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# Association of the mir-499 polymorphisms with oral cavity and oropharyngeal squamous cell carcinoma in an Iranian population

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## ABSTRACT

**Background:** Oral squamous cell carcinoma (SCC) is the most common oral malignancy. Some evidence indicated that there is a correlation between microRNA single nucleotide polymorphisms and the risk of oral cancer. The aim of the current study was to investigate the association between mir-499 polymorphism with the risk of oral cavity and oropharyngeal SCC in a subset of Iranian Population.

**Materials and Methods:** In this case–control pilot study total of 112 participants including 56 histopathlogically confirmed oral and oropharyngeal SCC patients and 56 age- and sex-matched controls were included The mir-499 rs3746444 T/C polymorphism was detected using polymerase chain reaction-restriction fragment length polymorphism method. The comparisons of the distribution of the allele and genotype frequencies were performed using Chi-square test, and $P < 0.05$ was considered as statistically significant.

**Results:** The result of the present study indicated that the frequency distribution of mir-499 was not significantly different between cases and controls ($P > 0.05$). We also did not find any significant association between the risk of the cancer and mir-499 polymorphisms in the recessive (Odds ratio [OR]: 6.60; 95% confidence interval [CI]: 0.77–56.74; $P = 0.11$) and dominant (OR: 1; 95% CI: 0.37–2.74; $P = 1$) inheritance models even after adjustment for smoking.

**Conclusion:** The results of the present study indicated that the polymorphisms of mir-499 are not associated with the risk of oral and oropharyngeal SCC in Iranian population. However, further large scale studies are needed to validate our findings.

**Key Words:** Head and neck squamous cell carcinoma, microRNA, polymorphism

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## INTRODUCTION

Head and neck cancers (HNCs) represent the sixth most common cancer in the world with more than 600,000 new patients diagnosed annually and a 5-year survival rate of 40%–50%.[1,2] It has been estimated that squamous cell carcinoma (SCC) of the head and neck SCC (HNSCC) originating from mucosal surfaces of the oral cavity, oropharynx, and larynx accounts for more than 90% of HNCs.[1] The etiology of HNSCC has been considered to be multifactorial with cigarette smoking, alcohol use, and human

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papillomavirus as its three main predictors.[3-6] There is a geographic variability in the incidence of HNSCC which is possibly related to demographic and lifestyle differences.[6] A higher incidence of the disease has been observed in South-East Asia, Pacific regions, Latin America, and some parts of Central and Eastern Europe.[7]

Some evidence suggested that there is a correlation between genetic susceptibility and the risk of HNSCC. It has been reported that the risk of HNSCC in the first-degree relatives of patients with the disease is about two times higher than general population.[8,9] Moreover, only a small proportion of people exposed to HNSCC risk factors develop the disease.[10] In recent years, a great deal of attention devoted to the study of molecular mechanisms contributing to the etiology of this cancer.

MicroRNAs are small, noncoding RNAs playing an important role in several biological processes including cell proliferation, differentiation, cell cycle progression, and apoptosis.[11-13] Recently, accumulated evidence revealed that single nucleotide polymorphisms (SNPs) of microRNAs are associated with the risk of some cancers.[14-20] It has been reported that SNPs occurring in microRNAs lead to occurrence of malignancies through affecting microRNA biogenesis, stability of mature microRNA molecules, efficiency of target gene regulation, and the specificity of targets.[21]

Mir-499 is a microRNA which involves in posttranscriptional gene regulation. A number of reports have indicated that there is an association between mir-499 polymorphism and the risk of many cancers such as lung cancer, breast cancer, gastric cancer, and HNC.[22-25] It has been suggested that miR-499 polymorphism increases the risk of breast and prostate cancer among Iranians.[26,27] However, we could not find any study that has assessed the association between the polymorphism of this gene and the risk of oral cancers in the population.

Considering the high prevalence of cancer in developing countries and the lack of evidence regarding the association between microRNA polymorphism and oral cancers, we designed the current study to explore the association between mir-499 polymorphism and oral cavity and oropharyngeal SCC in a subset of Iranian population.

