RESEARCH ARTICLE

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Association of Interleukin-10 Genotypes and Oral Cancer Susceptibility in Selected Malaysian Population: A Case-Control Study

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Abstract

Background: Interleukin-10 (IL10) genotypes have been closely correlated to the susceptibility for oral squamous cell carcinoma. More than half of oral cancers in the world occur in Asia with estimated 168,850 new cases were diagnosed in this geographical region alone. Considering the rising numbers of oral cancer cases in Malaysia, association of IL10 A1082G gene polymorphism was correlated. **Methodology:** 41 oral squamous cell carcinoma (OSCC) cases and 48 healthy controls of comparable age, gender, and with habits like smoking, alcohol consumption and betel quid chewing were selected. In this case-control study, samples were collected from the Oral Cancer Research and Coordinating Centre (OCRCC), Faculty of Dentistry, University of Malaya, Malaysia. Genotyping conditions were evaluated by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). The PCR products were subjected to digestion by MnlI enzyme (NEB, UK) to screen for the IL10 A-1082G. Digested DNA products were analyzed by electrophoresis on 4% (w/v) agarose gel, stained with ethidium bromide and imaged under UV illumination. Chi-square test and Fisher's Exact test were used in statistical analysis. **Results:** AG genotypes were present in 81.3% and 86.0% of healthy control and OSCC cases respectively (OR=0.468, 95% CI=0.133-1.653). No significant association was found between IL10 A1082G polymorphism with risk habits, clinico-pathological parameters and 5-years overall survival. The findings also show no significant correlation between the IL10 genotype and features of OSCC within the case group as measured by tumor size, lymph node involvement, stage, invasive front, grading, depth, pattern of invasion. **Conclusion:** This study suggests that functional polymorphism AG of IL10 A1082G may have no influence with OSCC susceptibility. However, further investigation with larger sample sizes can be conducted to provide additional evidence to support the lack of association of IL10 A1082G polymorphism in oral cancer.

Keywords: Genotype- interleukin-10- oral cancer- oral squamous cell carcinoma- polymorphism

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Introduction

Oral cancer also known as mouth cancer is a ceaseless, purposeless, uncoordinated growth of the tissue in the oral cavity. Though it can occur in any sub-sites of oral cavity, most common sub-sites are lip, tongue and oral cavity (Azizah et al., 2016). Around the globe, new oral cancers cases accounted for 300,373 and 145,353 cancer deaths in 2012 (Ferlay et al., 2015). The mortality to oral cancers in South East Asian region (SEA) has been increasing every year. Oral squamous cell carcinomas (OSCC) accounts for 90% of oral cancers are aggressive with a high recurrence rate and metastasis to lymph nodes. Study reported OSCC as worldwide eight common human malignancies with more than 4,260,000 patients and 128,000 deaths each year (Peterson, 2005). In Malaysia, oral cancer is a serious health problem. It ranks one among the common cancer having high incidence of mortality in the Indian ethnic population in Malaysia (Azizah et al., 2016). Many epidemiological studies revealed several risk factors like tobacco, alcohol, betel quid chewing, nutritional deficiency and environmental factors associated with oral cancer. In recent years, several lines of evidence showed cytokine like Interleukin 10 (IL10) plays critical role in the etiology and progression of various types of cancers (Yu et al., 2013).

Oral cancer progression is a complex process that involves host-tumor interactions, which occur via

