Behind XRCC1 Arg399Gln polymorphism: protection factor of laryngeal cancer

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Abstract

Laryngeal cancer is the second most common head and neck cancer worldwide, which has been considering a serious global health problem due to the high morbidity and mortality. Tumour risk factors include the DNA repair gene polymorphisms, but their contribution for metastasis and/or second primary tumour development has been seldom investigated. Objective (s): The present study evaluated the possible association between the DNA repair gene polymorphisms and laryngeal cancer risk, metastasis and/or second primary tumour in a hospital-based case-control study that comprised 149 laryngeal cancer patients and 448 controls from Heliópolis Hospital, São Paulo, Brazil. Design: The single nucleotide polymorphisms (SNPs) of the genes XRCC1 (Arg194Trp; Arg399Gln), XPD (Lys751Gln) and XRCC3 (Thr241Met) were analysed by TaqMan SNP Genotyping Assays. Results: The heterozygous genotype (OR 0.63, 95% CI 0.41-0.96) as well as the mutated homozygous genotype (OR 0.29, 95% CI 0.13-0.66) of XRCC1 (Arg399Gln) decreased the laryngeal cancer risk, even though none of the genes polymorphisms was associated with metastasis and/or second primary tumour development. Conclusion: The determination of the XRCC1 (399Gln) genotype might be applied as a molecular predictor of laryngeal cancer among individuals who are highly exposed to cigarette smoking carcinogens and improve the prognostic of the disease.

Behind *XRCC1* Arg399Gln polymorphism: protection factor of laryngeal cancer

Running title: *XRCC1* polymorphism and laryngeal cancer

Abstract

Laryngeal cancer is the second most common head and neck cancer worldwide, which has been considering a serious global health problem due to the high morbidity and mortality. Tumour risk factors include the DNA repair gene polymorphisms, but their contribution for metastasis and/or second primary tumour development has been seldom investigated.**Objective (s)** : The present study evaluated the possible association between the DNA repair gene polymorphisms and laryngeal cancer risk, metastasis and/or second primary tumour in a hospital-based case-control study that comprised 149 laryngeal cancer patients and 448 controls from (Blinded for Review). **Design:** The single nucleotide polymorphisms (SNPs) of the genes *XRCC1* (Arg194Trp; Arg399Gln), *XPD* (Lys751Gln) and *XRCC3* (Thr241Met) were analysed by TaqMan SNP Genotyping Assays. **Results:** The heterozygous genotype (OR 0.63, 95% CI 0.41-0.96) as well as the mutated homozygous genotype (OR 0.29, 95% CI 0.13-0.66) of *XRCC1*(Arg399Gln) decreased the laryngeal cancer risk, even though none of the genes polymorphisms was associated with metastasis and/or second primary tumour development. **Conclusion:** The determination of the*XRCC1* (399Gln) genotype might be applied as a molecular predictor of laryngeal cancer among individuals who are highly exposed to cigarette smoking carcinogens and improve the prognostic of the disease.

Keywords. *XRCC1* Arg399Gln, laryngeal cancer risk, metastasis, second primary tumour.

Key points:

- Laryngeal cancer has been considering a serious global health problem due to the high morbidity and mortality.

- Variants of XRCC1-rs25487 were associated with decreased risk of laryngeal cancer.

- Variants of DNA repair genes did not contribute to metastasis and second primary tumor.

- Heterozygous genotype XRCC1 Arg399Gln was associated with large primary tumors.

- The determination of the *XRCC1* (399Gln) genotype might be applied as a molecular predictor of laryngeal cancer.

