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Education, tobacco smoking, alcohol consumption, and IL-2 and IL-6 gene polymorphisms in the survival of head and neck cancer

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Abstract

The association of education, tobacco smoking, alcohol consumption, and interleukin-2 (IL-2 +114 and -384) and -6 (IL-6 -174) DNA polymorphisms with head and neck squamous cell carcinoma (HNSCC) was investigated in a cohort study of 445 subjects. IL-2 and IL-6 genotypes were determined by real-time PCR. Cox regression was used to estimate hazard ratios (HR) and 95% confidence intervals (95%CI) of disease-specific survival according to anatomical sites of the head and neck. Mean age was 56 years and most patients were males (87.6%). Subjects with 5 or more years of schooling had better survival in larynx cancer. Smoking had no effect on HNSCC survival, but alcohol consumption had a statistically significant effect on larynx cancer. IL-2 gene +114 G/T (HR = 0.52; 95%CI = 0.15-1.81) and T/T (HR = 0.22; 95%CI = 0.02-3.19) genotypes were associated with better survival in hypopharynx cancer. IL-2 +114 G/T was a predictor of poor survival in oral cavity/oropharynx cancer and larynx cancer (HR = 1.32; 95%CI = 0.61-2.85). IL-2 -384 G/T was associated with better survival in oral cavity/oropharynx cancer (HR = 0.80; 95%CI = 0.45-1.42) and hypopharynx cancer (HR = 0.68; 95%CI = 0.21-2.20), but an inverse relationship was observed for larynx cancer. IL-6 -174 G/C was associated with better survival in hypopharynx cancer (HR = 0.68; 95%CI = 0.26-1.78) and larynx cancer (HR = 0.93; 95%CI = 0.42-2.07), and C/C reduced mortality in larynx cancer. In general, our results are similar to previous reports on the value of education, smoking, alcohol consumption, and IL-2 and IL-6 genetic polymorphisms for the prognosis of HNSCC, but the risks due to these variables are small and estimates imprecise.

Key words: Smoking; Alcohol; Interleukin; Head and neck cancer; Cancer prognosis; Survival analysis

Introduction

Tumors of the oral cavity, pharynx and larynx, collectively defined as head and neck cancer, are a significant cause of morbidity and mortality, with over 500,000 new cases estimated for 2008 worldwide (1). Annually about 22,000 new cases are diagnosed in Brazil, the majority in the South and Southeast regions (2). Squamous cell carcinoma is the most frequent histological type. Prognosis varies according to anatomical subsites. The relative 5-year

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survival rate is around 50-70%, considering all sites and clinical stages, and all forms of treatment (3). In Brazil, the 5-year survival rate for oral and oropharyngeal cancer is still below 50% (4).

Prognostic factors, which have been examined in relation to head and neck cancer survival, include clinical tumor stage, patient education, tobacco smoking, and alcohol consumption (5-7). More recently, some genetic markers have been studied in relation to head and neck cancer survival, including gene polymorphisms related to invasion and metastasis, inflammation and DNA repair (8-10).

Interleukins (IL) are a type of cytokines, which are small secreted proteins related to the inflammation process and angiogenesis (11). IL-2 is an immunoregulatory cytokine and high serum levels of its receptor, sIL-2Rα, have been correlated with poor head and neck cancer survival and can be considered an independent prognostic biomarker in these tumors (12). IL-2 DNA polymorphisms have been recently associated with prostate tumor formation (13) and low risk of gastric cancer (14), but not with risk of chronic lymphocytic leukemia (15). Associations have also been identified between IL-6 DNA polymorphisms and the risk of colorectal, lung and oral cancer (16-18). However, investigations of the role of interleukins in cancer prognosis are rare. IL-2 +114 and -384, and IL-6 -174 were not associated with prognosis of gastric cardia or esophageal cancer (19). IL-6 -174 has been correlated with poor survival in breast, bladder, and prostate cancer (20-22).

In this study, we addressed the role of education, tobacco smoking, alcohol consumption, and IL-2 +114 and -384, and IL-6 -174 genetic polymorphisms in the prognosis of head and neck squamous cell carcinoma (HNSCC).

