

# Estimation of Salivary Magnesium Levels in Patients with Oral Squamous Cell Carcinoma

## Abstract

India holds the highest rank in terms of incidence of oral cancer. Early detection is essential to reduce the mortality rate results in late cancer diagnosis. Recent studies emphasize micronutrients or trace elements as harbingers in oral squamous cell carcinoma (OSCC). These trace elements play a dual role in cancer cell proliferation and act as anti-cancer agents. Magnesium (Mg) forms an essential component of the micronutrient family of the body. It has many functions, including regulating the cell cycle and proliferation, metabolic activities, and other physiological functions. Impaired Mg homeostasis is associated with various pathological conditions. It has gained much importance in the past decade due to its complex relationship with cancer, as alterations in serum and salivary Mg have been reported in cancer patients. This study aimed to estimate salivary Mg levels in patients with OSCC and compare them with those of healthy individuals for Mg to serve as a diagnostic tool. A case-control study was performed on 36 subjects with 18 per group of cases, with OSCC and control being healthy individuals. Unstimulated saliva was collected from each individual and subjected to analysis of salivary magnesium using the xylydyl blue method. The mean salivary Mg levels in patients with OSCC and healthy individuals did not exhibit a statistically significant difference using the ANOVA test. The comparison of salivary Mg in patients with OSCC and healthy individuals did not provide sufficient evidence of Mg as an alleged diagnostic tool or a potential biomarker for OSCC.

**Keywords:** Biomarkers, Diagnosis, Micronutrients, Trace elements

## Introduction

Globally, India holds the highest rank in terms of incidence of oral cancer, with a higher prevalence in males.<sup>[1, 2]</sup> A delay in cancer diagnosis results in a high mortality rate. Hence early detection is essential to reduce the mortality rate.<sup>[3, 4]</sup> Consumption of alcohol and tobacco in smoking or non-smoking forms is the main contributor to oral cancer. Tobacco consists of many carcinogenic components, of which polycyclic hydrocarbons and nitrosamines are the key players. Consumption of alcohol is linked to a higher risk of oral cancer even in patients who are non-smokers.<sup>[5]</sup> Recent studies emphasize micronutrients or trace elements as harbingers in oral squamous cell carcinoma (OSCC). These trace elements play a dual role in cancer cell proliferation and act as anti-cancer agents, thus proving their versatile nature.<sup>[6]</sup> Many researchers have evaluated the correlation of trace elements like copper (Cu), zinc (Zn), calcium (Ca), iron (Fe), and magnesium (Mg) with cancer mortality.<sup>[7-10]</sup>

In living cells, the most abundant cation

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present is Mg. It acts as a secondary messenger controlling intracellular signaling, cell growth, and cell survival rate.<sup>[11]</sup> It performs various physiological functions, including cell-energy metabolism, activation of cell-cytoskeleton, maintaining cell membrane integrity, synthesis of proteins, antioxidation, and replication of DNA. Mg's physiological levels are required in DNA repair and maintenance of genomic stability concerning cancer.<sup>[12, 13]</sup> There is a complex relationship between magnesium levels in the body and cancer occurrence. It has been found that both excess and deficiency of magnesium can impact carcinogenesis.<sup>[14, 15]</sup> The potential role of Mg in cell energy metabolism is solely responsible for increasing cell proliferation. Thus, neoplastic cells show significant Mg influx, leading to cancer progression.<sup>[16]</sup>

Although histopathological examination remains the gold standard for diagnosing OSCC, recent studies focus on parallel investigations. The emphasis is solely made on the examination of certain salivary variables to determine the occurrence of

**Madhura Shekatkar<sup>1</sup>,  
Supriya Kheur<sup>1\*</sup>,  
Shantanu  
Deshpande<sup>2</sup>,  
Swapnali Sakhare<sup>3</sup>,  
Gauri Kumbhar<sup>1</sup>,  
Mohit Kheur<sup>4</sup>,  
Avinash Sanap<sup>3</sup>**

<sup>1</sup> Department of Oral Pathology and Microbiology, Dr. D. Y. Patil Dental College and Hospital, Dr. D. Y. Patil Vidyapeeth, Pimpri, Pune, India. <sup>2</sup> Department of Pediatric and Preventive Dentistry, Terna Dental College and Hospital, Navi Mumbai, India. <sup>3</sup> Regenerative Medicine Laboratory, Dr. D. Y. Patil Dental College and Hospital, Pimpri, Dr. D. Y. Patil Vidyapeeth, Pimpri, Pune, India. <sup>4</sup> Department of Prosthodontics, M.A. Rangoonwala College of Dental Sciences and Research Centre, Pune, India.