Introduction

Laryngeal cancer is a disease that impairs several physiological functions such as swallowing, speech, and respiration, which contributes to a poor prognosis and a high relapse rate [1–3]. The malignancy in the larynx is the second most common head and neck cancer worldwide [4] and represents one-fourth of head and neck neoplasms in the United States [3], where 13,430 new cases were estimated for 2016 [2]. In Brazil, 7,650 new cases were estimated for 2020 (INCA 2020 estimation). A total of 4,532 laryngeal cancer patients died during 2019 in Brazil (INCA- Brasil, 2021) and 3,620 deaths were estimated for 2016 in the United States[2]. About 60% of the cases are diagnosed in advanced tumour stages, which might be one factor for the 5-year survival rate declination over the past 40 years [2]. Furthermore, the risk of second primary tumour development increases according to the time of laryngeal cancer diagnosis, for example, 14% in 5 years, 26% in 10 years and 26% in 15 years [5].

Because the disease incidence varies considerably across different populations, it has been suggested that the malignancy development is under several environmental factors and lifestyle behaviours, such as cigarette smoking and alcohol consumption [5]. However, studies have indicated that heritable factors also contribute to the laryngeal cancer risk [6]. These genetic factors include polymorphisms in the DNA repair genes [1,7], wherein the DNA repair system protects DNA from environmental injuries, aids to maintaining the genome integrity and prevents the carcinogenesis mechanism [8].

The differences in DNA repair ability among individuals are genetically determined [9] and evidences have implied a critical link between the individual DNA damage repair and cancer development, progression, and therapeutic response [1]. The most studied DNA repair polymorphic genes are *XRCC1* (Arg194Trp – re1799782; Arg399Gln – rs25487) that participates of base excision repair (BER) pathway, *XPD* (Lys751Gln – rs13181) that is involved in nucleotide excision repair (NER) pathway, and *XRCC3* (Thr241Met – rs861539) that participates in homologous recombination repair (HRR) pathway. Some epidemiological studies have reported that the variant alleles of these genes are implicated in the laryngeal cancer risk[10], but the results remain inconclusive[11,12]. Moreover, few investigations have been conducted about these polymorphic genes and the metastasis and second primary tumour. The aim of this study was to evaluate whether SNPs in the DNA repair genes *XRCC1* , *XRCC3* and *XPD* are biomarkers for the laryngeal cancer risk, metastasis and/or a second primary tumour.

Materials and methods

A hospital-based case-control study was carried out to explore the role of DNA repair gene polymorphisms in laryngeal cancer risk and the development of metastasis and/or second primary tumour. A total of 149 laryngeal cancer patients were recruited from the Department of Head and Neck Surgery and Otolaryngology at Heliópolis Hospital, São Paulo, Brazil. The patients were followed up by 5 years or until the death. Among patients, 10 were diagnosed with metastasis, 13 developed second primary tumour, and 3 were detected with both events. Regarding the anatomic tumour site, glottis carcinoma affected 65.1% of patients, and supraglottic cancer was detected in 34.9% of patients. Only patients who underwent surgery had a clinical

staging evaluation that the advanced stage (pTIII/IV) of the tumour was observed in the majority of the patients, whereas 30.5% of laryngeal cancer patients presented the malignancy in the early stages (pTI/II). Therefore, 95% of the individuals received curative treatment for a primary tumour, and 5% of patients received palliative care. The treatments that were used for the laryngeal cancer patients included surgery (37.7%), chemotherapy/radiotherapy (22.7%) and combined treatments (37.6%).

The control group was recruited from the same hospital and in the same period of HNC patients, which was composed by 448 patients without cancer diagnosis and familial laryngeal cancer history who were selected according to the frequency-matched with laryngeal cancer patients for gender and age within five years. The control group included only individuals recently diagnosed with diseases unrelated to tobacco or alcohol, such as digestive system disorders, injuries and diseases of the musculoskeletal system and connective tissue, genitourinary system or circulatory system. The research has been carried out in accordance with the World Medical Association Declaration of Helsinki, and all subjects provided written informed consent. Briefly, the study was approved by the Ethics Committee for Research Projects Analysis (Blinded by Review).