## MATERIALS AND METHODS

### Subjects

This case–control pilot study was approved by ethics committee of Islamic Azad University, Isfahan (Khorasgan) branch, Isfahan, Iran (Research number: 23810201951011), and informed consent was obtained from all participants after a full description of the study objectives. Study participants were recruited from Al-Zahra and Sayed-al-Shohada hospitals, affiliated to Isfahan University of Medical Sciences, Isfahan, Iran. All adult patients between 30 and 70 years with a histopathologically confirmed diagnosis of oral and oropharyngeal SCC were defined as cases. The control group consisted of patients without a history of cancer who were matched with case patients for age and sex. All patients with cervical metastases of unknown origin, primary tumors outside the upper aerodigestive tract, and primary tumors of the nasopharynx, larynx, hypopharynx, and sinonasal tract were excluded from the study. A self-administered researcher-made questionnaire was used to collect individuals' demographics and smoking habits.

### DNA extraction and genotyping

A 2.5 mL sample of whole blood was collected from each individual. Genomic DNA was extracted using commercial DNA Extraction kit (Iraizol#1004; RNA Biotechnology Company, Isfahan, Iran) according to the manufacturer's instructions.

The mir-499 rs3746444 T/C polymorphism was detected using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The polymorphism is situated in the stem region opposite to the mature miR-499 sequence, resulting in a change from A:U pair to G:U mismatch in the stem structure of miR-499 precursor.[28]

The primers used for amplification of the microRNA were 5'-CAAAGTCTTCACTTCCCTGCCA-3' and 5'-GATTTTAACTCCTCTCCACGTGATC-3'. Twenty-five microliters of the PCR reaction system consisted of 12.5 μl Taq 2X Master Mix with 1.5 mM MgCl2 (AMPLIQON# 5200300-A180303; Denmark), 10 μM of each BsmI-F and BsmI-R primers, and 10 ng genomic DNA. The cycling condition comprised an initial denaturation at 95°C for 5 min, followed by 35 cycles of amplification at 95°C for 30 s, 62°C for 30 s, and 72°C for 30 s, with an elongation step at 72ºC for 7 min. The PCR products were then digested by

*Bcl*I (Catalog # R0160S, NEW ENGLAND BioLabs) and separated in a 3% agarose gel. Homozygous C/C alleles of mir-499 were represented by a DNA band with a size of 146 bp, and the homozygous T/T alleles were represented by DNA bands with sizes of 120 and 26 bp. Heterozygotes C/T displayed a combination of the alleles (146, 120, and 26 bp).

### Statistical analysis

The comparisons of the distribution of the allele and genotype frequencies were performed using Chi-square test. The Statistical Package for Social Sciences version 20.0 (SPSS Inc., Chicago, IL, USA) was used to calculate the Chi-square value. $P < 0.05$ was considered as statistically significant.

## RESULTS

A total of 112 participants were recruited for the study comprising 56 case patients and 56 age- and sex-matched controls. Characteristics of the study population are presented in Table 1. There was a significant difference between cases and controls in terms of smoking ($P < 0.001$).

**Table 1: Basic characteristics of cases and matched controls**

| Variables | Case (*n*=56), *n* (%) | Control (*n*=56), *n* (%) | *P* |
|---|---|---|---|
| Age* | | | |
| Sex | | | |
| Male | 39 (69.6) | 39 (69.6) | 1.00 |
| Female | 17 (30.4) | 17 (30.4) | |
| Smoking status | | | |
| Yes | 23 (41.1) | 4 (7.1) | <0.001 |
| No | 33 (58.9) | 52 (92.9) | |

*The variable is presented as mean±SD. SD: Standard deviation

The genotypes and allele frequencies of mir-499 polymorphisms in cases and controls are summarized in Table 2. There was no significant difference between case and control groups in terms of mir-499 genotypes. We did not find any significant differences between cases and controls in terms of mir-499 polymorphisms in the recessive (Odds ratio [OR]: 6.60; 95% confidence interval [CI]: 0.77–56.74; $P = 0.11$) and dominant (OR: 1; 95% CI: 0.37–2.74; $P = 1$) inheritance models. Our findings also indicated that there was no significant association between mir-499 genotypes and the risk of cancer after adjustment for smoking suggesting no confounding by smoking [Table 2].

