

Tumor-associated macrophages: Harbingers of aggressiveness in oral squamous cell carcinoma

Anupama Mukherjee, Anita Spadigam, Anita Dhupar

Department of Oral and Maxillofacial Pathology, Goa Dental College and Hospital, Panaji, Goa, India

Abstract

Background: The significance of the tumor microenvironment (TME) as a modulator of tumor behavior was acknowledged by Hanahan and Weinberg in 2011 as the emerging hallmarks and enabling characteristics of the hallmarks of cancer. Subsequently, the role of inflammation, in conferring aggressiveness to a tumor, was regarded as a fundamental process in the evolution of the TME. Tumor-associated macrophages (TAMs) are distinctly polarized inflammatory cells and key shapers of a protumorigenic microenvironment.

Aims: The aim of the study was to evaluate the distribution of TAMs and the expression of CD-163 as a marker to evince tumor aggressiveness, in oral squamous cell carcinoma (OSCC).

Settings and Design: A retrospective institutional study was approved by the institutional ethics committee, and random sampling was carried out. Cases fulfilling the inclusion criteria were subjected to S(site), T(tumor), N(node), M(metastasis), P(pathology) STNMP staging along with immunohistochemical evaluation of CD-163.

Methods: Samples for this study included 58 archival cases of OSCC. Demographic details were recorded, and the STNMP stage ascertained, following which, each case was reevaluated histopathologically for the invasive front. Cases with sufficient stroma and demonstrating the invasive front were further subjected to immunohistochemical evaluation of CD-163 for TAMs. The density of CD-163-positive cells was assessed by three pathologists, independently, in a double-blinded evaluation using Image-J®.

Statistical Analysis: Descriptive data were evaluated and analyzed statistically using Spearman's/Pearson's correlation tests based on the distribution of data.

Results: The density of TAMs was noted to be directly proportional to the STNMP stage. In addition, a strong positive, statistically significant correlation was noted between the density of TAMs and tumor size, nodal status and STNMP stage.

Conclusion: The crucial role of the tumor microenvironment must be considered when evaluating OSCC. TAMs prove to be a reliable marker for assessing tumor aggressiveness and could aid in improved prognostication of OSCC, while also being potential targets for therapy.

Keywords: Oral squamous cell carcinoma, tumor aggressiveness, tumor-associated macrophages, tumor microenvironment

Address for correspondence: Dr. Anupama Mukherjee, F-1, Oasis, Street 1, Nagali Hills Colony, Dona Paula, Panaji - 403 004, Goa, India.

E-mail: dranupamamukherjee@hotmail.com

Submitted: 17-Jul-2020, **Accepted:** 28-Oct-2020, **Published:** 14-May-2021

INTRODUCTION

Cancer continues to be a global disease and is

currently the second leading cause of death.^[1] Annually approximately 300,000 cases of oral cancer are reported

Access this article online	
Quick Response Code:	Website: www.jomfp.in
	DOI: 10.4103/jomfp.JOMFP_302_20

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Mukherjee A, Spadigam A, Dhupar A. Tumor-associated macrophages: Harbingers of aggressiveness in oral squamous cell carcinoma. *J Oral Maxillofac Pathol* 2021;25:46-50.

globally, of which India accounts for nearly 10.4% of these cases.^[2] Oral squamous cell carcinoma (OSCC) ranks as one of the top three cancers in India.^[3]

While the genesis and evolution of cancer is attributed to multiple, variable factors, the several dispositions of the histopathological features are broadly clustered into grades of differentiation and harnessed with clinical staging for the purpose of standardizing therapeutic approaches.^[4,5]

While tumor grading and staging have been the universally accepted harbingers for prognosis and therapy, tumor grading alone fails to be descriptive of the biological behavior of the tumor. It has been noted that cases within the same grade or stage often demonstrate a variable outcome.^[5,6]

This variability could probably be attributed to the several anatomical and tumoral factors, which though descriptive of tumor aggressiveness, are yet to be included in the routinely used grading and staging systems.^[7,8]