Genomic DNA was obtained from peripheral blood lymphocytes by the salting-out extraction method, and genomic DNA was analysed by spectrophotometry (NanoDrop 2000, Thermo Fisher Scientific^{TM}, Wilmington, DE). The samples were genotyped for *XRCC1 rs25487* (C_622564_10), *rs1799782 (C_11463404_10)* , *XRCC3 rs861539* (C_8901525_10), and*XPD rs13181* (C_3145033_10) using TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA). Genotype analysis was carried out by quantitative polymerase chain reaction (qPCR), where amplifications were performed on a Step One-Plus Real-Time PCR apparatus (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. Samples were run in a 96-well format, wherein 20 ng/μL genomic DNA were used. Each plate contained three quality control samples, which included known genotypes of each SNP and a negative sample as external controls.

Hardy-Weinberg equilibrium was calculated using the χ^2-test. The association between SNPs in DNA repair genes and laryngeal cancer risk was evaluated among the case and control groups. Multivariate logistic regression analysis were used to identify whether the DNA repair gene polymorphisms were able to predict metastasis and/or second primary tumours within laryngeal cancer patients, which were adjusted for the gender, skin colour, age, educational level, cigarette smoking and alcohol consumption. All statistical analyses considered Odds Ratio (OR) and 95% Confidence Interval (CI). The association analyses were performed under different genetic models, considering "a" as the less frequent allele with the following models: genotype (AA versus Aa versus aa), dominant (AA versus Aa+aa), recessive (AA+Aa versus aa) and allele/multiplicative (A versus a) models. All statistical analyses were performed on Statistical Package for the Social Sciences (SPSS) software, version 18.0.

Results

Table 1 shows the sociodemographic data and the cigarette smoking and alcohol consumption habits of the laryngeal cancer patients and the control group. Gender, skin colour, age, educational level, cigarette smoking and alcohol consumption were significantly varied ($p<0.05$).

The genotype frequencies were within the Hardy-Weinberg equilibrium for all four investigated polymorphisms (data not shown). Among the variants of the DNA repair genes, only the SNP rs25487 of *XRCC1* was statistically associated with the laryngeal cancer, wherein the tumour risk decreased 37% for the heterozygous genotype (OR 0.63, 95% CI 0.41-0.96) and 71% for the mutated homozygous genotype (OR 0.29, 95% CI 0.13-0.66), as shown in Table 2. The dominant model confirmed the same association (Data not shown).

None of the four SNPs analysed were statistically significant associated with the metastasis and/or second primary tumour under the genotype, dominant and recessive models (Table 3).

Discussion

The proper functioning of cells and all biological systems depends on the maintenance of the genome integrity. In the larynx, as well as the head and neck region, the precise DNA repair mechanisms is expected due to the

continuously organ exposed to exogenous and endogenous DNA-damaging factors [13]. This hospital-based case-control study analysed whether four polymorphisms (in BER (*XRCC1*- 1799782 and *XRCC1*- rs25487), NER (*XPD*- rs13181) and HRR (*XRCC3*- rs861539) genes) play a role in the development, metastasis and/or second primary tumours of laryngeal cancer.

The variant allele of *XRCC1* -rs25487 (Arg399Gln) decreased the laryngeal cancer risk for the heterozygous genotype (OR 0.63, 95% CI 0.41-0.96) as well as for the mutated homozygous genotype (OR 0.29, 95% CI 0.13-0.66), results that was found in different models of inheritance. The same association was also found in a Turkish sample of oral squamous cell carcinoma [14]. However, the lack of association of the SNP *XRCC1* -rs25487 with laryngeal cancer was reported by the meta-analysis of Chen and colleagues[6], which was carried out without the evaluation of the Brazilian population that is one the most diverse populations worldwide[15]. Future studies performed in a large Brazilian population could confirm our findings.