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multiple molecular and cellular factors within the tumor microenvironment. The genetic abnormalities of human cancer to a certain extent depend on geographical location, cultural and environmental backgrounds and also cell lines in which it is expressed. IL10 operates through the Janus kinase–signal transducer and activator of transcription (JAK–STAT) signaling pathway by blocking nuclear factor-kappa-B (NF-κB) nuclear translocation. Disruption of the interactions of IL10 with its receptors (IL10RA and IL10RB) and α-2-macroglobulin (A2M) may lead to enhanced inflammation, which could promote tumor growth, blockage of the A2M-APP interaction may lead to cancerous cellular proliferation through free APP, blockage of A2M-KLK13 (Hk13) interaction can increase free Hk13, which can promote cancer cell growth, metastasis and invasion through damage in the extracellular matrix (Acuner-Ozbabacan et al., 2014). The balance of immune responses between Th1/Th2 determines outcomes of diseases. Th1 cells produce interferon (IFN) γ, IL2, IL12 and tumor necrosis factors α cytokines, which are involved in the cell-mediated pro-inflammatory response (Lippitz, 2013). Th2 cells secrete IL4, IL5, IL6, IL10 and IL13 cytokines, which mediate anti-inflammatory humoral response and immune suppression via the inhibition of Th1 cytokine production. TGFβ1, a member of the TGFβ family that is predominantly secreted by regulatory T-cells (Tregs), is another multi-functional cytokine. It promotes tumor progression by inducing mesenchymal transition, tumor escape by antagonizing IL2 functions and inducing immune suppression, tumor invasion and metastasis. Previous study concluded, that there was a highly significant contributions from IL6 and TNF-α in occurrence of OSCC than other interleukins like IL4, 8 and 10 (Vairaktaris et al., 2008).

IL10 also known as human cytokine synthesis inhibitory factor (CSIF), is an anti-inflammatory cytokine identified in 1989. It mainly modulates immune response and if not present, inflammation becomes possible. The main source of IL10 production is from macrophage and is encoded by a gene located on chromosome 1 at 1q31-32 which has five exons and four introns (Ghazaleh Shoja E. Razavi and Allen, 2014). IL10 has multiple functions, such as inhibition of cytokine production, T-cells proliferation, angiogenesis and regulation of inflammatory responses. The dynamic role of IL10 in tumorigenesis and tumor inhibition is of debate. Elevated levels of IL10 production have been identified in oral cancers and solid tumors (de Vries, 1995; Fortis et al., 1996; Tsai et al., 2014). Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation among humans. It is the difference of a nucleotide, namely adenine (A), thymine (T), cytosine (C) and guanine (G), between a pair of chromosomes. The etiology of a specific cancer might be associated with genetic variants. IL10 polymorphisms like A1082G/AA/AG/GG genotypes may not only contribute to differential expression levels of IL10 among individuals but may also lead the risk oral cancer susceptibility (Hussain et al., 2016).

Apart from oral cancers, studies showed IL10 polymorphisms to various cancer types like breast carcinoma, hepatocellular carcinoma (Tseng et al., 2006), cervical carcinoma (Trabace et al., 2002), renal cell carcinoma (Havranek et al., 2005), multiple myeloma (Savage et al., 2004), and malignant melanomas (Palli et al., 2005). Human papilloma virus (HPV) infection, in advanced stage of oral cancer patients has high risk of mortality and morbidity. Previous studies showed IL10 expression levels were significantly higher in individuals with HPV-16 infected high grade of cervical neoplasia, when compared with normal cervical epithelium. This suggests IL10 may play a role in progression of HPV-associated cervical pre-cancers (Cheong et al., 2017).

To our knowledge, no studies to date have examined the association between genetic polymorphisms in IL10 gene and oral cancer in Malaysian population. This study, investigates the association of IL10 promoter genotypes with oral cancer susceptibility and also the association among the IL10 genotypes with individual risk factors (smoking, alcohol consumption and betel quid chewing habit) in selected Malaysian population.

Materials and Methods

Study population and sample collection

Eighty-nine GenomiPhi amplified DNA blood nucleotide acid samples, derived from 41 oral cancer patients and 48 healthy controls were obtained by convenient sampling method from Malaysian Oral Cancer Database and Tissue Bank System (MOCDTBS) which was coordinated by the Oral Cancer Research and Coordinating Centre (OCRCC), Faculty of Dentistry, University of Malaya, Malaysia. Data were collected using a structured questionnaire and electronically stored in the MOCDTBS (Zain et al., 2013). Patients' socio-demographic information, such as age, gender, ethnicity and risk habits (smoking, drinking and betel quid chewing habits) and clinico-pathological data such as site, tumor size (pT), lymph node metastasis (pN), staging (pTNM), borders grading, pattern of invasion and depth of invasion was collected. Informed consent was obtained from all the individuals involved in study. The ethical approval for this study was obtained from the Medical Ethics Committee from Faculty of Dentistry, University of Malaya (DF OS1713/0037[L]).