### Address for correspondence:

Supriya Kheur,  
Department of Oral Pathology and Microbiology,  
Dr. D. Y. Patil Dental College and Hospital,  
Pimpri, Pune, India.  
E-mail: supriya.kheur@dpu.edu.in

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OSCC. However, the results are sometimes the least likely.<sup>[13]</sup> Saliva is a substantial fluid that is important in the human body. It is required to maintain the integrity of oral structures and protect the oral mucosa from infection.<sup>[17]</sup> The oral cavity is continuously bathed by saliva. Therefore, saliva could represent the changes associated with the oral cavity at cellular and molecular levels through the variation in its composition.<sup>[18, 19]</sup> Molecular components found in other body fluids like serum and urine are also present to a certain extent in the saliva; thus, saliva has been used as a diagnostic tool for various diseases.<sup>[20]</sup> Saliva is composed of 98% water, while the remaining 2% consists of electrolytes, glycoproteins, and antibacterial substances like immunoglobulins and lysosomal enzymes.<sup>[19, 21]</sup> It contains a broad spectrum of biomarkers to detect various diseases. Saliva collection is a non-invasive procedure; its easily stored and transported, making it economical and efficient.<sup>[22]</sup> Recent studies and technologies have shown that saliva can be used as a tool for the diagnosis of cancer, immunodeficiency, hormone imbalances, and liver function.<sup>[5]</sup> Salivary electrolyte composition varies in individuals due to age, oral health status, and adverse habits like smoking and consumption of alcohol. In OSCC, the oral cavity environment is compromised, impacting the salivary composition and alteration of certain micro-nutrients. Assessing the altered levels of such micro-nutrients in saliva can diagnose various oral cancers.<sup>[21]</sup>

Saliva lies nearby of oral tissues, and any modification in oral tissues certainly reflects in the salivary composition, and estimating salivary Mg in patients with OSCC can be a sensitive aid in diagnosis.<sup>[16]</sup> Thus, the present study aimed to evaluate salivary magnesium levels in patients with OSCC and determine whether it can serve as a biomarker in the diagnosis and prognosis of OSCC.

## Materials and Methods

This case-control study was performed on patients diagnosed with OSCC. Patients visiting the oral pathology and microbiology department for biopsy procedures were taken as subjects after microscopic evaluation for the presence of OSCC. The Institutional Ethics Committee at Dr. D. Y. Patil Dental College and Hospital, Pune, India, approved the experimental protocol (DYPDCH/IEC/123/129/19) for undertaking this study.

The sample consisted of 36 individuals, calculated assuming a confidence interval level (almost 95%). Out of the 36 participants, 18 patients were those diagnosed with OSCC histopathologically, and the remaining 18 were otherwise healthy patients visiting the hospital for routine dental procedures. The study participants were subjected to detailed clinical examination to identify other potential lesions. A detailed case history was obtained from each individual before sample collection. The entire procedure of the study was explained to every participating subject, and informed consent was taken from everyone accordingly. The study was conducted between June 2020 and June 2021.

Sample collection was performed in the morning from 10:00 AM to 12:00 PM to avoid diurnal variation. Participants were exempted from consuming any food or drinks for 2 hours before the collection period.<sup>[9]</sup> Whole unstimulated saliva around 3 ml was collected from every individual where participants were asked to pool saliva on the floor of the mouth for a minute, and by the drooling method, the accumulated saliva was collected in a sterile glass tube. The collected samples were subjected to centrifugation at 2500 rpm for 15 mins at 4 degrees Celsius. The supernatants were stored at -20 degrees for further analysis of Mg. Salivary Mg was estimated using the xylydyl blue method and the values were obtained in mg/dl as described previously.<sup>[23]</sup> The obtained mean values were compared among the cases and controls.