With cancer now being regarded as a “Rogue Organ,”^[9] the tumor microenvironment (TME) has become a significant entity in the “Hallmarks of Cancer.”^[10] The TME comprises numerous cells and stromal components that eventually impact tumor behavior and modulate host responses. The various cells that constitute the TME include cancer-associated fibroblasts, immune and inflammatory cells, endothelial cells, pericytes, adipocytes, mesenchymal stem cells, myocytes and associated muscle tissue along with neural cells and tissues.^[9,11] The immune cells which constitute “tumor-promoting inflammation” (TPI)^[12] play a pivotal role in establishing and maintaining a protumorigenic environment.^[9,13] Tumor-associated macrophages (TAMs) are one of the primary cell types constituting TPI. Under the influence of the tumor tissue, along with a milieu of cytokines, an alternate activation of infiltrating monocytes/resident macrophages occurs, thus resulting in the presence of M-2 polarized macrophages in the tumor-associated stroma.^[13,14]

Unlike their classical antitumorigenic counterparts, these Type-2 or M-2 macrophages demonstrate an anti-inflammatory response, participate in tissue remodeling along with tumor cell activation. The synthetic activity of M-2 macrophages alters tumor cell behavior and confers a protumorigenic role to these cells. As a net effect, M-2 macrophages facilitate evasion of host responses, increase angiogenesis, encourage lymphangiogenesis, potentiate therapeutic resistance and eventually promote tumor growth, invasion and metastasis.^[9,11,14-17] While experimental

data have established the role of TAMs in the tumor milieu, translation of these data to clinical applications remains unexplored.

METHODS

The aim of this study was to assess the aggressiveness of OSCC by evaluating and quantifying a key component of the TME, the TAMs. The study was approved by the institutional ethics committee, and sample size was determined *a priori*. Fifty-eight archival specimens of OSCC from the department of oral and maxillofacial pathology were re-evaluated histopathologically using hematoxylin- and eosin-stained sections for the invasive front of the tumor and to reconfirm the tumor grade. These cases of well, moderate and poorly differentiated OSCC were staged according to the STNMP staging system and subjected to immunohistochemical staining for CD-163, a marker specific for TAMs.^[11,18]

Four micro sections were floated on to annexed, positively charged slides. The slides were rested at room temperature for 10 min and placed on a hotplate at 60 degrees centigrade for 30 min. Sections were allowed to cool and rested overnight following which heat-induced epitope retrieval was carried out using the Dako PT Link (Model no. PT105311T).

Immunohistochemical staining was carried out using the One-Step Polymer Horseradish Peroxidase immunohistochemistry (IHC) Technique. The antibody used was a monoclonal mouse anti-CD163 concentrate ([MRQ-26]-Cell Marque Rocklin, California, USA). Validation and standardization of the antibody was carried out, following which an ideal incubation period of 30 min, at a dilution of 1:100 was determined. Human placental tissue was used as a positive control. 3,3'-diaminobenzidine was used for visualization of the reactivity, and the slides were counterstained with Harris' hematoxylin. To demonstrate the absence of M-2 macrophages in normal oral tissues and inflammatory lesions of the oral cavity, normal mucosa obtained from uninflamed third molar extraction cases and mucocoeles were also subjected to IHC staining.

Immunohistochemical staining was evaluated by assessing five standardized high power for each case, in a double-blinded evaluation by three observers. Image-J[®] analysis was used to tag and label positive cells. Based on the above evaluation, an average count was determined, and each case was assigned a final score of 1+, 2+, 3+ and 4+ based on the density of TAMs [Figure 1].

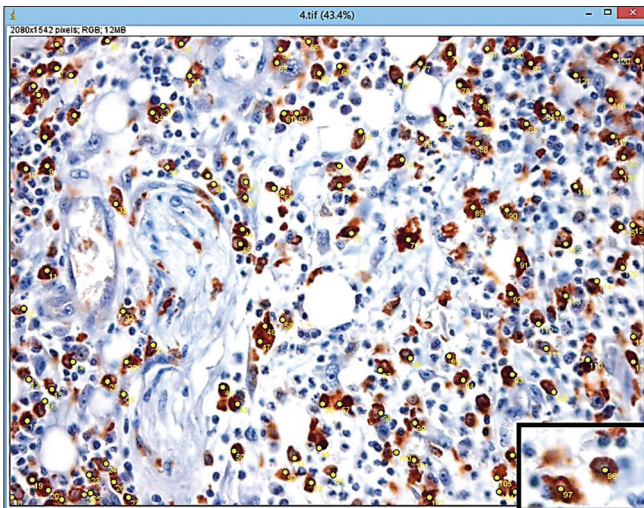


Figure 1: Photomicrograph showing the assessment of each standardized field using Image J. Evaluation was carried out in a zig-zag manner and included placing numbered yellow blebs onto the cells positive for CD-163 ($\times 100$) inset shows two labeled cells positive for CD-163

