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ORIGINAL ARTICLE

Role of Interleukin-6 Gene Variants in the Development of Oral Squamous Cell Carcinoma

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OBJECTIVE

Oral squamous cell carcinoma (OSCC), with its low survival rates and increasing incidence, is due to various etiological factors including environmental, genetic, and epigenetic changes. Interleukin 6 (IL-6) is a cytokine with both pro- and anti-inflammatory effects. Therefore, we investigated the possible association of the IL-6 gene variants with risk for OSCC in a Turkish cohort.

METHODS

This study included 42 patients with OSCC and 110 age-and gender-matched healthy controls. Three variants (-174G/C [rs1800795], -572G/C (rs1800796), and -597G/A [rs1800797]) in the IL-6 promoter region were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

RESULTS

There was a significant difference in the genotype and allele frequencies of the IL-6 -174G/C variant between OSCC patients and controls. While the IL-6 -174G/C G/C genotype was higher in the patient group than in the control group, the G/G and C/C genotypes were lower in the patients compared to the control group (p=0.016, OR:0.653, 95% CI: 0.38-1.11). The genotype and allele distributions of -572G/C and -597G/A variants of the IL-6 gene were not statistically different between OSCC patients and the control group.

CONCLUSION

Our current investigation is focused on the role of variants of IL-6 gene on OSCC. To the best of our knowledge, this study is the first study to evaluate the genotype and allele frequencies of IL-6 -174G/C, -572G/C, and 597G/A variants in patients with OSCC in a Turkish population. The results support that the IL-6 -174G/C variant may play an important role in susceptibility to OSCC in our population.

Keywords: Interleukin 6; oral squamous cell carcinoma; PCR-RFLP; variant.

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) is classified among the most common solid tumors, accounting for 4% of all malignancies.[1] OSCC is more common in men (male:female =2:1). Many risk factors have been associated with the development of OSCC, including low intake of fresh fruits-vegetables, alcoholism, and cigarette smoking.[2] Many studies have shown that the inflammatory process leads to an increase in the risk of developing cancer. The chronic inflammatory process causes permanent tissue damage and changes the microenvironment by affecting inflammatory cells and cytokines. These changes lead to a cascade of events such as tumorigenesis, then malignant invasion, and finally metastasis.

Interleukin 6 (IL-6), which is among the most studied cytokine types, is both an important pro-inflammatory and anti-inflammatory mediator. It is also considered to induce immune reactions and host defense.[3] IL-6 plays a role in various biological reactions through stimulation of B and T lymphocytes, production of acute-phase protein synthesis in the liver, regulation of hematopoiesis. IL-6 also acts as a pyrogen, activates the coagulation system, and suppresses the synthesis of tumor necrosis factor-alpha (TNF-α) and IL-1β *in vitro*.[4] The gene coding for IL-6 is located on chromosome 7p21-24 and consists of four introns and five exons. The data showed that IL-6 expression can be partially genetically modulated by variants located at positions in the promoter region of IL-6 (rs1800795, rs1800796, and rs1800797 [also known as IL-6 -174G/C, -572G/C, and -597G/A]).[5] The IL-6 -174G/C variant is one of the most studied polymorphisms. The IL-6 gene functional -174G/C variant can increase the transcriptional activity of the IL-6 promoter, leading to IL-6 upregulation in stress or infection. Some studies have shown that the IL-6 -174G/C gene variant is significantly associated with certain cancers.[6]

There was no previous study showing the association between IL-6 -174G/C, -572G/C, and -597G/A variants and OSCC in the Turkish population. Therefore, we aimed to investigate the role of IL-6 gene variants in the pathogenesis of OSCC in the Turkish population.

