, L, Mansueto, G, et al. Squamous cell carcinoma of the lower lip: FAS/FASL expression, lymphocyte subtypes and outcome. Int J Immunopathol Pharmacol 2005; 18(1):59-64.
48. Hu, Y, He, MY, Zhu, LF, Y, et al. Tumor-associated macrophages correlate with the clinicopathological features and poor outcomes via inducing epithelial to mesenchymal transition in oral squamous cell carcinoma. J Exp Clin Cancer Res 2016; 35(1):12.
49. Christopher, V, Murthy, S, Sr, A, Singh, S, Cp, A, Shivaram, SK and Neethupriya. Morphometry As a

Diagnostic Tool for Potentially Malignant Lesions. J Clin Diagn Res 2015; 9(12):ZC22-5.

50. Lo Muzio, L, Falaschini, S, Farina, A, et al. Bcl-2 as prognostic factor in head and neck squamous cell carcinoma. Oncol Res 2005; 15(5):249-55.
51. Carinci, F and Carls, PF. Prognostic value of histological and biological markers in pharyngeal squamous cell carcinoma: a case-control study. Br J Cancer 2000; 82(9):1613-4.
52. Lo Muzio, L, Campisi, G, Farina, A, et al. Effect of p63 expression on survival in oral squamous cell carcinoma. Cancer Invest 2007; 25(6):464-9.
53. Tosi, L, Rinaldi, E, Carinci, F, et al. Akt, protein kinase C, and mitogen-activated protein kinase phosphorylation status in head and neck squamous cell carcinoma. Head Neck 2005; 27(2):130-7.
54. Chaisuparat, R, Limpiwatana, S, Kongpanitkul, S, Yodsanga, S and Jham, BC. The Akt/mTOR pathway is activated in verrucous carcinoma of the oral cavity. J Oral Pathol Med 2016.
55. Colella, G, Izzo, G, Carinci, F, et al. Expression of sexual hormones receptors in oral squamous cell carcinoma. Int J Immunopathol Pharmacol 2011; 24(2S):129-32.
56. Doll, C, Arsenic, R, Lage, H, Jöhrens, K, Hartwig, S, Nelson, K and Raguse JD. Expression of Estrogen Receptors in OSCC in Relation to Histopathological Grade. Anticancer Res. 2015;35(11:5867-72.
57. Lo Muzio, L, Campisi, G, Farina, A, Rubini, C, Pannone, G, Serpico, R, Laino, G, De Lillo, A and Carinci, F. P-cadherin expression and survival rate in oral squamous cell carcinoma: an immunohistochemical study. BMC Cancer 2005; 5:63.
58. Carinci, F, Stabellini, G, Calvitti, M, Pelucchi, S, Targa, L, Farina, A, Pezzetti, F and Pastore, A. CD44 as prognostic factor in oral and oropharyngeal squamous cell carcinoma. J Craniofac Surg 2002; 13(1):85-9.
59. Nogami, T, Kuyama, K and Yamamoto, H. Histopathological and immunohistochemical study of malignant transformation of oral leukoplakia, with special reference to apoptosis-related gene products and proliferative activity. Acta Otolaryngol 2003; 123(6):767-75.
60. Chen, C, Zhang, Y, Loomis, MM, et al. Genome-Wide Loss of Heterozygosity and DNA Copy Number Aberration in HPV-Negative Oral Squamous Cell Carcinoma and Their Associations with Disease-Specific Survival. PLoS One 2015; 10(8):e0135074.
61. El-Deftar, MF, El Gerzawi, SM, Abdel-Azim, AA and Tohamy, SM. Prognostic significance of ploidy and S-phase fraction in primary intraoral squamous cell carcinoma and their corresponding metastatic lymph nodes. J Egypt Natl Canc Inst 2012; 24(1):7-14.
62. Siebers, TJ, Bergshoeff, VE, Otte-Holler, I, Kremer, B, Speel, EJ, van der Laak, JA, Merkx, MA and Slootweg, PJ. Chromosome instability predicts the progression of premalignant oral lesions. Oral Oncol 2013; 49(12):1121-8.
63. Scapoli, L, Girardi, A, Rubini, C, Martinelli, M, Spinelli, G, Palmieri, A, Lo Muzio, L and Carinci, F. LOH at PDCD4, CTNNB1, and CASP4 loci contributes to stage progression of oral cancer. Int J Immunopathol Pharmacol 2011; 24(2S):89-93.
64. Zhang, L, Poh, CF, Williams, M, Laronde, DM, Berean, K, Gardner, PJ, Jiang, H, Wu, L, Lee, JJ and Rosin, MP. Loss of heterozygosity (LOH) profiles--validated risk predictors for progression to oral cancer. Cancer Prev Res (Phila) 2012; 5(9):1081-9.
65. Cavenee, WK. Genetic driver events in premalignancy: LOH validated for marking the risk of oral cancer. Cancer Prev Res (Phila) 2012; 5(9):1073-4.
66. Poh, CF, Zhang, L, Anderson, DW, et al. Fluorescence visualization detection of field alterations in tumor margins of oral cancer patients. Clin Cancer Res 2006; 12(22):6716-22.
67. Petruzzi, M, Lucchese, A, Nardi, GM, Lauritano, D, Favia, G, Serpico, R and Grassi, FR. Evaluation of autofluorescence and toluidine blue in the differentiation of oral dysplastic and neoplastic lesions from non dysplastic and neoplastic lesions: a cross-sectional study. J Biomed Opt 2014; 19(7):76003.
68. Paderni, C, Compilato, D, Carinci, F, et al. Direct visualization of oral-cavity tissue fluorescence as novel aid for early oral cancer diagnosis and potentially malignant disorders monitoring. Int J Immunopathol Pharmacol 2011; 24(2S):121-8.
69. Contaldo, M, Poh, CF, Guillaud, M, et al. Oral mucosa optical biopsy by a novel handheld fluorescent confocal microscope specifically developed:

technologic improvements and future prospects. Oral Surg Oral Med Oral Pathol Oral Radiol 2013; 116(6):752-8.

70. Contaldo, M, Agozzino, M, Moscarella, E, Esposito, S, Serpico, R and Ardigo, M. *In vivo* characterization of healthy oral mucosa by reflectance confocal microscopy: a translational research for optical biopsy. Ultrastruct Pathol 2013; 37(2):151-8.
71. Carinci, F, Girardi, A, Palmieri, A, Martinelli, M, Scapoli, L, Avantaggiato, A, Nardi, GM and Lauritano, D. Lab-test 1:peri-implantitis and bacteriological analysis. European Journal of Inflammation 2012; 10(1S2):91-93.
72. Lauritano, D, Cura, F, Gaudio, RM, Pezzetti, F, Andreasi Bassi, M and Carinci, F. Polymerase Chain Reaction to Evaluate the Efficacy of Silica Dioxide Colloidal Solutions in the Treatment of Chronic Periodontitis: A Case Control Study. J Biol Regul Homeost Agents 2015; 29(3S1):131-5.
73. Lauritano, D, Cura, F, Candotto, V, Gaudio, RM, Mucchi, D and Carinci, F. Evaluation of the Efficacy of Titanium Dioxide with Monovalent Silver Ions Covalently Linked (Tiab) as an Adjunct to Scaling and Root Planing in the Management of Chronic Periodontitis Using Pcr Analysis: A Microbiological Study. J Biol Regul Homeost Agents 2015; 29(3S1):127-30.
74. Lauritano, D, Cura, F, Candotto, V, Gaudio, RM, Mucchi, D and Carinci, F. Periodontal Pockets as a Reservoir of Helicobacter Pylori Causing Relapse of Gastric Ulcer: A Review of the Literature. J Biol Regul Homeost Agents 2015; 29(3S1):123-6.
75. Scapoli, L, Girardi, A, Palmieri, A, Martinelli, M, Cura, F, Lauritano, D, Pezzetti, F and Carinci, F. Interleukin-6 Gene Polymorphism Modulates the Risk of Periodontal Diseases. J Biol Regul Homeost Agents 2015; 29(3S1):111-6.
76. Scapoli, L, Girardi, A, Palmieri, A, Martinelli, M, Cura, F, Lauritano, D and Carinci, F. Quantitative Analysis of Periodontal Pathogens in Periodontitis and Gingivitis. J Biol Regul Homeost Agents 2015; 29(3S1):101-10.
77. Lauritano, D, Petruzzi, M, Nardi, GM, Carinci, F, Minervini, G, Di Stasio, D and Lucchese, A. Single Application of a Dessicating Agent in the Treatment of Recurrent Aphthous Stomatitis. J Biol Regul Homeost Agents 2015; 29(3S1):59-66.
78. Carinci, F, Scapoli, L, Girardi, A, Cura, F, Lauritano, D, Nardi, GM and Palmieri, A. Oral microflora and periodontal disease: new technology for diagnosis in dentistry. Ann Stomatol (Roma) 2013; 4(2):170-3.
79. Scapoli, L, Girardi, A, Palmieri, A, Testori, T, Zuffetti, F, Monguzzi, R, Lauritano, D and Carinci, F. Microflora and periodontal disease. Dent Res J (Isfahan) 2012; 9(S2):S202-6.
80. Scapoli, L, Girardi, A, Palmieri, A, Carinci, F, Testori, T, Zuffetti, F, Monguzzi, R and Lauritano, D. IL6 and IL10 are genetic susceptibility factors of periodontal disease. Dent Res J (Isfahan) 2012; 9(S2):S197-201.