RESULTS

Appraisal of the clinical data revealed a distribution of cases between the age group of 21 years to 76 years with a mean age of 52.51 years. Maximum cases were reported in the age group of 41–50 years and 61–70. A distinct male predominance was noted, and the most common site to be involved was the tongue (53.44%), followed by buccal mucosa (36.20%), floor of mouth (8.62%) and alveolus (1.72%).

In the present study, cells demonstrating a membranous and/or cytoplasmic positivity for CD-163 were identified as TAMs. As seen in Figure 2, neither the normal oral mucosa nor mucocele demonstrated the presence of CD-163-positive cells, suggestive of the absence of these cells in the stroma associated with normal oral epithelium and inflammatory lesions of the oral cavity.

Samples of OSCC, subjected to CD-163 staining, were evaluated and scored as 1+, 2+, 3+ and 4+ based on the density of TAMs.

TAMs showed both intratumoral and stromal distribution, with cells predominantly distributed at the tumor invasive front and perivascular regions. A distinct trend was noted in the distribution of cells across the STNMP stages [Figure 3]. An increase in the STNMP stage was associated with an increase in the density of CD-163(+) cells, while a larger number of cases, in the lower stages, demonstrated fewer CD-163(+) cells. Using the Spearman-Rho correlation test, with a P value of 0.005 assigned statistical significance, we

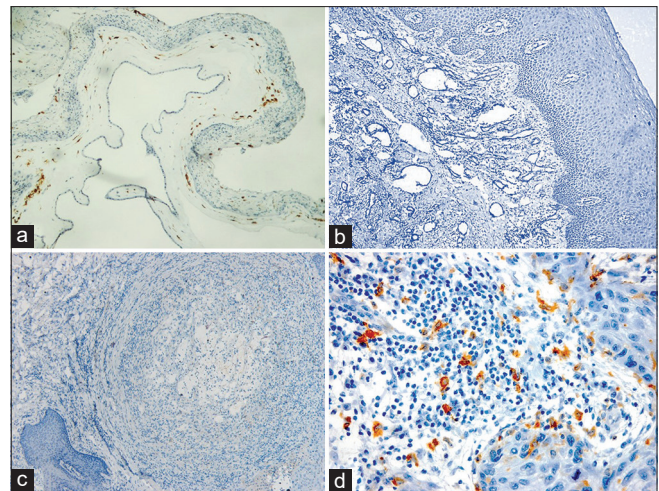


Figure 2: Photomicrograph of immunohistochemistry stained sections showing (a) placental tissue (positive control) ($\times 40$) (b) normal mucosa showing no expression of CD-163 (scored as 0) ($\times 40$) (c) mucocele showing no expression of CD-163 (scored as 0) ($\times 40$) (d) CD-163 positive tumor-associated macrophages in the stroma of oral squamous cell carcinoma ($\times 100$)

further observed a significant strong positive correlation between the expression of CD-163 and tumor size, the nodal status and the STNMP stage respectively.

DISCUSSION

Decades of research have focused on understanding the mechanisms of malignant transformation of cells and categorizing disease characteristics with the hope of developing modalities to prevent or halt initiation, promotion and/or progression of tumors. Limited success has been achieved for OSCC, as is reflected in its variable and poor prognosis, along with a high recurrence rate (35%).^[19]

Evaluation and assessment of tumors of the oral cavity based on site-specificity is warranted due to the regional variations in mucosal thickness, the content of submucosa and presence or absence of mucoperiosteum. These factors may influence the interaction of tumor cells with lymphatics and vessels, thus impacting interpretation of tumor aggressiveness, staging and prognostication.^[8] Therefore, the STNMP staging system accounts for the variability of individual sites, thus providing a holistic evaluation of OSCC.^[20]