MATERIALS AND METHODS

Study Population

Forty-two patients with OSCC (27 male, 15 women, mean age: 63.05±13.25 years) and 110 healthy controls (72 male, 38 women, mean age: 56.02±11.03 years) were included in this study. The patients were recruited from the Department of Medical Oncology, Faculty of Medicine, Gaziosmanpaşa University, Tokat, Turkey. All patients with OSCC were pathologically confirmed. Patients with oral precancerous diseases such as oral submucous fibrosis, leukoplakia, erythroplakia, or verrucous hyperplasia were not included in the study. The age-and gender-matched control group (n=110) consisted of healthy individuals with no personal or family history of cancer. Informed consent was obtained from each participant interviewed to gather detailed information on demographic information. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Hitit University, Faculty of Medicine (2017/200).

Genotyping

Genomic DNA was extracted from whole venous blood samples using a commercial DNA isolation kit (Sigma-Aldrich, Taufkirchen, Germany). The IL-6 gene -174G/C, -572G/C, and -597G/A variants were analyzed by polymerase chain reaction (PCR) based restriction fragment length polymorphism analysis. The IL-6 -174G/C variant was analyzed as previously described by Tseng et al.[7] using forward (F) 5′-TTG TCA AGA CAT GCC AAA GTG CGG AG-3′ and reverse (R) 5′-GTG CAA TGT GAC GTC CCT TAG CAT-3′ primers. The amplification conditions consisted of an initial melting step of 5 min at 94°C; followed by 40 cycles of 30 s at 94°C, 30 s at 56°C, and 1 min at 72°C. After amplification, the 156 bp PCR product was digested with Fast Digest BseL-I restriction endonuclease (Fermentas) at 37°C for 30 min and analyzed on a 3% agarose gel stained with ethidium bromide. Two fragments (139 and 17 bp) for G allele and three fragments (117, 22, and 17 bp) for C allele were observed.

The primers used for IL-6-572G/C and -597G/A variants were (F): 5′-CAG CAG CCA ACC TCC TCT AA-3′ and (R) 5′-AAA CCA GAC CCT TGC ACA AC-3′. The cycling conditions for IL-6 -572G/C and -597G/A variants were 40 cycles of 30 s at 95°C, 30 s at 62°C, and 1 min at 72°C. For the IL-6-572G/C, the G to C transversion was identified by digestion with BsrBI, producing for the G allele fragments of 150 and 74 bp. For the IL-6 -597GA, the G to A transition was identified with FokI, the A allele yields fragments of 136 and 88 bps. A second PCR was performed to confirm samples whose results were not clear.

STRING Analysis

STRING is a proteomics database focused on networks and interactions of proteins of a wide variety of species. In this study, IL-6 protein was examined by the STRING analysis, and the proteins it interacts with were investigated.

Statistical Analysis

All statistical analyses were performed with the Statistical Package for the Social Science for Windows (version 22.0; SPSS Inc., Chicago, IL, USA) Statistical Program. The χ^2 test was used to measure significance of differences in the allele frequency and the genotype distribution between the groups. Odds ratio (OR) and 95% confidence intervals (CI) were calculated. A value of $p<0.05$ was considered statistically significant.

RESULTS

A total of 152 subjects (42 OSCC patients and 110 healthy controls) were included in this study. The sex, age, smoking, age of onset of smoking, duration, daily cigarette consumption, alcohol consumption, frequency of alcohol consumption, family history, response of treatment, patient status, disease status, and disease area of OSCC patients were analyzed. The demographic and clinical characteristics of the study participants are shown in Table 1. Allelic and genotypic distributions of IL-6 gene -174 G/C, -572G/C, and -597G/A variants are presented in Table 2.

IL-6 gene -174G/C Variant (rs1800795)

There was a significant difference in the genotype distribution of the IL-6 -174G/C variant between OSCC patients and controls. IL-6 -174G/C G/C genotype was higher in the patients compared to the control group and G/G and C/C genotypes were lower in the patients compared to the control group (p=0.016, OR:0.653, 95%CI: 0.38-1.11). No significant difference was observed in patients and healthy controls in terms of allele frequencies of the IL-6 -174G/C variant.