Material and Methods

From November 1998 to December 2002, 586 individuals diagnosed with HNSCC were recruited at eight hospitals from three cities in Brazil (São Paulo, Goiânia, and Porto Alegre) and were followed up until June 30, 2005. The study was approved by the Ethics Committee of each clinical center and written informed consent was obtained from all participants. All head and neck cancer cases were confirmed by histology as squamous cell carcinoma. Using the 10th revision of the International Classification of Diseases (ICD-10) (23), tumors were grouped into three categories, according to anatomical subsites, considering their distinct prognosis: oral cavity/oropharynx, hypopharynx, or larynx. The ICD-10 coding used for subsite classification was that used by Hashibe et al. (24): oral cavity/oropharynx (C00.3-C00.9, C01.9, C02.0-C02.3, C02.4, C02.8, C02.9, C03.0, C03.1, C04.0, C04.1, C04.8, C04.9, C05.0, C05.1, C05.8, C05.9, C06.0-C06.2, C06.8, C06.9, C09.0, C09.1, C09.8, C09.9, C10.0-C10.4, C10.8, C10.9, C14.0, C14.2, and C14.8), hypopharynx (C12.9, C13.0-C13.2, C13.8, and C13.9), or larynx (C32.0-C32.3, C32.8, and C32.9).

Patient hospital records were reviewed to obtain additional information about tumor clinical stage and treatment, and patient vital status. Tumor clinical stage was classified according to the tumor-node-metastases system (TNM) (25) as clinical stage (CS) I to IV. As the effect of tumor clinical stage on head and neck cancer survival is well known, we only included patients with advanced clinical stages (CS III and IV). As a consequence, the sample size was restricted to 445 subjects.

Information on patient vital status was also assessed by linking to population mortality and cancer registry databases. Cause of death was validated through death certificates obtained from the São Paulo State Death Registry (for patients from São Paulo city) and from the Population Cancer Registry of Goiânia (for patients from Goiânia). In Porto Alegre, death certificates were included in hospital medical records; these were also the only source of patient vital status information at that center. Of the 445 individuals, 111 were alive and 267 had died by the end of the study, with 224 deaths being related to head and neck cancer, and 67 were lost during follow-up.

All patients underwent face-to-face interviews immediately after diagnosis by a trained interviewer who used a structured questionnaire to obtain information on variables, which could impact head and neck cancer survival, such as education, tobacco smoking, and alcohol consumption. Formal education was measured in years of schooling. Tobacco smoking was measured by calculating the average number of cigarette packs smoked per day multiplied

Table 1. Primers for interleukin polymorphism genotyping.

Gene/SNP position		Forward primer (5'-3')	Reverse primer (5'-3')
IL-2 +114	Primers	5'-GCACCTACTTCAAGTTCTACAAAGAA-3'	5'-AAAGGAAATATACTTACATTAATTCCATTCAAAATCATCTG-3'
rs 2069763	Probes	5'-ATCCAGCAGTAAATG-3'	5'-TAAATCCAGAAGTAAATG-3'
IL-2 -384	Primers	5'-GCTCTTGTCCACCACAATATGCTAT-3'	5'-GCCTTCTGTATGAAACAGTTTTTCCT-3'
rs 2069762	Probes	5'-ATTTTCTTTGTCATAAAACT-3'	5'-TTTCTTTGTCCTAAAACT-3'
IL-6 -174	Primers	5'-GACGACCTAAGCTGCACTTTTC-3'	5'-GGGCTGATTGGAAACCTTATTAAGATTG-3'
rs 1800795	Probes	5'-CTTTAGCATGGCAAGAC-3'	5'-CTTTAGCATCGCAAGAC-3'

Source: Ref. 19.

by the number of years smoking (pack-years) (26). Alcohol consumption prior to head and neck cancer diagnosis was measured in grams of ethanol per milliliter per day (g ethanol/day), considering the equivalence of ethanol in different beverages: beer, wine, and spirits (27).

Blood DNA was extracted at the Institute of Tropical Medicine, University of São Paulo, using the Qiagen kit™ and genotyping procedures were conducted at the Cell Therapy Center Faculty of Medicine of Ribeirão Preto, University of São Paulo. Genetic polymorphisms were determined by real-time polymerase chain reaction (PCR) using TaqMan SNP genotyping assays. These assays included two locus-specific PCR primers that flank the single nucleotide polymorphism (SNP) of interest, and two allele-specific oligonucleotide TaqMan® probes (Applied Biosystems, USA). Each probe used a different fluorescent reporter dye at the 5' end, and a non-fluorescent quencher with a minor groove binder (MGB) at the 3' end. PCR primers amplify a specific locus on the genomic DNA template, and each fluorescent dye-labeled hybridization probe reports the presence of its associated allele in the DNA sample, as an allele-specific PCR, in a single tube (28). The primer design is unique and specific for the SNP, as assays are functionally tested against a small subset of genomic DNA by the supplier. All primers and probes were designed using the Assay-by-Design service offered by Applied Biosystems. These primers and probes are designed to be amplified under the following universal cycling conditions: 10 min at 95°C, followed by 40 cycles of 94°C for 15 s and 60°C for 1 min. All primers are depicted in Table 1. Genomic DNA obtained from whole blood samples was diluted at 1 ng/µL and 5.0 ng was used for each 15 µL real-time PCR using the TaqMan Master Mix (Applied Biosystems).

Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS Inc., USA) and the STATA (Stata Corp., USA) software. Frequencies and percentages were used for categorical variables and means and standard deviations were calculated for continuous variables. Life tables were constructed for 1-, 3-, and 5-year survival according to anatomical site. Median follow-up was calculated for each site. Kaplan-Meier curves were constructed for each site and a log-rank test was used to compare these survival curves. At the end of follow-up, living patients, those dead from other cause (not head and neck cancer), and those lost during follow-up were considered censored.

Cox proportional hazards regression was used to estimate hazard ratios (HR) and the respective 95% confidence intervals (95%CI) for disease-specific survival including education (classified into three strata), tobacco smoking (continuous variable according to consumption in pack-years), alcohol consumption (continuous variable according to g ethanol/day), and interleukin genetic polymorphisms according to specific subsite adjusted for age and gender.

Results

Patient characteristics are shown in Table 2. Most patients were diagnosed with oral/oropharynx cancer. Patient mean age was 56 years and males were predominant. Almost 70% of the patients had less than 5 years of

Table 2. Patient distribution by tumor anatomical site, social and demographic characteristics, tobacco smoking, alcohol consumption, tumor clinical stage, and genotypic frequencies of IL-2 and IL-6 polymorphisms.

	Cases (total = 445)	
	N	%
Anatomical site		
Oral/oropharynx	286	64.3
Hypopharynx	54	12.1
Larynx	105	23.6
Age (years)		
≤50	143	32.1
51-60	154	34.6
61-70	111	24.9
>70	37	8.3
Mean (standard deviation)	56.0 (10.3)	
Gender		
Male	390	87.6
Female	55	12.4
Education (years)		
<1	82	18.4
1-4	228	51.2
5 or more	135	30.3
Tobacco consumption (pack-years)		
Mean (standard deviation)	40.1 (27.4)	
Alcohol consumption (g ethanol/day)		
Mean (standard deviation)	147.8 (182.3)	
Clinical stage		
CS III	106	23.8
CS IV	339	76.2
IL-2 +114[a]		
G/G	190	47.3
G/T	181	45.0
T/T	31	7.7
IL-2 -384[b]		
G/G	66	16.1
G/T	159	38.7
T/T	186	45.3
IL-6 -174[c]		
G/G	253	58.0
G/C	150	34.4
C/C	33	7.6

[a]43 missing; [b]34 missing; [c]9 missing.

schooling. Mean cumulative tobacco smoking was 40.1 pack-years and mean daily alcohol consumption was 147.8 g ethanol/day. Most cases were diagnosed at advanced tumor clinical stage (category CS IV). IL-2 +114 G/G, IL-2 -384 T/T, and IL-6 -174 G/G were the most common genotypes.

Table 3 shows post-diagnosis disease-specific survival rates for the entire group of patients with overall HNSCC, and for specific subsites (oral cavity/oropharyngeal, hypopharyngeal and laryngeal cancer) at 1, 3, and 5 years. Figure 1 shows the survival curves for three HNSCC anatomical sites. Differences between the curves are clear (log-rank test, P = 0.001). The best median survival time was observed for larynx tumors and the poorest for hypopharynx tumors.

Table 4 shows adjusted HR's for oral cavity/oropharynx, hypopharynx and larynx tumors according to education, tobacco smoking, alcohol consumption, IL-2 +114, IL-2 -384, and IL-6 -174 adjusted for age and gender.

Higher education was positively associated with better survival for laryngeal cancer, but with poor survival for hypopharyngeal cancer. Tobacco smoking did not show any effect on survival in oral/oropharyngeal, hypopharyngeal, or laryngeal tumors.