In an attempt to improve prognostication of OSCC, various indicators such as site and size of the tumor, grade of OSCC and the nodal status have been proposed.^[21] Microenvironmental characteristics such as tumor-associated inflammation are accounted for in proposed grading systems but are yet to feature as definitive prognostic markers.^[7,22]

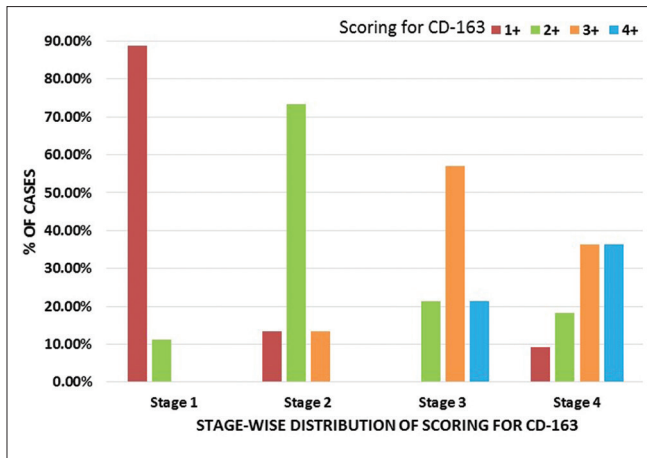


Figure 3: A positive correlation was noted between the STNMP stage and density of tumor-associated macrophages. Most cases in Stage III and IV demonstrated a higher density of CD-163 positive cells (scoring 3+ and 4+). Cases in Stage I and II showed a decreased density of CD-163 positive (scoring 1+ and 2+, respectively). Thus, a distinct trend is evident since cases with a higher STNMP stage demonstrated a higher density of tumor-associated macrophages suggestive of an aggressive phenotype. Few cases in each stage demonstrate a variation from this trend, highlighting individual variability of each tumor, host and microenvironment

TAMs are induced in response to various signals such as interleukin (IL)-4, IL-10 and IL-13, along with a proposed disruption of the Notch signaling pathway.^[23,24] These cells perform immunosuppressive functions, stimulate angiogenesis and enhance tumor cell invasion. CD-163 is an effective marker to distinguish TAMs (M-2 phenotype) from the M-1 phenotype of macrophages, since both populations have distinct roles in eliciting an anti-inflammatory or a pro-inflammatory response, respectively.^[11,18]

As reported by Lúcio *et al.*, an increase in TAMs was noted with an increase in tumor size.^[25]

This could be explained by the known association of tumor diameter to concomitant nodal metastases, local recurrence and poor survival.^[8,20] Authors have regarded the interaction of tumor cells and macrophages as one of the mechanisms for the polarization of the latter into TAMs, thus fortifying the association of tumor bulk and density of TAMs.^[16]

The increase in CD-163 positive cells in patients with a higher N-status could reflect the establishment of an increasingly tumor-promoting microenvironment, facilitating lymph node metastasis.^[11,26]

Findings similar to our study have been reported by Weber and Wang, wherein an increase in TAM counts was reported in cases with nodal metastasis.^[11,27,28] This can be interpreted as the establishment of a highly tumor-promoting microenvironment, in such patients,

leading to lymph node metastasis. These CD-163(+) protumorigenic macrophages (M-2) have been regarded as crucial accomplices that validate the “seed and soil” theory of metastasis.^[26,29]

A prominent finding noted in the present study was the lack of correlation between the density of TAMs and the grade of OSCC. Similar observations have been reported by Kumar *et al.*, Weber *et al.* and Fujita *et al.*^[26,27,30,31]

The grade of OSCC is based largely on the degree of differentiation of oral keratinocytes and is often regarded as the basis of prognosis. The grade however does not translate into the biological behavior of the tumor, since the tumor-host relationship and TME remain unaccounted for. Similar findings were noted in the present study with a weak positive correlation between the grade of OSCC and density of TAMs, which was not statistically significant.

The statistically significant strong positive correlation of M-2 macrophages with the overall STNMP stage fortifies the need for a holistic staging system that accounts for the numerous variables in the oral cavity.

The above findings corroborate the proposition of the TME being a determinant of tumor aggressiveness along with the role of the TPI and particularly the TAMs, in conferring a malignant phenotype to the tumor.