IL-6 gene-572G/C Variant (rs1800796)

The frequencies of the G/G, G/C, and C/C genotypes of IL-6 -572G/C were 76.2%, 19.0%, and 33.3% in OSCC patients and 74.5%, 21.8%, and 3.6% in controls, respectively. The G and C allele frequencies were 85.71% and 14.28% in the OSCC patients, and 84.54% and 14.54% in controls, respectively. The genotype and allele distributions of IL-6 gene -572G/C variant were

Table 1 Baseline demographical and clinical features of the patients with OSCC

Characteristics	Patient group (n=42)		Control group (n=110)	
	n	%	n	%
Gender				
Male	27	64.3	72	65.4
Female	15	35.7	38	34.5
Age, mean±SD, years	63.05±13.25		56.02±11.03	
Smoking				
Yes	5	11.9		
No	30	71.4		
Ex-smoking	5	16.7		
Smoking onset age, mean±SD	18.92±2.84			
years smoking duration				
10-20 years	2	15.4		
20-30 years	5	46.2		
>30 years	3	38.5		
Daily cigarette consumption				
One package	4	33.3		
>One package	8	66.7		
Alcohol consumption				
Yes	8	19.0		
No	34	81.0		
Frequency of alcohol consumption				
Daily	5	62.5		
Social drinker	3	37.5		
Family History				
Yes	35	83.3		
No	7	16.7		
Response to treatment				
Yes	29	70.7		
No	12	29.3		
Patients status				
Living	35	83.3		
Exitus	7	16.7		
Disease state				
Remission	27	65.9		
Stabile	2	4.9		
Metastatic	12	29.3		
Disease area				
Intra-oral	3	2.0		
Floor of the mouth	3	2.0		
Buccal	1	0.7		
Roof of the mouth	3	2.0		
Tongue	12	7.9		
Lip	16	10.5		
Oral mucosa	1	0.7		
Tonsil	2	1.3		
Cheek mucosa	1	0.7		

OSCC: Oral squamous cell carcinoma

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Table 2 Genotype and allele distribution of IL-6 gene variants in groups

	Patient group n=42		Control group n=110		p	OR (95% CI)
	n	%	n	%		
IL-6 -174G/C variant (rs1800795)						
Genotypes						
G/G	14	33.3	61	55.5	**0.016**	0.653 (0.38-1.11)
G/C	24	57.1	35	31.8		
C/C	4	9.5	14	12.7		
Alleles						
G	52	61.90	157	71.36	>0.05	
C	32	38.09	63	28.63		
IL-6 -572G/C variant (rs1800796)						
Genotypes						
G/G	32	76.2	82	74.5	>0.05	1.032 (0.50-2.18)
G/C	8	19.0	24	21.8		
C/C	2	33.3	4	3.6		
Alleles						
G	72	85.71	186	84.54	>0.05	
C	12	14.28	32	14.54		
IL-6 -597G/A variant (rs1800797)						
Genotypes						
G/G	27	64.3	82	74.5	>0.05	0.646 (0.33-1.28)
G/A	14	33.3	27	24.5		
A/A	1	2.4	1	0.9		
Alleles						
G	68	80.95	191	86.81	>0.05	
A	16	19.04	29	13.18		

The results that are statistically significant are typed in bold. OR: Odds ratio; CI: Confidence interval

not statistically different between OSCC patients and the control group.

IL-6 gene -597G/A Variant (rs1800797)

The frequencies of the G/G, G/A, and A/A genotypes of IL-6 -597G/A were 64.3%, 33.3%, and 2.4% in the OSCC patients and 74.5%, 24.5%, and 0.9% in controls, respectively. G and A allele frequencies were 80.95% and 19.04% in the OSCC patients, and 86.81% and 13.18% in controls, respectively. There was no significant difference between patients and controls in terms of genotype and allele distribution of the IL-6 gene -597G/A variant.