IL-2 +114 G/T and T/T were associated with better prognosis in hypopharyngeal cancer. IL-2 -384 G/T showed better survival in oral cavity/oropharynx and hypopharynx tumors, but increased the risk of death in laryngeal cancer. IL-6 -174 C/C showed worse survival in oral cavity and hypopharyngeal cancer, but better survival in laryngeal cancer.

Table 3. Median survival time and probability of survival 1, 3, and 5 years after diagnosis for head and neck squamous cell carcinoma (HNSCC) and according to specific subsite.

	Cumulative survival proportion			Median survival time (months)
	1 year	3 years	5 years	
HNSCC	0.71	0.50	0.39	36.59
Oral cavity/oropharynx	0.69	0.48	0.37	30.09
Hypopharynx	0.59	0.33	0.27	18.69
Larynx	0.84	0.67	0.52	67.86

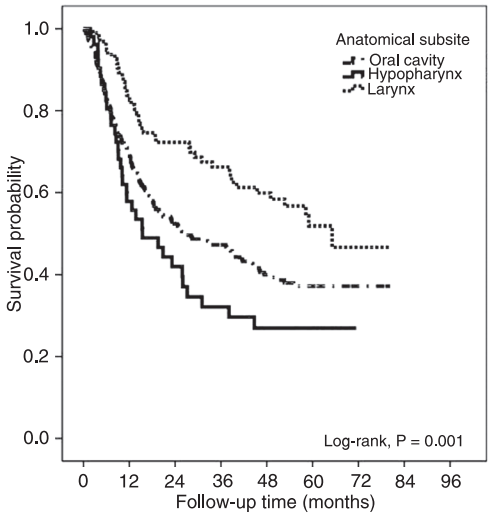

Figure 1. Survival of patients with head and neck squamous cell carcinoma related to specific anatomical sites: oral cavity, hypopharynx and larynx. Log-rank test for equality survival curves hypothesis (Kaplan-Meier or product limit estimator).

Discussion

Low socioeconomic status (SES) has been associated with the risk of head and neck cancer (29). The relationship between SES and disease prognosis is less well known. We observed different effects of educational levels on head and neck cancer, depending on tumor specific anatomical site. In oral cavity/oropharynx and hypopharyngeal tumors, the risk of death increased with more years of schooling, whereas in laryngeal cancer, it was associated with better survival. These results, however, were imprecise.

Tobacco smoking and alcohol consumption are well-known risk factors for head and neck cancer, but the implications of these two factors in disease prognosis are not completely clear. Some studies have identified an effect, although not statistically significant, of these variables on head and neck cancer (30,31). In our study, we observed that tobacco smoking had no effect on prognosis in oral cavity/oropharynx, hypopharynx, or laryngeal cancer. Alcohol consumption showed a statistically significant association with poor survival in laryngeal cancer, but the HR (considering the increase of risk according to each g ethanol/day) was very close to 1.

A statistically significant association was observed between high serum IL-2 levels and shorter survival time in head and neck cancer patients (32). In our study, we found that IL-2 +114 was associated with poor survival in oral cavity/oropharynx cancer, although amino acid sequence did not change (codon 38, CTG>CTT; Leu>Leu). Instead, this polymorphism was associated with increased survival in patients with hypopharyngeal tumors, while its effect was unclear in laryngeal cancer. The IL-2 polymorphism -384 T/T was associated with low survival in all anatomical subsites and has been reported to show lower IL-2 levels compared to the G allele, although there are many conflicting data showing opposite results, which indicate that other factors may contribute to the effect of this polymorphism on IL-2 expression levels (33).

Some studies have found an association between IL-6

-174 genetic polymorphisms and the risk of prostate, colorectal, and lung tumors (15-17). Few studies have found IL-6 -174 as a worse prognostic factor in breast, bladder, and prostate cancer (20-22), principally with the presence of the C allele. In our study, IL-6 -174 G/C and G/G genotypes were associated with reduced mortality in patients with oral and hypopharynx cancer. Moreover, the same genotypes were associated with a better survival in larynx cancer. It has been reported that G/G homozygotes produce higher levels of serum IL-6 when compared to C/G heterozygotes or C/C homozygotes (G/G>G/C>C/C) (34).