CONCLUSION

Immune cells infiltrating tumor tissues show functional plasticity and may adopt an antitumoral or protumoral activity. Macrophages have been regarded as a double-edged sword, with the potential to express both pro- and anti-tumor activity (the macrophage balance), which varies depending on the M-2 and M1-macrophage populations. The former population prevails in established neoplasia. TAMs have been prominently recognized to be associated with the development and progression of OSCC.

Since the evaluation of the nodal status has been regarded as the single most accurate predictor of prognosis, the correlation of CD-163 with the nodal status, as observed in this study, highlights its potential role as a probable prognostic indicator and warrants further investigation and validation.

It has also emerged that solely assessing the grade or degree of differentiation of a tumor may not provide a complete understanding of its aggressiveness. Since tumor grade is not an indicator of biological behavior, the inclusion of stromal factors may address the variable outcomes of

similarly graded cases. The presence and density of these cells could be used as an indicator to identify significantly aggressive cases.

TAMs are now being recognized as possible targets for therapy though there is limited research regarding the same for OSCC. The inhibition of TAMs through targeting cytokines and tumor progression pathways associated in the recruitment of these cells, along with targeting the tumor cell-TAM malignancy cycle, may allow for improved therapeutic approaches.^[32]

Financial support and sponsorship

This study was financially supported by Goa Cancer Society, Goa

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.
- India Globocan 2018, WHO IARC India cancer fact sheet 2018. The Global Cancer Observatory, <https://gco.iarc.fr/today/data/factsheets/populations/356-india-fact-sheets.pdf>.
- Abdulla R, Adyanthaya S, Kini P, Mohanty V, Souza ND. Clinicopathological analysis of oral squamous cell carcinoma among the younger age group in coastal Karnataka, India: A retrospective study. *J Oral Maxillofac Pathol* 2018;22:180-7.
- Koch W M, Ridge JA, Forastiere A, Manola J. Comparison of clinical and pathological staging in head and neck squamous cell carcinoma: Results from intergroup study ECOG 4393/RTOG 9614. *Archives of Otolaryngology - Head and Neck Surgery*, 135:851-8. <https://doi.org/10.1001/archoto.2009.123>. [Last accessed on 2020 Nov 26].
- Doshi NP, Shah S A, Patel K B, Jhabuawala M F, Histological grading of oral cancer: A comparison of different systems and their relation to lymph node metastasis. *Natl J Community Med* 2011;2:136-42.
- Hadler-Olsen E, Wirsing AM. Tissue-infiltrating immune cells as prognostic markers in oral squamous cell carcinoma: A systematic review and meta-analysis. *Br J Cancer* 2019;120:714-27.
- Yazdi I, Khalili M. Grading of oral cancer: Comparison of different systems with respect to lymph node metastasis in tongue squamous cell carcinoma. *Arch Iran Med* 1999;2:87-92.
- Kim S, Smith B, Haffty B. Chapter 2 - Prognostic factors in patients with head and neck cancer. *Harrisson*. 2009;960.
- Balkwill FR, Capasso M, Hagemann T. The tumor microenvironment at a glance. *J Cell Sci* 2012;125:5591-6.
- Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. *Cell* 2011;144:646-74. doi: 10.1016/j.cell.2011.02.013. PMID: 21376230.
- Yamagata Y, Tomioka H, Sakamoto K, Sato K, Harada H, Ikeda T, *et al*. CD163-positive macrophages within the tumor stroma are associated with lymphangiogenesis and lymph node metastasis in oral squamous cell carcinoma. *J Oral Maxillofac Surg* 2017;75:2144-53.
- Bondar T, Medzhitov R. The origins of tumor-promoting inflammation. *Cancer Cell* 2013;24:143-4.