The XRCC1 protein interacts with three DNA repair enzymes (DNA ligase III, DNA polymerase and PARP) to repair single-strand DNA breaks in the BER pathway [6,16]. The variant allele (*XRCC1*399Gln) has been shown to be associated with a noticeably reduced DNA repair ability, as indicated by the persistence of DNA adducts[17–19], elevated levels of sister chromatid exchanges[17,20], increased RBC glycophorin A[21], *p53* mutations [22]and prolonged cell cycle delay [19,20]. These events supports the supposition of this variant allele increases the tumour risk.

However, our study showed a protective effect of *XRCC1* 399Gln for the laryngeal cancer development. This effect might be a response a gainst severe and continuous damages that DNA can suffer, which triggers the interruption of the cell cycle avoiding the damage genome transfer to new cells while regulated signalling pathways actives the apoptotic processes. The same occurrences were reported for lung cancer, the other anatomic site that is constantly and directly exposed to genotoxic compounds [23].

We found no association between DNA repair polymorphic genes and the metastases and/or second primary tumour events. This result suggests that the DNA repair mechanisms play a key role on the genome integrity, even when the oncogenic transformations are sustained in the tumour cells. Studies reported that during the carcinogenesis mechanism, the surveillance system denominated DNA damage responses (DDRs) is impaired, which contributes to the uncontrolled tumour cells proliferation and the progression of the disease as metastases and second primary tumours[24]. Then, our findings propose that other intrinsic factors, such as DDRs, loss of p53 function, cell cycle regulation, are involved in metastasis and second primary tumours, as previously reported for the prostate cancer progression [25].

Conclusion

In conclusion, the heterozygous (Arg/Gln) and mutated homozygous (Gln/Gln) genotypes of *XRCC1*-rs25487 were associated with a decreased risk of laryngeal cancer, but none association was found between the polymorphic genes evaluated and the metastasis and/or second primary tumour events in a sample of the Brazilian population. In that regard, the determination of the *XRCC1* (399Gln) genotype might be applied as a molecular predictor of laryngeal cancer among individuals who are highly exposed to cigarette smoking carcinogens and improve the prognostic of the disease.

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Tables

Table 1: Comparison of sociodemographic data among laryngeal patients and controls

Variables	**Categories**	**Controls N**	**Controls %**	**LC patients N**	**LC patients %**	**OR**	**95% CI Inferior**	**95% Supe**
Gender	Female	71	15.8	16	10.7	1	-	-
	Male	377	84.2	133	89.3	1.72	1.19	2.48
	Total	448	100	149	100			
Skin color	White	258	58.4	107	71.8	1	-	-
	Non-white	184	41.6	42	28.2	0.67	0.52	0.86
	Total	442	100	149	100			
Age (years)	[?]50 years	184	41.2	39	26.2	1	-	-
	> 50 years	263	58.8	110	73.8	1.45	1.13	1.87
	Total	447	100	149	100			
Educational level	0 to 8 years	262	61.5	92	64.4	1	-	-
	8 to 11 years	104	24.4	38	25.5	0.99	0.74	1.32
	> 11 years	60	14.1	15	10.1	0.58	0.39	0.87
	Total	426	100	149	100			
Cigarette smoking	Never	119	26.7	8	5.4	1	-	-
	Only in the past	125	28.1	35	23.5	3.20	2.02	5.09
	Still smoke	201	45.2	106	43.0	8.45	5.56	12.85
	Total	445	100	149	100			
Alcohol consumption	Never	107	24.0	30	20.1	1	-	-
	Only in the past	153	34.4	55	36.9	1.52	1.08	2.16
	Still drink	185	41.6	64	43.0	1.91	1.37	2.65
	Total	445	100	149	100			

N = number of subjects; LC patients = laryngeal cancer patients; OR =*odds ratio* ; 95% CI = 95% confidence interval; * = p<0.05.