STRING Analysis

Analyzing the IL-6 protein with the STRING database, predicted the functional partners of the protein with high confidence (score: 0.7) was found as follows: Signal transducer and activator of transcription 3 (STAT3), Proto-Oncogene C-Jun (JUN), Interleukin-4 (IL-4), Interleukin-10 (IL-10), Interleukin-6 Receptor (IL6R), IL-6 Cytokine Family Signal Transduce (IL6ST), Interleukin 13 (IL13), Suppressor of Cytokine Signaling 3 (SOCS3), Interleukin 1B (IL1B), and C-X-C Motif Chemokine Ligand 8 (CXCL8). The interaction network of these proteins is shown in Figure 1.

DISCUSSION

Cancer development is a multistep process attributed to the accumulation of the risk factors in addition to the genetic susceptibility.[8] The molecular changes responsible for the initiation and progression of malignancy in normal epithelial cells are not yet well known. Other factors associated with the inflammatory response, angiogenic process, immune reactions, and thrombotic activity have also been associated with an increased risk of developing oral cavity cancer. The key element in this cancer development

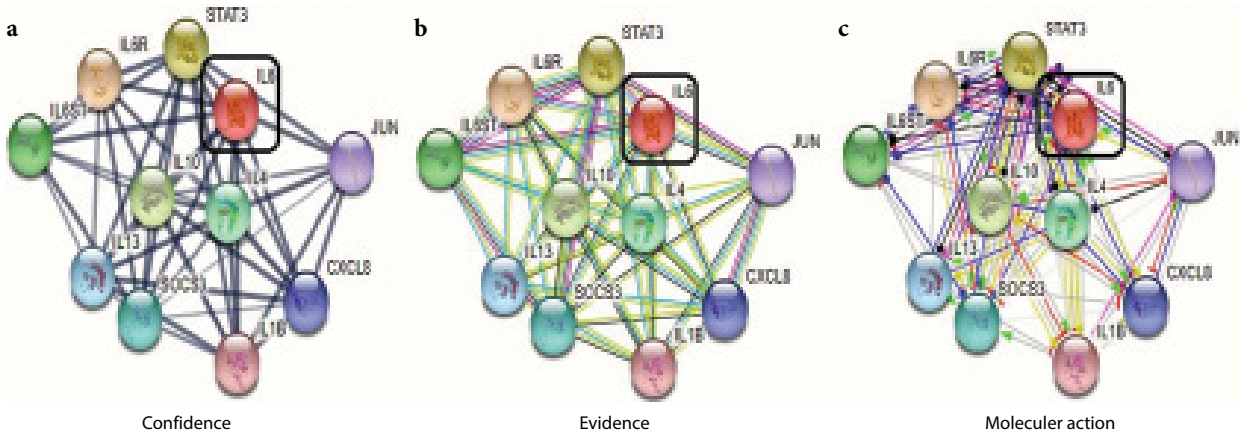

Fig. 1. Interactions of IL-6 protein, according to STRING database predictions. (a) Confidence network: stronger associations are represented by thicker lines, (b) this network represents the types of evidences for the association, (c) presentation of the different modes of action, involving in the protein–protein interactions.
SOCS3: Suppressor of Cytokine Signaling 3; STAT3: Signal transducer and activator of transcription 3; IL6R: Interleukin-6 Receptor; IL6ST: IL-6 Cytokine Family Signal Transduce; IL-4-6-10-13: Interleukin-4-6-10-13; IL1B: Interleukin 1B; CXCL8: C-X-C Motif Chemokine Ligand 8.

process is cytokines produced by activated innate immune cells that stimulate growth and progression in tumor cells. Recent studies have shown that genetic markers such as single-nucleotide polymorphisms (SNPs) may be associated with increased risk for specific diseases. The pathogenesis of OSCC is attributed to many factors, such as environmental, infections, diet, primarily tobacco, alcohol consumption, and viral agents. In addition, genetic and epigenetic changes are also effective in the development of malignant lesions of the oral cavity.