- Chanmee T, Ontong P, Konno K, Itano N. Tumor-associated macrophages as major players in the tumor microenvironment. *Cancers (Basel)* 2014;6:1670-90.
- Quatromoni JG, Eruslanov E. Tumor-associated macrophages: Function, phenotype, and link to prognosis in human lung cancer. *Am J Transl Res* 2012;4:376-89.
- Mori K, Hiroi M, Shimada J, Ohmori Y. Infiltration of m2 tumor-associated macrophages in oral squamous cell carcinoma correlates with tumor malignancy. *Cancers (Basel)* 2011;3:3726-39.
- Hu Y, He MY, Zhu LF, Yang CC, Zhou ML, Wang Q, *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;35:1-19.
- Franklin RA, Liao W, Sarkar A, Kim MV, Bivona MR, Liu K, *et al*. The cellular and molecular origin of tumor-associated macrophages. *Science*. 2014 May 23;344:921-5. doi: 10.1126/science.1252510. Epub 2014 May 8. PMID: 24812208; PMCID: PMC4204732.
- Lau SK, Chu PG, Weiss LM. CD-163. *Am J Clin Pathol* 2004;122:794-801.
- Thavarool SB, Muttath G, Nayanar S, Duraisamy K, Bhat P, Shringarpure K, *et al*. Improved survival among oral cancer patients: Findings from a retrospective study at a tertiary care cancer centre in rural Kerala, India. *World J Surg Oncol* 2019;17:1-7.
- Rapidis A, Harvey PW. STNMP A new classification for oral cancer. 2016;(AUGUST 1977).
- Jadhav KB, Gupta N. Clinicopathological prognostic implicators of oral squamous cell carcinoma: Need to understand and revise. *N Am J Med Sci* 2013;5:671-9.
- Anneroth G, Hansen LS. A methodologic study of histologic classification and grading of malignancy in oral squamous cell carcinoma. *Eur J Oral Sci* 1984;92:448-68.
- Röszer T. Understanding the mysterious M2 macrophage through activation markers and effector mechanisms. *Mediators Inflamm* 2015;2015:16-8.
- Wang YC, He F, Feng F, Liu XW, Dong GY, Qin HY, *et al*. Notch signaling determines the M1 versus M2 polarization of macrophages in antitumor immune responses. *Cancer Res* 2010;70:4840-9.
- Lúcio PS, Ribeiro DC, Aguiar MC, Alves PM, Nonaka CF, Godoy GP. Tumor-associated macrophages (TAMs): Clinical-pathological parameters in squamous cell carcinomas of the lower lip. *Braz Oral Res* 2016;30:e95.
- Kumar AT, Knops A, Swendseid B, Martinez-Outschoom U, Harshyne L, Philp N, *et al*. Prognostic significance of tumor-associated macrophage content in head and neck squamous cell carcinoma: A meta-analysis. *Front Oncol* 2019;9:1-10.
- Weber M, Büttner-Herold M, Hyczel P, Moebius P, Distel L, Ries J, *et al*. Small oral squamous cell carcinomas with nodal lymphogenic metastasis show increased infiltration of M2 polarized macrophages--an immunohistochemical analysis. *J Craniomaxillofac Surg* 2014;42:1087-94. doi: 10.1016/j.jcms.2014.01.035. Epub 2014 Jan 21. PMID: 24556525.
- Wang S, Sun M, Gu C, Wang X, Chen D, Zhao E, *et al*. Expression of CD163, interleukin-10, and interferon-gamma in oral squamous cell carcinoma: Mutual relationships and prognostic implications. *Eur J Oral Sci* 2014;122:202-9.
- Bastida-Ruiz D, Van Hoesen K, Cohen M. The dark side of cell fusion. *Int J Mol Sci* 2016 Apr 28;17:638. doi: 10.3390/ijms17050638. PMID: 27136533; PMCID: PMC4881464.
- Weber M, Iliopoulos C, Moebius P, Büttner-Herold M, Amann K, Ries J, *et al*. Prognostic significance of macrophage polarization in early stage oral squamous cell carcinomas. *Oral Oncol* 2016;52:75-84.
- Fujita Y, Okamoto M, Goda H, Tano T, Nakashiro K, Sugita A, *et al*. Prognostic significance of interleukin-8 and CD163-positive cell-infiltration in tumor tissues in patients with oral squamous cell carcinoma. *PLoS One* 2014;9:1-17.
- Petruzzi MN, Cherubini K, Salum FG, de Figueiredo MA. Role of tumour-associated macrophages in oral squamous cells carcinoma progression: An update on current knowledge. *Diagn Pathol* 2017;12:1-7.