Statistical analysis to determine the difference in salivary Mg was performed using Unpaired t-Test and One-way ANOVA in IBM SPSS software version 20. Data were expressed as Mean±SD, and significance levels were determined; the p-value of <0.05 was considered statistically significant.

## Results and Discussion

A total of 36 individuals were enrolled in the present study. Out of which, 18 individuals were histopathologically proven cases of OSCC, and 18 were healthy individuals taken as the control group. After performing a detailed case history, it was observed that all OSCC patients reported an adverse habit history of consumption of tobacco in smoking or non-smoking form. The summary of habit history is mentioned in (Table 1).

**Table 1. Types of adverse habits among OSCC patients.**

Type of habit	Number of patients
Tobacco chewing only	6
Tobacco chewing, areca nut consumption, Mishri, Pan consumption	1
Tobacco chewing and smoking	3
Tobacco chewing and alcohol	4
Mishri only	1
Areca nut only	2
Tobacco chewing and Mishri	1

Mean salivary Mg levels were more significant in OSCC patients than in the control group. However, a statistically significant difference was not evident according to the unpaired t-test. The salivary Mg levels in OSCC and control groups are presented in (Tables 2-4).

**Table 2. Salivary Mg levels in OSCC and control groups estimated from the present study.**

Groups	Mean	Standard Deviation	Median	95% Confidence Interval [Lower bound]	95% Confidence Interval [Upper bound]
OSCC	0.5778	0.36551	0.5	0.3960	0.7595
Control	0.4333	0.28901	0.45	0.2896	0.5771

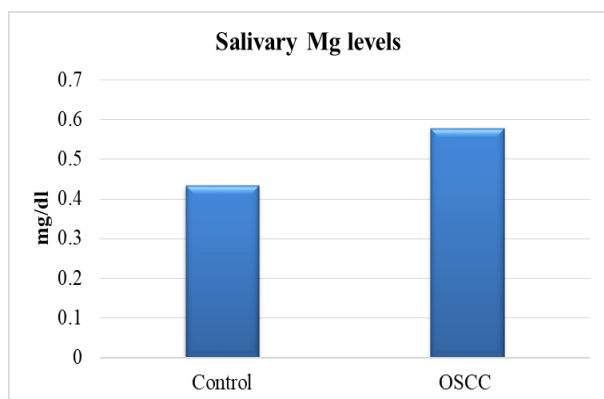
**Table 3. Statistical data obtained using unpaired t-test.**

Groups	Standard error Difference	Significance	95% Confidence Interval of the Difference	
			Lower	Upper
Control	0.10983	0.197	-.36764	.07876
OSCC	0.10983	0.198	-.36808	.07919

**Table 4. Statistical data obtained using One-way ANOVA.**

Groups	No. of subjects	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Control	18	0.4333	0.28901	0.06812	0.2896	0.5771	0.10	1.00
OSCC	18	0.5778	0.36551	0.08615	0.3960	0.7595	0.20	1.60
Total	36	0.5056	0.33290	0.05548	0.3929	0.6182	0.10	1.60

GA graphical representation of mean salivary Mg levels is shown in (Figure 1).



**Figure 1. Graphical representation of salivary Mg levels in study groups.**

The spread of oral cancer is turning into an alarming epidemic. OSCC is the leading cause of mortality in terms of oral cancer. The etiological association of OSCC is usually with carcinogens in tobacco and its related products consumed by people through the oral cavity. To name a few are nitrosamines and polynuclear aromatic hydrocarbons.<sup>[24]</sup> Recent studies point towards the association of various parallel factors playing an important role in carcinogenesis despite all these facts. These parallel factors are nothing but trace elements mentioned above that undergo alterations during the progression of OSCC.<sup>[6, 9]</sup> Assessing the alterations in the body fluids is a critical step toward early diagnosis. A higher incidence of OSCC is witnessed in industrialized countries where tobacco and alcohol consumption is more considerable. Consumption of tobacco in smoking or non-smoking form and

areca nut has shown alterations in body fluids of micro-elements such as Mg, Zn, Cu, and Ca.<sup>[18, 25, 26]</sup>