Polymerase Chain Reaction (PCR)

PCR was carried out to amplify the targeted SNP region as described previously (Tsai et al., 2014). Briefly, 0.5 μL of DNA template was mixed with oligonucleotide forward primer (5'-CTC GCT GCA ACC CAA CTG GC-3') and reverse primer (5'- TCT TAC CTA TCC CTA CTT CC-3') and GoTaq(R) Green Master Mix (Promega, USA). The following thermo cycler conditions were performed: initial denaturation at 94°C for 3 min; 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds; and then a final extension at 72°C for 10 min.

Genotyping conditions

The PCR products were subjected to digestion by MnlI enzyme (NEB, UK) to screen for the IL10 A1082G. The enzymatic mixture consisted of 1 μL restriction enzyme, 1

μL 10× buffer, 6 μL PCR products and 2 μL distilled water; the mixture was incubated for 5 h at 37°C for digestion. Digested DNA products were analyzed by electrophoresis on 4% (w/v) agarose gel, stained with ethidium bromide and imaged under UV illumination.

Statistical analysis

Pearson's Chi-square test was used to compare the distribution of the IL10 genotypic and allelic frequencies between case and control groups. The odds ratios (OR) together with 95% confidence intervals (CI) were calculated to assess the relative risk conferred by a particular allele and genotype. Socio-demographic and clinico-pathologic data between groups were compared using Chi-square test and Fisher's Exact Test. Result was significant at a statistical level when the p-value was less than 0.05.). A log- rank test and Kaplan-Meier curve were used to determine significant differences in the survival distribution for the different IL10 genotypes AA and AG.

Results

There was no significant difference between oral cancer and control groups in the distribution of genotypic frequency (p=0.231) and the OR for AG was 0.468 (95% CI=0.133-1.653) compared to those who carried the AA wild-type genotype. The socio-demographic characteristics of 41 patients with oral cancer and 48 healthy controls are summarized in Table 1. The gender distribution between the control group and case group were similar. However, there was significant higher proportion of patients older than 50 years in case group (80.5%) compared to the control group (47.9%). The mean age for the control group was 46.23 ± 13.86 years of age while that of the case group was 57.88 ± 12.75 years of age. The p-value of 0.002 shows that increasing age above 50 years is significantly correlated with incidence of oral cancer. Ethnic distribution was selected to be approximately similar across both groups.

There were more individuals in the case group with alcohol consumption (p=0.024) and areca chewing habits (p<0.001) but smoking habits (p=0.648) remained lower in this group compared to the control group. Approximately half (47.1%) of the oral cancer cases were found to have invaded adjacent structures (T4). The majority of cases exhibited no lymph node invasion (57.6%) and all cases showed no metastasis. The majority (55.9%) of oral cancer cases were recorded at stage 4, with 63.6% exhibiting a non-cohesive invasion front and 57.1% were graded as moderately differentiated. Depth of invasion tended to be more than 8mm (60.0%) with more non-cohesive pattern of invasion seen (66.7% in type 3 and 4 group). The findings show no significant correlation between the IL10 genotype and features of oral cancer within the case group as measured by tumor size, lymph node involvement, stage, invasive front, grading, depth, pattern of invasion (Table 2). There was no significant correlation found between the IL10 A1082G genotype with smoking, alcohol consumption, and betel quid chewing habit in the control and case groups (Table 3). The survival distributions for the genotypes were also found to be not statistically significant (Figure 1).

Discussion

Globally, the prevalence of oral cancer distribution was 11% in SEA (South-east Asia). In Malaysia, the reported mortality rate was predicted to be increase from 253 in 2012 to 336 by 2020 giving a possible increase of 32.8% in mortality rate (Cheong et al., 2017). This potential increase may be due to the increase in use of tobacco smoking, smokeless tobacco and betel quid chewing which is highly associated with oral cancers. However, the chances of a true burden to oral cancers may be significantly under-reported due to various social and cultural factors in SEA countries.