## DISCUSSION

In the present study, we investigated the association between mir-499 polymorphism and the risk of oral cavity and oropharyngeal SCC. A number of studies have suggested the association between mir-499 polymorphism and the susceptibility to some kind of cancers. Hashemi *et al*. reported an association between the risk of prostate cancer and mir-499 polymorphism among the Iranian population.[26] Omrani *et al*. also suggested that the gene polymorphism both in recessive and dominant inheritance models is associated with a higher risk of breast cancer in Iranian individuals.[27] The result of the study by Wang *et al*. showed that mir-499 A > G polymorphism is associated with increased risk of hepatocellular carcinoma.[29] However, Zhang

**Table 2: Genotype and allelic frequencies of miR499 rs3746444 variants polymorphisms in case and controls**

| 38 polymorphisms | Control (*n*=56), *n* (%) | Case (*n*=56), *n* (%) | OR (95%CI) | Adjusted OR | *P* |
|---|---|---|---|---|---|
| Codominant | | | | | |
| TT | 9 (16.1) | 9 (16.1) | 1 | 1 | |
| CT | 46 (82.1) | 41 (73.2) | 0.89 (0.32-2.46) | 0.68 (0.22-2.15) | 0.19 |
| CC | 1 (1.8) | 6 (10.7) | 6 (0.60-60.44) | 5.30 (0.42-66.56) | |
| Recessive | | | | | |
| TT + CT | 50 (89.3) | 55 (98.2) | 1 | 1 | 0.11 |
| CC | 6 (10.7) | 1 (1.8) | 6.60 (0.77-56.74) | 7.24 (0.69-75.43) | |
| Dominant | | | | | |
| CC + CT | 47 (83.9) | 47 (83.9) | 1 | 1 | 1.00 |
| TT | 9 (16.1) | 9 (16.1) | 1 (0.37-2.74) | 1.29 (0.41-4.02) | |
| Allele | | | | | |
| C | 53 (47.3) | 48 (42.9) | 1 | 1 | 0.50 |
| T | 59 (52.7) | 64 (57.1) | 1.19 (0.71-2.03) | 1.13 (0.63-2) | |

OR: Odds ratio; CI: Confidence interval

*et al*. did not find any association between the risk of hepatocellular carcinoma and the polymorphism in the gene.[30] The study on Greek population by Dikaiakos *et al*. also did not find any association between the risk of colorectal cancer and mir-499 polymorphism.[31] The results of several meta-analyses and systematic review studies have also indicated that the polymorphism of mir-499 can increase the risk of some cancers, especially among Asians.[32,33]

Recently, a limited number of studies have assessed the association between mir-499 polymorphism and the risk of oral cancer; although, there is not any consensus between the results of these studies. In the present study, we did not find any association between mir-499 polymorphism and the risk of oral and oropharyngeal cancer. Hou *et al.* reported that there is an inverse association between the risk of oral SCC and mir-499 polymorphism.[34] It has also been reported that mir-499 polymorphism was associated with a decreased risk of SCC of the head and neck among non-Hispanic white population.[25] On the contrary, the result of a study by Zhang *et al*. showed that miR-499 polymorphism contributes to genetic susceptibility to oral SCC. According to the results of this study, people with CC genotype had an increased risks of oral cancer compared to those with wild TT genotype.[35] Tandon *et al*. in a study on Indian population found that genetic polymorphisms of miR-499 contribute to the risk of oral SCC.[36] The discrepancies in the results of these studies are possibly due to differences in studied populations and sample size. Surprisingly, it has also been suggested that the interaction between microRNAs genetic polymorphism and environmental risk factors is associated with an increased risk of oral cancer.[19] Thus, it is of great importance to investigate the role of mir-499 polymorphism in the pathogenesis of oral cancer in combination with other possible risk factors. Our study contains several limitations comprising small sample size and lack of information on confounding variables such as alcohol and tobacco use. As a result, there is a need to do further studies among Iranian population with the consideration of possible confounders.

## CONCLUSION

In summary, we did not find any association between genetic polymorphism of mir-499 and the risk of oral cavity and oropharyngeal SCC. Further, large-scale studies among Iranian population are warranted to explore the association between microRNAs polymorphisms and underlying mechanisms.

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### Conflicts of interest

The authors of this manuscript declared that they have no conflicts of interest, real or perceived, and financial or non-financial in this article.