Saliva is an essential body fluid. Its close approximation with oral cavity saliva exhibits micro-molecular changes associated with oral tissues. The non-invasive collection of saliva as a sample for investigation has further enhanced the potential use of saliva as a diagnostic tool in any diseased condition.<sup>[21]</sup> Of all the trace elements found in the body, Mg counts as the most abundant mineral essential during the cell cycle. Its regulatory role during protein synthesis and DNA replication has been investigated.<sup>[9, 27]</sup>

In the present study, we evaluated the salivary magnesium levels of patients with OSCC and compared them with those obtained from an equal number of healthy controls to study its association with the disease. The mineral composition found in individuals consuming tobacco-based products and those without adverse habits is variable. Dzielulska *et al.*<sup>[10]</sup> studied salivary mineral composition in patients with oral cancer and found no significant difference in the salivary magnesium levels between oral cancer patients and healthy control following the results observed in the present study. Kolte *et al.*<sup>[28]</sup> emphasized that smoking influences the salivary mineral composition, increasing the saliva's magnesium levels. These findings are applicable in the present study, where patients with OSCC have been noted to have tobacco-related adverse habits. The increased magnesium levels in our study group can be associated with tobacco consumption in smoking or non-smoking form. Similar findings were noted by Kode *et al.*<sup>[25]</sup> while evaluating the levels of trace elements in the saliva of patients with oral submucous fibrosis (OSMF). Shpitzer *et al.*<sup>[29]</sup> found higher magnesium concentrations in the saliva of 25 patients with OSCC associated with the lateral aspect of the tongue, which correlated with the present study. However, contrasting results were obtained by Al-Rawi<sup>[30]</sup> and his associates, where statistically significant lower salivary magnesium levels were estimated in patients with oral cancer. Estimation of salivary magnesium levels in patients with premalignant lesions has concluded with a significantly lower level of mean salivary magnesium in the diseased state than in the healthy state.<sup>[9, 13]</sup> In a quantitative serum analysis for magnesium in patients with OSMF and OSCC, Hosthor *et al.* found lower serum magnesium levels in their diseased subjects.<sup>[31]</sup> The conflicting results can be explained based on adverse habits (consumption of tobacco) of patients with OSCC and fewer subjects involved.<sup>[9, 10]</sup>

Although Mg is considered an epitome concerning the cell cycle and proliferation, its potential role in the progression of OSCC remains unfolded. The variation in results between studies can be attributed to inconsistent experimental observations and epidemiologic data regarding different cancers.<sup>[32]</sup> In the present study, the lower levels of Mg in the control group can be attributed to lower dietary intake. Furthermore, aside from dietary factors, an individual's lifestyle and socioeconomic status impact Mg levels.<sup>[32]</sup> The conflicting results among various authors can be assigned to the variation in the number of involved subjects, the stage of

the OSCC of the patient, the method of collection of saliva, and its analysis for Mg levels.<sup>[33]</sup> In the present study, the sample size was 18 per group, comparatively less than other studies. Thus, the author recommends using a greater sample size and standardizing saliva collection and analysis for salivary Mg.

## Conclusion

The present study did not find any significant difference in the mean salivary Mg levels between the OSCC and healthy group in the present study. In conclusion, the author would like to emphasize that the comparison of salivary Mg in patients with OSCC and healthy individuals did not provide sufficient evidence of Mg to serve as an alleged diagnostic tool or a potential biomarker for OSCC. Differences in the cancer cases studied so far, and the techniques used to evaluate the Mg levels can be accounted for in this conflict. Moreover, an individual's nutritional status also held a potential role in the varying levels of Mg and was left unattended by a majority of the studies. Thus, the author suggests future studies focus on the existing lacunae and evaluate the nutritional socioeconomic status of the individual while estimating both serum and salivary Mg concentrations in an optimal sample size.

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## Conflict of interest

None.

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None.

## Ethics statement

The Institutional Ethics Committee at Dr. D. Y. Patil Dental College and Hospital, Pune, India, approved the experimental protocol (DYPDCH/IEC/123/129/19) for undertaking this study.

Informed consent was obtained from all individual participants included in the study.