In the studied individuals, polymorphisms in the promoter region could influence the expression levels of IL10 for pathogenesis of various types of cancer. Specified genetic factors influence or regulate the level of expression of IL10 gene like polymorphisms at the location A1082G in the promote region of the gene (Linnet al., 2003). Previous study reported high expression levels of IL10 A1082G polymorphism have been observed in OSCC (Karcher et al., 1999). In a study done in Taiwanese population AG and GG genotypes of IL10 A1082G were significantly associated with a higher susceptibility for oral cancer. While it is known that smoking, betel quid chewing habits contribute to carcinogenesis, the AG and GG genotypes of IL10 A1082G in combination with these established risk factors may also contribute to carcinogenesis. At the same time, no obvious association between IL10 A1082G genotype and alcohol drinking was found (Tsai et al., 2014). Another case control study in India Hussain et al., (2016) revealed that among AA, AG, GG genotypes, an increase AG genotype cases as compared to control was found to be statistically significant. Thus the G allele of IL10 may be involved in the progression of OSCC. Polymorphisms in the IL10 gene promoter may leave a close relation with changes in IL10 expression, thus leading to occurrence of cancers with poor prognosis.

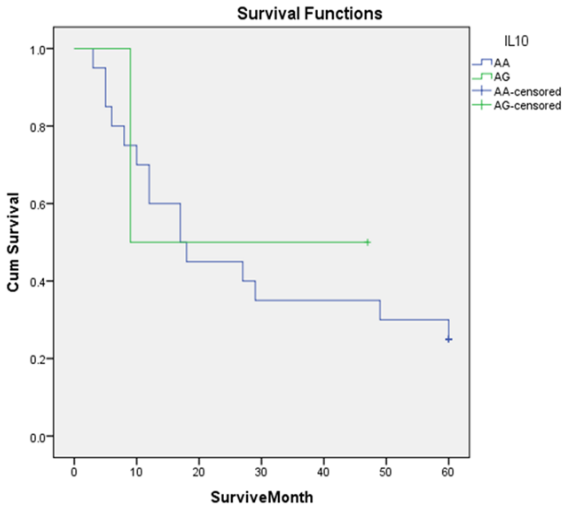

Figure 1. Five Years Overall Survival Analysis Using Kaplan-Meier Procedure, p=0.801 (Log-Rank test)

Table 1. Comparison of Sociodemographic, Clinicopathological Data and IL-10 Genotyping Status of Study Population

Variables	Control n	(%)	Case n	(%)	OR (95% CI)	p-value*
Gender						
Male	17	35.40%	16	39.00%	reference	
Female	31	64.60%	25	61.00%	0.857 (0.362-2.03)	0.725
Age						
≤50	25	52.10%	8	19.50%	reference	
>50	23	47.90%	33	80.50%	4.484 (1.721-11.684)	0.002
Race						
Indian	12	25.00%	12	29.30%	reference	
Malay	18	37.50%	11	26.80%	0.611 (0.204-1.830)	0.378
Chinese	6	12.50%	5	12.20%	0.833 (0.199-3.484)	0.803
Other	12	25.00%	13	31.70%	1.083 (0.353-3.322)	0.889
Smoke						
No	33	68.80%	30	73.20%	reference	
Yes	15	31.30%	11	26.80%	0.807 (0.321-2.028)	0.648
Drink						
No	43	89.60%	29	70.70%	reference	
Yes	5	10.40%	12	29.30%	3.559 (1.133-11.179)	0.024
Chew						
No	42	87.50%	19	46.30%	reference	
Yes	6	12.50%	22	53.70%	8.105 (2.828-23.228)	<0.001
IL-10						
AA	39	81.30%	37	86.00%	reference	
AG	9	18.70%	4	14.00%	0.468 (0.133-1.653)	0.231
Site (n = 36)						
Buccal + Gingiva			24	66.70%		
Tongue + FOM			12	33.30%		
Size (n = 34)						
T1			6	17.60%		
T2			9	26.50%		
T3			3	8.80%		
T4			16	47.10%		
Lymph Node (n = 33)						
N0			19	57.60%		
N1			2	6.10%		
N2			11	33.30%		
N3			0	0.00%		
N4			1	3.00%		
Metastasis (n = 11)						
M0			11	100.00%		
Stage (n = 34)						
Stage 1			6	17.60%		
Stage 2			7	20.60%		
Stage 3			2	5.90%		
Stage 4			19	55.90%		
Invasive Front (n = 11)						
Cohesive			4	36.40%		
Non-cohesive			7	63.60%		