## REFERENCES

1. Vigneswaran N, Williams MD. Epidemiologic trends in head and neck cancer and aids in diagnosis. Oral Maxillofac Surg Clin North Am 2014;26:123-41.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74-108.
3. Smith EM, Rubenstein LM, Haugen TH, Hamsikova E, Turek LP. Tobacco and alcohol use increases the risk of both HPV-associated and HPV-independent head and neck cancers. Cancer Causes Control 2010;21:1369-78.
4. Smith EM, Rubenstein LM, Haugen TH, Pawlita M, Turek LP. Complex etiology underlies risk and survival in head and neck cancer human papillomavirus, tobacco, and alcohol: A case for multifactor disease. J Oncol 2012;2012:1-9.
5. Rautava J, Syrjänen S. Biology of human papillomavirus infections in head and neck carcinogenesis. Head Neck Pathol 2012;61:3-15.
6. Michmerhuizen NL, Birkeland AC, Bradford CR, Brenner JC. Genetic determinants in head and neck squamous cell carcinoma and their influence on global personalized medicine. Genes Cancer 2016;7:182-200.
7. John K, Wu J, Lee BW, Farah CS. MicroRNAs in head and neck cancer. Int J Dent 2013;2013:1-12.
8. Foulkes WD, Brunet JS, Kowalski LP, Narod SA, Franco EL. Family history of cancer is a risk factor for squamous cell carcinoma of the head and neck in Brazil: A case-control study. Int J Cancer 1995;63:769-73.
9. Yu GP, Zhang ZF, Hsu TC, Spitz MR, Schantz SP. Family history of cancer, mutagen sensitivity, and increased risk of head and neck cancer. Cancer Lett 1999;146:93-101.
10. Sturgis EM, Castillo EJ, Li L, Zheng R, Eicher SA, Clayman GL, *et al*. Polymorphisms of DNA repair gene XRCC1 in squamous cell carcinoma of the head and neck. Carcinogenesis 1999;20:2125-29.
11. He L, Hannon GJ. MicroRNAs: Small RNAs with a big role in gene regulation. Nat Rev Genet 2004;5:522-31.
12. Pillai RS. MicroRNA function: Multiple mechanisms for a tiny RNA? RNA 2005;11:1753-61.
13. Croce CM, Calin GA. MiRNAs, cancer, and stem cell division. Cell 2005;122:6-7.
14. Xu Q, Dong Q, He C, Liu W, Sun L, Liu J, *et al.* A new polymorphism biomarker rs629367 associated with increased risk and poor survival of gastric cancer in Chinese by up-regulated miRNA-let-7a expression. PLoS One 2014;9:1-11.