## References

- Borse V, Konwar AN, Buragohain P. Oral cancer diagnosis and perspectives in India. *Sens Int.* 2020;1:100046. doi:10.1016/J.SINTL.2020.100046
- Sharma S, Satyanarayana L, Asthana S, Shivalingesh KK, Goutham BS, Ramachandra S. Oral cancer statistics in India on the basis of first report of 29 population-based cancer registries. *J Oral Maxillofac Pathol.* 2018;22(1):18-26. doi:10.4103/JOMFP.JOMFP\_113\_17
- Sankaranarayanan R, Mathew B, Jacob BJ, Thomas G, Somanathan T, Pisani P, et al. Early findings from a community-based, cluster-randomized, controlled oral cancer screening trial in Kerala, India. *Cancer.* 2000;88(3):664-73. doi:10.1002/(SICI)1097-0142(20000201)88:3
- Chuang SL, Su WW, Chen SL, Yen AM, Wang CP, Fann JC, et al. Population-based screening program for reducing oral cancer mortality in 2,334,299 Taiwanese cigarette smokers and/or betel quid chewers. *Cancer.* 2017;123(9):1597-609. doi:10.1002/CNCR.30517
- Ayinampudi BK, Narsimhan M. Salivary copper and zinc levels in oral pre-malignant and malignant lesions. *J Oral Maxillofac Pathol.* 2012;16(2):178-82. doi:10.4103/0973-029X.98452
- Shetty SR, Babu S, Kumari S, Shetty P, Hegde S, Karikal A. Status of trace elements in saliva of oral precancer and oral cancer patients. *J Cancer Res Ther.* 2015;11(1):146-9. doi:10.4103/0973-1482.137973
- Kaur J, Jacobs R, Huang Y, Salvo N, Politis C. Salivary biomarkers for oral cancer and pre-cancer screening: a review. *Clin Oral Investig.* 2018;22(2):633-40. doi:10.1007/S00784-018-2337-X
- Goldoni R, Scolaro A, Boccari E, Dolci C, Scarano A, Inchingolo F, et al. Malignancies and biosensors: A focus on oral cancer detection through salivary biomarkers. *Biosensors.* 2021;11(10):396. doi:10.3390/BIOS11100396
- Rezazadeh F, Salehi S, Rezaee M. Salivary level of trace element in oral lichen planus, a premalignant condition. *Asian Pac J Cancer Prev.* 2019;20(7):2009-13. doi:10.31557/APJCP.2019.20.7.2009
- Dziewulska A, Janiszewska-Olszowska J, Bachanek T, Grocholewicz K. Salivary mineral composition in patients with oral cancer. *Magnes Res.* 2013;26(3):120-4. doi:10.1684/mrh.2013.0346
- Castiglioni S, Maier JA. Magnesium and cancer: a dangerous liaison. *Magnes Res.* 2011;24(3):92-100. doi:10.1684/mrh.2011.0285
- Gröber U, Schmidt J, Kisters K. Magnesium in prevention and therapy. *Nutrients.* 2015;7(9):8199-226. doi:10.3390/nu7095388
- Nola Fuchs P, Rogić D, Vidović-Juras D, Sušić M, Milenović A, Brailo V, et al. Salivary analytes in patients with oral squamous cell carcinoma. *Coll Antropol.* 2011;35(2):359-62.
- Biswal BN, Das SN, Das BK, Rath R. Alteration of cellular metabolism in cancer cells and its therapeutic prospects. *J Oral Maxillofac Pathol.* 2017;21(2):244-51. doi:10.4103/jomfp.JOMFP
- Al Alawi AM, Majoni SW, Falhammar H. Magnesium and human health: perspectives and research directions. *Int J Endocrinol.* 2018;2018. doi:10.1155/2018/9041694
- Gupta AA, Shekatkar M, Raj AT, Kheur S. Potential role of magnesium in cancer initiation and progression. *Pathol Oncol Res.* 2020;26(3):2001-2. doi:10.1007/s12253-019-00750-w
- Zalewska A, Waszkiewicz N, López-Pintor RM. The use of saliva in the diagnosis of oral and systemic diseases. *Dis Markers.* 2019;2019:1-2. doi:10.1155/2019/9149503
- Khulbe G, Tantradi P, Ammanagi R, Byahatti S. Estimation of salivary copper, zinc, iron and copper to zinc ratio in oral submucous fibrosis patients and its comparison with healthy individuals. *J Indian Acad Oral Med Radiol.* 2019;31(4):333. doi:10.4103/jiaomr.jiaomr
- Zhang CZ, Cheng XQ, Li JY, Zhang P, Yi P, Xu X, et al. Saliva in the diagnosis of diseases. *Int J Oral Sci.* 2016;8(3):133-7. doi:10.1038/IJOS.2016.38
- Khurshid Z, Zafar MS, Khan RS, Najeeb S, Slowey PD, Rehman IU. Role of salivary biomarkers in oral cancer detection. *Adv Clin Chem.* 2018;86:23-70. doi:10.1016/BS.ACC.2018.05.002
- Lee YH, Wong DT. Saliva: an emerging biofluid for early detection of diseases. *Am J Dent.* 2009;22(4):241-8.
- Feng Y, Li Q, Chen J, Yi P, Xu X, Fan Y, et al. Salivary protease spectrum biomarkers of oral cancer. *Int J Oral Sci.* 2019;11(1):1-1. doi:10.1038/s41368-018-0032-z
- Aziz NZ, Arathi K, Prasad BG, Desai D, Shetty SJ, Shahid M. Evaluation of magnesium levels in blood and saliva of oral squamous cell carcinoma and potentially malignant disorders by xylylidyl blue method. *J Oral Maxillofac Pathol.* 2018;22(1):147-8. doi:10.4103/JOMFP.JOMFP\_34\_17
- Jethwa AR, Khariwala SS. Tobacco-related carcinogenesis in head and neck cancer. *Cancer Metastasis Rev.* 2017;36(3):411-23. doi:10.1007/S10555-017-9689-6
- Kode MA, Karjodkar FR. Estimation of the serum and the salivary trace elements in OSMF patients. *J Clin Diagn Res.* 2013;7(6):1215-8. doi:10.7860/JCDR/2013/5207.3023
- Saavedra JA, Novo DL, Mesko MF, Vasconcellos AC, Da Silva KD, Zuñiga GR, et al. Comparison of Salivary Electrolytes Profile in Oral Potentially Malignant Disorders and Oral Squamous Cell Carcinoma. *Asian Pac J Cancer Prev.* 2022;23(3):1031-9. doi:10.31557/APJCP.2022.23.3.1031