Table 1. Continued

Variables	Control n	(%)	Case n	(%)	OR (95% CI)	p-value*
Grading (n = 21)						
Well differentiated			9	42.90%		
Moderately differentiated			12	57.10%		
Depth (n = 10)						
0 - 4mm			3	30.00%		
>4 - 8mm			1	10.00%		
>8mm			6	60.00%		
Pattern of Invasion (n = 6)						
Type 1			1	16.70%		
Type 2			1	16.70%		
Type 3			2	33.30%		
Type 4			2	33.30%		
Type 1 & 2			2	33.30%		
Type 3 & 4			4	66.70%		
Survival Months (n = 11)						
0 - 20			8	72.70%		
21 - 40			1	9.10%		
41 - 60			1	9.10%		
60 - 80			1	9.10%		
Survival Status (n = 26)						
Alive			3	11.50%		
Deceased			12	46.20%		
Ltf			11	42.30%		
Chemotherapy (n = 36)						
No			35	97.20%		
Yes			1	2.80%		
Radiotherapy (n = 36)						
No			36	100.00%		
Yes			0	0%		

OR, Odds Ratio; CI, Confidence Interval; *Based on Chi Square Test; p-value less than 0.05 is taken as significant

Table 2. Comparison of Clinicopathological Data with IL-10 Genotypes

Variables	IL-10 genotypes				OR (95% CI)	p-value
	AA (n)	(%)	AG (n)	(%)		
Tumour Size						
T1	5	16.70%	1	25.00%		
T2	9	30.00%	0	0%		
T3	2	6.70%	1	25.00%		
T4	14	46.70%	2	50%	-	0.442*
Lymph Node						
N0	17	58.60%	2	50.00%		
N1	2	6.90%	0	0%		
N2	9	31.00%	2	50.00%		
N3	0	0%	0	0%		
N4	1	3.40%	0	0%	-	0.840*
Stage						
Stage 1	6	20.00%	0	0%		
Stage 2	6	20.00%	1	25.00%		
Stage 3	2	6.70%	0	0%		
Stage 4	16	53.00%	3	75.00%	-	0.704*

Table 2. Continued

Variables	IL-10 genotypes				OR (95% CI)	p-value
	AA (n)	(%)	AG (n)	(%)		
Invasive Front						
Cohesive	4	44.40%	0	0%	reference	
Non-cohesive	5	55.60%	2	100.00%	NIL (0.876-2.237)	0.491†
Grading						
Well Differentiated	8	44.40%	1	33.30%	reference	
Moderately Differentiated	10	55.60%	2	66.70%	1.600 (0.122-20.993)	0.719*
Depth						
0 - 4mm	3	33.30%	0	0%		
>4 - 8mm	1	11.10%	0	0%		
>8mm	5	55.60%	1	100.00%	-	0.690*
Pattern of Invasion						
Type 1	1	20.00%	0	0%		
Type 2	1	20.00%	0	0%		
Type 3	1	20.00%	1	100.00%		
Type 4	2	40.00%	0	0%	-	0.494*
Type 1 & 2	2	40.00%	0	0.00%		
Type 3 & 4	3	60.00%	1	100.00%	-	1.000*
Survival Months						
0 - 20	7	70.00%	1	100.00%		
21 - 40	1	10.00%	0	0%		
40 - 60	1	10.00%	0	0%		
60 - 80	1	10.00%	0	0%	-	0.938*
Survival Status						
Alive	3	13.60%	0	0%		
Deceased	11	50.00%	1	25.00%		
Ltf	8	36.40%	3	75.00%	-	0.333*