IL-6 is produced by various endothelial cells, malignant cells and immune cell types primarily macrophages, dendritic cells, and B-cells.[9] IL-6 mostly stimulates the JAK1/STAT3 signaling pathway and acts as a pro-inflammatory factor.[10] IL-6 promotes carcinogenesis in a variety of ways, including apoptosis, survival, proliferation, angiogenesis, invasion, metastasis, and metabolism.[11] IL-6 level is elevated in plasma and cancer tissue where it plays a role in the proliferation and differentiation of malignant cells.[11] Circulating high IL-6 level is generally associated with poor prognosis and indicates a high degree of tumor aggressiveness, while low serum IL-6 is thought to respond better to treatment.[12,13]

There have been many studies investigating possible genetic mutations that could be used as a predictive and diagnostic tool for OSCC.[14] Many of these mutations have been identified in different tissues such as exfoliated oral epithelial cells,[15] saliva,[16] or serum.[17] It is well know that OSCC tumor cells produce different types of cytokines primarily IL-1, IL-6, IL-8, and TNF-α.[18] Lee et al.[19] found increased IL-1β, IL-6, and IL-8 in saliva in OSCC carcinogenesis. In addition, a significant overexpression of IL-6 and IL-8 was demonstrated in OSCC using microarray.[20] Wang et al.[21] showed a remarkable expression of IL-6R and IL-6 mRNA in tissue samples from OSCC patients compared to normal mucosa. Furthermore, Chen et al.[22] showed that overexpression of IL-6 in affected cancer cells correlated with aggressive clinical behavior, particularly in the male population with OSCC, so IL-6 can be used as a predictive independent the factor for survival.

The regulation and expression of the IL-6 gene are modulated by the binding of several transcriptional factors to the specific promoter site. The promoter region of IL-6 contains localized SNPs that can induce some modification at the level of specific DNA sequences thereby altering the binding of various transcription factors to these cis-acting elements, resulting in gene expression and synthesis of IL-6. This may also explain differences in susceptibility to various diseases such as cancer among different individuals.[23] IL-6 -174G/C is close to the binding sites of several transcriptional factors, including NF-κB and NF-IL-6.[24] IL-6 -174G/C promoter variant, a critical moderator of Th2 reactions, have been associated with high IL-6 production.[25] Oligonucleotides containing guanine at -174 position have a greater DNA-protein interac-

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tion than those containing cytosine. Therefore, they have a greater affinity for transcription factors and subsequently cause increased expression of the IL-6 gene.[24] During the inflammation, the C allele of the IL-6 -174G/C gene variant elevates the level of IL-6 protein. The IL-6 -174G/C variant was observed to be significantly associated with many malignities including breast, ovarian, prostatic, gastric, cervical, and colorectal cancer.[26] Vairaktaris et al.[27] reported that IL-6 -174G/C variant G/C- C/C genotypes and C allele were associated with an increased risk of OSCC. Furthermore, Singh et al.[26] found significant differences in G/C genotype and C allele frequencies of the IL-6 -174 G/C variant between OSCC patients and controls in the Indian population. Zafar et al.[28] demonstrated that there was a significant association between OSCC stages (III and IV) and IL-6 -174G/C variant. Hsu et al.[29] showed an association between an increased risk of oral precancerous lesions and IL-6 -174G/C C allele. However, in another study performed by Gaur et al.,[30] it was reported that IL-6 -174G/C CC genotype and C allele appeared to be protective in patients with tobocco-related OSCC. These inconsistencies can be attributed to various factors considered in these studies including race, population size, sex, and disease status. In this study, we found that the IL-6 -174G/C G/C genotype was significantly associated with an increased risk of OSCC (Table 2). Our results were consistent with many studies.