27. Mendes PM, Bezerra DL, Dos Santos LR, de Oliveira Santos R, de Sousa Melo SR, Morais JB, et al. Magnesium in breast cancer: what is its influence on the progression of this disease?. *Biol Trace Elem Res.* 2018;184(2):334-9. doi:10.1007/s12011-017-1207-8
28. Kolte AP, Kolte RA, Laddha RK. Effect of smoking on salivary composition and periodontal status. *J Indian Soc Periodontol.* 2012;16(3):350-3. doi:10.4103/0972-124X.100909
29. Shpitzer T, Bahar G, Feinmesser R, Nagler RM. A comprehensive salivary analysis for oral cancer diagnosis. *J Cancer Res Clin Oncol.* 2007;133(9):613-7. doi:10.1007/s00432-007-0207-z
30. Al-Rawi NH, Talabani NG. Quantitative analysis of trace elements in saliva of oral cancer patients from Iraq. *J Coll Dent.* 2005;17:32-5.
31. Hothor SS, Mahesh P, Priya SA, Sharada P, Jyotsna M, Chitra S. Quantitative analysis of serum levels of trace elements in patients with oral submucous fibrosis and oral squamous cell carcinoma: A randomized cross-sectional study. *J Oral Maxillofac Pathol.* 2014;18(1):46-51. doi:10.4103/0973-029X.131902
32. Qiao W, Lan XM, Ma HX, Chan JY, Lui VW, Yeung KW, et al. Effects of salivary mg on head and neck carcinoma via TRPM7. *J Dent Res.* 2019;98(3):304-12. doi:10.1177/0022034518813359
33. Khulbe G, Tantradi P, Ammanagi R, Byahatti S. Estimation of salivary copper, zinc, iron and copper to zinc ratio in oral submucous fibrosis patients and its comparison with healthy individuals. *J Indian Acad Oral Med Radiol.* 2019;31(4):333-8. doi:10.4103/jiaomr.jiaomr\_81\_19