OR, Odds Ratio; CI, Confidence Interval; * Chi-square test; † Fisher's Exact Test; p-value less than 0.05 is taken as significant

Knowing the expression levels of IL10 may contribute to the prediction of oral cancer. Thus, we selected a polymorphic site within the promoter region of the IL10 A1082G (RS 1800896) and studied its susceptibility to oral cancer in selected Malaysian population. In the current study, the gene-lifestyle interactions with personal risk habits for oral cancer were analyzed. From the results of our study, AG genotypes of IL10 A1082G were not associated with oral cancer susceptibility in this selected Malaysian population. There was no significant differential distribution of the IL10 A1082G genotypic frequencies among oral cancer cases and healthy controls. Consistent with our findings, IL10 A1082G was not found to be associated with oesophageal squamous cell carcinoma (Guo et al., 2005) and gastro-esophageal junction adenocarcinoma (Savage et al., 2004).

Our study was also consistent with a study done to determine the role of polymorphisms of the IL10 and

Table 3. Comparison of Genotype in IL10 A-1082G in OSCC Patients and Healthy Controls with Individuals under Smoking, Drinking and Chewing Criteria

Habit groups	Control n	(%)	Case n	(%)	OR (95% CI)	p value
Smokers	(n=15)		(n=11)			
AA	12	80.00%	10	90.90%	reference	
AG	3	20.00%	1	9.10%	0.400 (0.036-4.470)	0.446*
Drinkers	(n=5)		(n=12)			
AA	4	75.00%	11	91.70%	reference	
AG	1	25.00%	1	8.30%	0.364 (0.018-7.295)	0.515†
Chewers	(n=6)		(n=22)			
AA	6	100.00%	19	86.40%	reference -	
AG	0	0 %	3	13.60%	NIL (0.610-0.947)	0.338*

OR, Odds Ratio, CI, Confidence Interval; *Chi-square test; † Fisher's exact test; p-value less than 0.05 is taken as significant

TNF-alpha promoter genes in carcinogenesis of gastric cancer of Koreans (Lee et al., 2005). They found that there was no difference in genotypes between study group and healthy controls, concluding that IL10 A1082G genetic polymorphism may not be important contributors to gastric carcinoma in Korean.

Though various cancer cells produce IL10, and circulating concentrations of IL10 have been shown in 13 different cancer types including oral cancer, their biological pathway may illustrate the relative significance of each polymorphic site in the final clinical result. In a meta-analysis of IL10 A592C and T819C, it was found that the polymorphisms sites had no relationship with oral cancer risk. However, even though it is not significant, it was also shown that there is an increased risk by IL10 A1082G polymorphisms, but authors suggest to verify the results with a larger sample size (You et al., 2015). The underlying mechanism of IL10 affecting tumor progression is yet undefined. Hsu et al., (2015) also could not find an association between cytokine gene polymorphisms with oral cancer and did not detect any significant association of oral cancer with IL10 A1082G although, an elevated oral cancer risk involving IL10 A592C, T-819C and TGF-β1 polymorphisms was found in this population (Hsu et al., 2015).

In conclusion, it is suggestive that the functional polymorphism of IL10 A1082G may not have any influence with oral cancer susceptibility. However, further investigation with larger sample sizes can be conducted to provide additional evidence to support the lack of association of IL10 A-1082G polymorphism in oral cancer.

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References

Acuner-Ozbabacan E, Engin B, Guven-Maiorov E, et al (2014). The structural network of Interleukin-10 and its implications in inflammation and cancer. *BMC Genomics*, **15**, S2.