Table 2: Association between DNA repair gene polymorphisms and laryngeal cancer risk for the genotype model

Polymorphism	**Genotype**	**Controls N**	**Controls %**	**LC patients N**	**LC patients %**	χ^2	**p#**	**OR**	**95% CI Inferior**	**95% CI Superior**
XRCC1-194 (rs1799782)	CC (Arg/Arg)	402	89.7	133	89.3			1.00		
	CT (Arg/Trp)	43	9.6	15	10.1	0.03	0.99	0.90	0.46	1.74

	TT (Trp/Trp)	3	0.7	1	0.7			0.55	0.05	6.35
	Total	448	100	149	100					
XRCC1-399 (rs25487)	GG (Arg/Arg)	196	43.8	84	56.4			1.00		
	GA (Arg/Gln)	202	45.1	57	38.3	8.87	0.012*	0.63	0.41	0.96
	AA (Gln/Gln)	50	11.2	8	5.4			0.29	0.13	0.66
	Total	448	100	149	100					
XRCC3 (rs861539)	TT (Thr/Thr)	212	47.3	66	44.3			1.00		
	TC (Thr/Met)	182	40.6	58	38.9	2.19	0.34	1.03	0.67	1.61
	CC (Met/Met)	54	12.1	25	16.8			1.47	0.81	2.68
	Total	448	100	149	100					
XPD (rs13181)	AA (Lys/Lys)	216	48.2	72	48.3			1.00		
	AC (Lys/Gln)	191	42.6	63	42.3	0.01	1.00	0.86	0.56	1.32
	CC (Gln/Gln)	41	9.2	14	9.4			0.98	0.48	2.00
	Total	448	100	149	100					

LC patients = laryngeal cancer patients; N= number of subjects;χ^2 = chi square; p# = p-value ofχ^2 test; OR = odds ratio adjusted for the gender, skin colour, age, educational level, cigarette smoking and alcohol consumption; 95% CI = 95% confidence interval; p## = p-value of OR; * = p<0.05.

Table 3: Association between four DNA repair gene polymorphisms and metastasis and/or second primary tumour risk for the genotype model

Polymorphism	**Genotype**	**Primary tumour**	**Primary tumour**	**Metastasis and/or Second primary tumour**	**Metastasis and/or Second primary tumour**	**χ^2**	**p#**	**OR**	**95% CI**	**95% CI**
		N	**%**	**N**	**%**				**Inferior**	**Superior**
XRCC1-194 (rs1799782)	CC (Arg/Arg)	115	89.2	18	90.0	0.16	0.92	1.00		
	CT (Arg/Trp)	13	10.0	2	10.0			0.98	0.20	4.72
	TT (Arg/Trp)	1	0.8	0	0			0	0	0
	Total	129	100	20	100					

XRCC1-399 (rs25487)	GG (Arg/Arg)	74	57.0	10	50.0	0.45	0.80	1.00		
	GA (Arg/Gln)	48	37.0	9	45.0			1.39	0.53	3.66
	AA (Gln/Gln)	7	6.0	1	5.0			1.06	0.12	9.51
	Total	129	100	20	100					
XRCC3 (rs861539)	TT (Thr/Thr)	56	43.0	10	50.0	0.78	0.67	1.00		
	TC (Thr/Met)	52	40.0	6	30.0			0.65	0.22	1.90
	CC (Met/Met)	21	16.0	4	20.0			1.07	0.30	3.77
	Total	129	100	20	100					
XPD (rs13181)	AA (Lys/Lys)	62	48.0	10	50.0	0.05	0.97	1.00		
	AC (Lys/Gln)	55	43.0	8	40.0			0.90	0.33	2.45
	CC (Gln/Gln)	12	9.0	2	10.0			1.03	0.20	5.32
	Total	129	100	20	100					

N= number of subjects; χ^2 = chi square; p# = p-value of χ^2 test; OR = odds ratio adjusted for the gender, skin colour, age, educational level, cigarette smoking and alcohol consumption; 95% CI = 95% confidence interval; p## = p-value of OR.