The relationship between IL-6 -572G/C and circulating IL-6 levels has been inconclusive in previous genetic studies. Shin et al.[31] observed that the IL-6 -572 G/G genotype was associated with significantly higher levels of circulating IL-6 than the C/C or C/G genotype. Furthermore, Seow et al.[32] reported that the IL-6 -572G/C G allele was associated with increased IL-6 protein level in serum compared to the C allele. Zhang et al.[33] found that IL-6 -572C/G genotype distribution differed between patients with breast cancer and controls. However, there was no significant difference between IL-6 -572C/G genotypes and serum IL-6 levels in these patients and healthy controls. In studies in patients with hepatocellular carcinoma and gastric adenocarcinoma, the IL-6 -572 G/C genotype and allele frequency were not different between patients and controls.[34,35] Singh et al.[26] reported that the IL-6 -572G/C variant was not significantly associated with OSCC patients. In this study, there was no significant difference between OSCC patients and controls in terms of genotype and allele distribution of the IL-6 -572G/C variant.

IL-6 -597G/A is another variant in the promoter region of the IL-6 gene that can modulate the effect of the IL-6-174G/C on transcription activity.[24] De Michele et al.[36] observed that IL-6 -597G/A G/G genotype was significantly associated with worse disease and free survival in patients with breast cancer. In addition, they found that IL-6 -597G/A genotype distribution was significantly associated with the number of positive lymph nodes. Snoussi et al.[37] showed that IL-6 -597G/A heterozygous genotype was related to breast carcinoma. Wilkening et al.[38] found that patients with skin basal cell carcinoma had a higher IL-6 -597G/A G/G genotype. Gupta et al.[39] reported that IL-6 -597G/A variant G/G and G/A genotype have been reported to be more common in patients with OSCC than in controls. In this study, genotype and allele distributions of IL-6 -597G/A were not significantly different between OSCC patients and controls.

There are several limitations to this analysis. First, only three variants of IL-6 were evaluated. Second, the gene-gene and gene-environment interactions were not investigated. Finally, IL-6 expression level was not examined in this study.

CONCLUSION

Our current investigation is focused on the role of variants of IL-6 gene on OSCC. To the best of our knowledge, this study is the first study to evaluate the genotype and allele frequencies of IL-6 -174G/C, -572G/C, and -597G/A variants in patients with OSCC in a Turkish population. Our results support that the IL-6 -174G/C variant may play an important role in susceptibility to OSCC in our population.

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Ethics Committee Approval: The study was approved by the Hitit University, Faculty of Medicine Ethics Committee (no: 2017/200, date: 19/12/2017).