Cheong SC, Vatanasapt P, Yi-Hsin Y, et al (2017). Oral cancer in South East Asia. *Trans Res Oral Oncol*, **2**, 2057178X1770292.

de Vries JE (1995). Immunosuppressive and anti-inflammatory properties of interleukin 10. *Ann Med*, **27**, 537-41.

Ferlay J, Soerjomataram I, Dikshit R, et al (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*, **136**, E359-86.

Fortis C, Foppoli M, Gianotti L, et al (1996). Increased interleukin-10 serum levels in patients with solid tumours. *Cancer Lett*, **104**, 1-5.

Ghazaleh Shoja ER, Allen T (2014). Emerging role of interleukins in cancer treatment. *Immunome Res*, **1**.

Guo W, Wang N, Wang Y-M, et al (2005). Interleukin-10 -1082 Promoter Polymorphism is Not associated with Susceptibility to Esophageal Squamous Cell Carcinoma and Gastric Cardiac Adenocarcinoma in a Population of High-Incidence Region of North China. *World J Gastroenterol*, **11**, 858-62.

Havranek E, Howell W, Fussell H, et al (2005). An interleukin-10 promoter polymorphism may influence tumor development in renal cell carcinoma. *J Urol*, **173**, 709-12.

Hsu H-J, Yang Y-H, Shieh T-Y, et al (2015). TGF-β1 and IL-10 Single Nucleotide Polymorphisms as Risk Factors for Oral Cancer in Taiwanese. *Kaohsiung J Med Sci*, **31**, 123-9

Hussain SR, Ahmad MK, Mahdi AA, et al (2016). Association of interleukin-10 (A1082G) gene polymorphism with oral squamous cell carcinoma in north Indian population. *J Genet*, **95**, 249-55.

Lee JY, Kim HY, Kim KH, et al (2005). Association of polymorphism of IL-10 and TNF-A genes with gastric cancer in Korea. *Cancer Lett*, **225**, 207-14.

Lippitz BE (2013). Cytokine patterns in patients with cancer: a systematic review. *Lancet Oncol*, **14**, e218-e28.

Palli D, Saieva C, Luzzi I, et al (2005). Interleukin-1 gene polymorphisms and gastric cancer risk in a high-risk Italian population. *Am J Gastroenterol*, **100**, 1941-8.

Peterson PE (2005). Strengthening the prevention of oral cancer: the WHO perspective. *Community Dent Oral Epidemiol*, **33**, 397-9.

Savage SA, Abnet CC, Haque K, et al (2004). 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*, **13**, 1547-9.

Trabace S, Brioli G, Lulli P, et al (2002). Tumor necrosis factor gene polymorphism in migraine. *Headache J Head Face Pain*, **42**, 341-5.

Tsai CW, Chang WS, Lin KC, et al (2014). Significant association of Interleukin-10 genotypes and oral cancer susceptibility in Taiwan. Anticancer Res, 34, 3731-7.

Tseng LH, Lin MT, Shau WY, et al (2006). Correlation of interleukin-10 gene haplotype with hepatocellular carcinoma in Taiwan. *Tissue Antigens*, **67**, 127-33.

Vairaktaris E, Yapijakis C, Serefoglou Z, et al (2008). Gene expression polymorphisms of interleukins-1β, -4, -6, -8, -10, and tumor necrosis factors-α, -β: regression analysis of their effect upon oral squamous cell carcinoma. *J Cancer Res Clin Oncol*, **134**, 821-32.

You Y, Du X, Fan M, et al (2015). Association between IL-10 polymorphisms (-1082A/G, -592A/C and -819T/C) and oral cancer risk. *Int J Clin Exp Med*, **8**, 13187-94.

Yu Z, Liu Q, Huang C, et al (2013). The interleukin 10 −819C/T polymorphism and cancer risk: A HuGE review and meta-analysis of 73 studies including 15,942 cases and 22,336 controls. OMICS. *J Integr Biol*, **17**, 200-14.

Zain RB, Athirajan V, Ghani WM, et al (2013). An oral cancer biobank initiative: a platform for multidisciplinary research in a developing country. *Cell Tissue Bank*, **14**, 45-52.