Some limitations of our study should be considered, such as sample size. Even though the total sample was relatively large, 445, when tumors were stratified by anatomical origin, samples were reduced in size limiting the power of this study. Also, we were only able to genotype IL-2 +114 and IL-2 -384 polymorphisms in 90.3 and 92.4% of our sample, respectively. This affects the precision of our results, especially those related to genetic polymorphisms. A second possible limitation that we should not ignore was related to human papillomavirus (HPV). The presence of HPV infection may alter tumor profile and could have some implications in outcome. Individuals with HPV infection appear to have increased survival in oral cancer, but the reasons for this are unclear (35,36). A recent study on a group of patients enrolled in a randomized clinical trial has suggested an association with HPV-positivity as an independent prognostic factor among patients with oropharyngeal cancer (37). Moreover, in a recent report, the anti-apoptotic effect of HPV E6 expression was found to be mediated in part by up-regulation of osteoprotegerin and IL-6 in cutaneous squamous cell carcinoma (38). In a

Table 4. Cox proportional hazards model for disease-specific survival according to the study variables.

Variable	Oral cavity/oropharyngeal [adjusted HR (95%CI); P]	Hypopharyngeal [adjusted HR (95%CI); P]	Laryngeal [adjusted HR (95%CI); P]
Age (years)			
≤50	1.00	1.00	1.00
51-60	1.03 (0.66-1.61); 0.88	1.09 (0.24-4.90); 0.91	0.63 (0.22-1.81); 0.39
61-70	1.34 (0.78-2.31); 0.28	2.00 (0.33-12.04); 0.46	1.22 (0.44-3.35); 0.71
>70	1.40 (0.61-3.20); 0.42	13.09 (1.37-125.12); 0.03	1.03 (0.22-4.79); 0.97
Gender			
Female	1.00	NA	1.00
Male	1.19 (0.66-2.14); 0.57		2.50 (0.45-13.85); 0.29
Education (years)			
<1	1.00	1.00	1.00
1-4	1.10 (0.63-1.93); 0.74	2.44 (0.53-11.20); 0.25	0.70 (0.22-2.24); 0.55
≥5	1.16 (0.61-2.20); 0.64	4.86 (0.56-42.30); 0.15	0.39 (0.10-1.53); 0.18
Smoking[a]			
Pack-years	1.00 (0.99-1.01); 0.51	1.00 (0.97-1.01); 0.45	1.00 (0.98-1.01); 0.41
Alcohol consumption[b]			
g ethanol/day	1.00 (0.99-1.00); 0.87	1.00 (0.99-1.00); 0.78	1.00 (1.00-1.01); 0.01
IL-2 +114			
G/G	1.00	1.00	1.00
G/T	1.07 (0.72-1.57); 0.75	0.52 (0.15-1.81); 0.31	1.32 (0.61-2.85); 0.48
T/T	1.14 (0.58-2.23); 0.70	0.22 (0.02-3.19); 0.27	0.86 (0.15-4.80); 0.86
IL-2 -384			
G/G	1.00	1.00	1.00
G/T	0.80 (0.45-1.42); 0.45	0.68 (0.21-2.20); 0.52	2.42 (0.71-8.30); 0.16
T/T	1.11 (0.64-1.93); 0.71	1.95 (0.52-7.37); 0.32	1.32 (0.39-4.47); 0.66
IL-6 -174			
G/G	1.00	1.00	1.00
G/C	1.00 (0.68-1.49); 0.99	0.68 (0.26-1.78); 0.43	0.93 (0.42-2.07); 0.86
C/C	1.23 (0.69-2.18); 0.48	3.33 (0.51-23.01); 0.21	0.12 (0.01-1.52); 0.10

HR = hazard ratio; 95%CI = 95% confidence interval; NA = not available; [a]Pack-years = number of cigarette packs smoked per day versus number of years of smoking; HR calculated for each pack-year increase. [b]HR calculated for each g ethanol/day increase.

case-control study on women with cervical neoplasias, the authors showed that the level of E7-induced IL-2 production from the lymphocytes of patients with HPV 16 clearance was inversely correlated with the time relative to the last HPV DNA + test (39). In another study on women with cervical and vulvar cancer, the results suggested an increased risk of cancer among smokers modified by genetic variation in IL-2, cigarette components and decreased IL-2 levels (40). Confounding effects from tobacco use and tumor clinical stage at diagnosis have not been ruled out as potential reasons for the association between HPV and survival. In addition, we assumed that treatment was similar for all patients with head and neck cancer, but this could be an important confounding variable in studies on cancer survival. These limitations argue the case for a prudent interpretation of our results.

Overall, our study revealed the same inconsistent results as some earlier reports on the prognostic value of environmental factors, such as education, tobacco smoking, and alcohol consumption, as well as IL-2 and IL-6 gene polymorphisms in HNSCC.

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