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REFERENCES

1. Sun Y, Liu N, Guan X, Wu H, Sun Z, Zeng H. Immunosuppression induced by chronic inflammation and the progression to oral squamous cell carcinoma. Mediators Inflamm 2016;2016:5715719.
2. Vigneswaran N, Williams MD. Epidemiologic trends in head and neck cancer and aids in diagnosis. Oral Maxillofac Surg Clin North Am 2014;26(2):123–41.
3. Ishihara K, Hirano T. IL-6 in autoimmune disease and chronic inflammatory proliferative disease. Cytokine Growth Factor Rev 2002;13:357–68.
4. Jawa RS, Anillo S, Huntoon K, Baumann H, Kulaylat M. Interleukin-6 in surgery, trauma, and critical care part II: clinical implications. Interleukin-6 in surgery, trauma, and critical care part II: clinical implications. J Intensive Care Med 2011;26(2):73–87.
5. Li F, Xu J, Zheng J, Sokolove J, Zhu K, Zhang Y, et al. Association between interleukin-6 gene polymorphisms and rheumatoid arthritis in Chinese Han population: a case-control study and a meta-analysis. Sci Rep 2014;4:5714.
6. Zhai K, Yang Y, Gao ZG, Ding J. Interleukin-6-174G>C gene promoter polymorphism and prognosis in patients with cancer. Oncotarget 2017;8(27):44490–97.
7. Tseng LH, Chen PJ, Lin MT, Singleton K, Martin EG, Yen AH, et al. Simultaneous genotyping of single nucleotide polymorphisms in the IL-6, IL-10, TNFalpha and TNFbeta genes. Tissue Antigens 2002;59:280–86.
8. Kim MM, Califano JA. Molecular pathology of head-and-neck cancer. Int J Cancer 2004;112:545–54.
9. Choudhary MM, France TJ, Teknos TN, Kumar P. Interleukin-6 role in head and neck squamous cell carcinoma progression. World J Otorhinolaryngol Head Neck Surg 2016;2(2):90–7.
10. Heinrich PC, Behrmann I, Haan S, Hermanns HM, Müller-Newen G, Schaper F. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J 2003;374(Pt 1):1–20.
11. Kumari N, Dwarakanath BS, Das A, Bhatt AN. Role of interleukin-6 in cancer progression and therapeutic resistance. Tumour Biol 2016;37:11553–72.
12. Chen MF, Chen PT, Lu MS, Lin PY, Chen WC, Lee KD. IL-6 expression predicts treatment response and outcome in squamous cell carcinoma of the esophagus. Molecular cancer 2013;12(1):26.
13. Guo Y, Xu F, Lu T, Duan Z, Zhang Z. Interleukin-6 signaling pathway in targeted therapy for cancer. Cancer treatment reviews 2012;38(7):904–10.
14. Lippman SM, Hong WK. Molecular markers of the risk of oral cancer. N Engl J Med 2001;344:1323–26.
15. Mehrotra R, Gupta A, Singh M, Ibrahim R. Application of cytology and molecular biology in diagnosing premalignant or malignant oral lesions. Mol Cancer 2006;5:11.
16. Wu JY, Yi C, Chung HR, Wang DJ, Chang WC, Lee SY, et al. Potential biomarkers in saliva for oral squamous cell carcinoma. Oral Oncol 2010;46:226–31.
17. Feng XY, Li JH, Li JZ, Han ZX, Xing RD. Serum SCCA, Cyfra 21-1, EGFR and Cyclin D1 levels in patients with oral squamous cell carcinoma. Int J Biol Markers 2010;25(2):93–8.
18. Cohen RF, Contrino J, Spiro JD, Mann EA, Chen LL, et al. Interleukin-8 expression by head and neck squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 1995;121(2):202–9.
19. Lee LT, Wong YK, Hsiao HY, Wang YW, Chan MY, Chang KW. Evaluation of saliva and plasma cytokine biomarkers in patients with oral squamous cell carcinoma. Int J Oral Maxillofac Surg 2018;47(6):699–707.
20. Alevizos I, Mahadevappa M, Zhang X, Ohyama H, Kohno Y, Posner M, et al. Oral cancer *in vivo* gene expression profiling assisted by laser capture microdissection and microarray analysis. Oncogene 2001;20(43):6196–204.
21. Wang YF, Chang SY, Tai SK, Li WY, Wang LS. Clinical significance of interleukin-6 and interleukin-6 receptor expressions in oral squamous cell carcinoma. Head Neck 2002;24(9):850–58.
22. Chen CJ, Sung WW, Lin YM, Chen MK, Lee CH, Lee H, et al. Gender difference in the prognostic role of interleukin 6 in oral squamous cell carcinoma. Plos One 2012;7(11):e50104.
23. Banday MZ, Balkhi HM, Sameer AS, Chowdri NA, Haq E. Strong association of interleukin-6 –174G/C promoter single nucleotide polymorphism with a decreased risk of colorectal cancer in ethnic Kashmiri population: A case control study. Tumour Biol 2017;39(3):1010428317695940.
24. Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin-6 transcriptional regulation. J Biol Chem 2000;275(24):18138–44.
25. Savage SA, Abnet CC, Haque K, Mark SD, Qiao YL, Dong ZW, et al. Polymorphisms in interleukin – 2, – 6, and – 10 are not associated with gastric cardia or esophageal cancer in a high-risk Chinese population. Cancer Epidemiol Biomarkers Prev 2004;13(9):1547–9.
26. Singh PK, Chandra G, Bogra J, Gupta R, Kumar V, Jain A, et al. Association of interleukin-6 genetic polymorphisms with risk of OSCC in Indian population. Meta Gene 2015;4:142–51.
27. Vairaktaris E, Yiannopoulos A, Vylliotis A, Yapijakis C, Derka S, Vassiliou S, et al. Strong association of interleukin-6 -174 G>C promoter polymorphism with increased risk of oral cancer. J Biol Markers 2006;21:246–50.
28. Zafar M, Hadi NI, Baig S, Zehra N. Association between Interleukin 6 gene polymorphism and human papilloma virus infection in oral squamous cell carcinoma patients. BJMMR 2015;10(6):1–9.