15. Zhang Z, Zhou B, Gao Q, Wu Y, Zhang K, Pu Y, *et al.* A polymorphism at miRNA-122-binding site in the IL-1α 3'UTR is associated with risk of epithelial ovarian cancer. Fam Cancer 2014;13:595-601.
16. Liu Y, He A, Liu B, Zhong Y, Liao X, Yang J, *et al.* Rs11614913 polymorphism in miRNA-196a2 and cancer risk: An updated meta-analysis. Onco Targets Ther 2018;11:1121-39.
17. Dongdong WU, Song P, Bo FU, Ming LU, Zhao Q, Wang B. The association between Has-miRNA-149 polymorphism and susceptibility of digestive tract cancer. Chongqing Med 2016;45:5121-25.
18. Shen GR, Li WZ, Liu YC, Li XP, Yuan HY. Association between a microRNA-214 binding site polymorphism in the methylenetetrahydrofolate reductase gene and esophageal squamous cell carcinoma. Genet Mol Res 2016;15:1-8.
19. Chu YH, Tzeng SL, Lin CW, Chien MH, Chen MK, Yang SF. Impacts of microRNA gene polymorphisms on the susceptibility of environmental factors leading to carcinogenesis in oral cancer. PLoS One 2012;7:1-8.
20. Christensen BC, Avissar-Whiting M, Ouellet LG, Butler RA, Nelson HH, McClean MD, *et al.* Mature microRNA sequence polymorphism in MIR196A2 is associated with risk and prognosis of head and neck cancer. Clin Cancer Res 2010;16:3713-20.
21. Nikolić Z, Savić Pavićević D, Vučić N, Cidilko S, Filipović N, Cerović S, *et al.* Assessment of association between genetic variants in microRNA genes hsa-miR-499, hsa-miR-196a2 and hsa-miR-27a and prostate cancer risk in Serbian population. Exp Mol Pathol 2015;99:145-50.
22. Tian T, Shu Y, Chen J, Hu Z, Xu L, Jin G, *et al.* A functional genetic variant in microRNA-196a2 is associated with increased susceptibility of lung cancer in Chinese. Cancer Epidemiol Biomarkers Prev 2009;18:1183-87.
23. Catucci I, Yang R, Verderio P, Pizzamiglio S, Heesen L, Hemminki K, *et al.* Evaluation of SNPs in miR-146a, miR196a2 and miR-499 as low-penetrance alleles in German and Italian familial breast cancer cases. Hum Mutat 2010;31:1052-57.
24. Okubo M, Tahara T, Shibata T, Yamashita H, Nakamura M, Yoshioka D, *et al.* Association between common genetic variants in pre-microRNAs and gastric cancer risk in Japanese population. Helicobacter 2010;15:524-31.
25. Liu Z, Li G, Wei S, Niu J, El-Naggar AK, Sturgis EM, *et al.* Genetic variants in selected pre-microRNA genes and the risk of squamous cell carcinoma of the head and neck. Cancer 2010;116:4753-60.
26. Hashemi M, Moradi N, Ziaee SA, Narouie B, Soltani MH, Rezaei M, *et al.* Association between single nucleotide polymorphism in miR-499, miR-196a2, miR-146a and miR-149 and prostate cancer risk in a sample of Iranian population. J Adv Res 2016;7:491-98.
27. Omrani M, Hashemi M, Eskandari-Nasab E, Hasani SS, Mashhadi MA, Arbabi F, *et al.* Hsa-mir-499 rs3746444 gene polymorphism is associated with susceptibility to breast cancer in an Iranian population. Biomark Med 2014;8:259-67.
28. Xiang Y, Fan S, Cao J, Huang S, Zhang LP. Association of the microRNA-499 variants with susceptibility to hepatocellular carcinoma in a Chinese population. Mol Biol Rep 2012;39:7019-23.
29. Wang Z, Wu J, Zhang G, Cao Y, Jiang C, Ding Y, *et al.* Associations of miR-499 and miR-34b/c polymorphisms with susceptibility to hepatocellular carcinoma: An evidence-based evaluation. Gastroenterol Res Pract 2013;2013:1-8.
30. Zhang LH, Hao BB, Zhang CY, Dai XZ, Zhang F. Contributions of polymorphisms in miR146a, miR196a, and miR499 to the development of hepatocellular carcinoma. Genet Mol Res 2016;15:399-407.
31. Dikaiakos P, Gazouli M, Rizos S, Zografos G, Theodoropoulos GE. Evaluation of genetic variants in miRNAs in patients with colorectal cancer. Cancer Biomark 2015;15:157-62.
32. Qiu MT, Hu JW, Ding XX, Yang X, Zhang Z, Yin R, *et al.* Hsa-miR-499 rs3746444 polymorphism contributes to cancer risk: A meta-analysis of 12 studies. PLoS One 2012;7:1-7.
33. Chen C, Yang S, Chaugai S, Wang Y, Wang DW. Meta-analysis of hsa-mir-499 polymorphism (rs3746444) for cancer risk: Evidence from 31 case-control studies. BMC Med Genet 2014;15:126-137.
34. Hou YY, Lee JH, Chen HC, Yang CM, Huang SJ, Liou HH, *et al.* The association between miR-499a polymorphism and oral squamous cell carcinoma progression. Oral Dis 2015;21:195-206.
35. Zhang E, Xu Z, Duan W, Huang S, Lu L. Association between polymorphisms in pre-miRNA genes and risk of oral squamous cell cancer in a Chinese population. PLoS One 2017;12:1-10.
36. Tandon D, Dewangan J, Srivastava S, Garg VK, Rath SK. MiRNA genetic variants: As potential diagnostic biomarkers for oral cancer. Pathol Res Pract 2018;214:281-9.