Turk J Oncol 2022;37(4):371–78
doi: 10.5505/tjo.2022.3624

29. Hsu HJ, Yang YH, Shieh TY, Chen CH, Kao YH, Yang CF, et al. Role of cytokine gene (interferon-γ, transforming growth factor-β1, tumor necrosis factor-α, interleukin-6, and interleukin-10) polymorphisms in the risk of oral precancerous lesions in Taiwanese. Kaohsiung J Med Sci 2014;30(11):551–58.
30. Gaur P, Mittal M, Mohanti B, Das S. Functional variants of IL4 and IL6 genes and risk of tobacco-related oral carcinoma in high-risk Asian Indians. Oral Dis 2011;17(7):720–6.
31. Shin KK, Jang Y, Koh SJ, Chae JS, Kim OY, Park S, et al. Influence of the IL-6 -572C>G polymorphism on inflammatory markers according to cigarette smoking in Korean healthy men. Cytokine 2007;39(2):116–22.
32. Seow A, Ng DP, Choo S, Eng P, Poh WT, Ming T, et al. Joint effect of asthma/atopy and an IL-6 gene polymorphism on lung cancer risk among lifetime non-smoking Chinese women. Carcinogenesis 2006;27:1240–44.
33. Zhang Z, Chen Z, Chen D, Lin Y, Jiang Y, Wang Q. Association between IL-6-572 C/G polymorphism and breast cancer susceptibility. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2016;32(12):1667–70.
34. Liu S, Qiu X, Zeng X, Bai H, Bei C, Yang Y. Relationship between IL6-572G/C polymorphism and hepatocellular carcinoma in men. Zhonghua Gan Zang Bing Za Zhi 2012;20(6):463–67.
35. Gatti LL, Burbano RR, Zambaldi-Tunes M, de-Lábio RW, de Assumpção PP, de Arruda Cardoso-Smith M, et al. Interleukin-6 polymorphisms, Helicobacter pylori infection in adult Brazilian patients with chronic gastritis and gastric adenocarcinoma. Arch Med Res 2007;38(5):551–5.
36. DeMichele A, Gray R, Horn M, Chen J, Aplenc R, Vaughan WP, et al. Host genetic variants in the interleukin-6 promoter predict poor outcome in patients with estrogen receptor-positive, node-positive breast cancer. Cancer Res 2009;69(10):4184–91.
37. Snoussi K, Strosberg AD, Bouaouina N, Ben Ahmed S, Chouchane L. Genetic variation in pro-inflammatory cytokines (interleukin-1beta, interleukin-1alpha and interleukin-6) associated with the aggressive forms, survival, and relapse prediction of breast carcinoma. Eur Cytokine Netw 2005;16(4):253–60.
38. Wilkening S, Hemminki K, Rudnai P, Gurzau E, Koppova K, Kumar R, et al. Case-control study in basal cell carcinoma of the skin: single nucleotide polymorphisms in three interleukin promoters pre-analysed in pooled DNA. Br J Dermatol 2006;155(6):1139–44.
39. Gupta MK, Sagar N, Pant R, Banarjee M. Cytokine gene polymorphisms and their association with oral squamous cell carcinoma (OSCC): a North Indian study. EJPMR 2016:3(8):550–58.