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Association between superoxide dismutase 2, glutathione peroxidase 1, xeroderma pigmentosum group d gene variations, and head and neck squamous cell cancer susceptibility

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Abstract: As oxidative stress is implicated in the pathogenesis of head and neck squamous cell cancer (HNSCC), the functions of antioxidant enzyme systems and DNA repair proteins are critical in the development of cancer. To investigate the role of genetic polymorphisms of the antioxidant superoxide dismutase 2 (*SOD2*) Val16Ala, glutathione peroxidase 1 (*GPX1*) Pro198Leu, and the DNA repair Xeroderma Pigmentosum Group D (*XPD*) Lys751Gln genes under exogenous risk factors, including smoking and alcohol consumption, in HNSCC carcinogenesis, we conducted a case-control study on 139 unrelated cases and 265 non-cancer controls. Polymorphisms were analyzed in additive, dominant and recessive genetic models, individually and in an interaction model. Carriers of the T allele of *SOD2* were associated with an increased risk for HNSCC in males and smokers; similarly, the T allele of *GPX1* was associated with elevated risk in the overall and smoker subgroup. A 12.47-fold increased risk was observed for the carriers of *GPX1* TT, *SOD2* CT and *XPD* CC genotypes for HNSCC. This is the first study presenting the potential roles of *SOD2*, *GPX1* and *XPD* polymorphisms in interaction and under three genetic models in the development of HNSCC. The results suggest that these polymorphisms slightly modify the risk in HNSCC development individually but are significantly higher when they functioned and were evaluated together.

Keywords: head and neck squamous cell cancer; gene polymorphism; *GPX1*; *SOD2*; *XPD*

INTRODUCTION

Head and neck squamous cell cancer (HNSCC) is an aggressive, life-threatening disease with high mortality, which develops from the mucosal epithelium in the oral cavity, pharynx and larynx[1]. The development of HNSCC is related to both lifestyles such as tobacco smoking and alcohol consumption, as well as genetic factors [2, 3].

Tobacco smoke contains high concentrations of free radicals, reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical, and hydrogen peroxide (H_2O_2), together with other carcinogenic compounds, and it can induce DNA damage and consequently plays an important role in the occurrence of HNSCC [4]. ROS and oxidative stress are implicated in the pathogenesis of HNSCC. The role of the antioxidant enzyme system in the process of detoxification has gained much attention as it is critical in limiting the oxidative burden [5]. The biological effects of ROS are mainly controlled by enzymatic antioxidant defense mechanisms through the activities of superoxide dismutase (SOD) and glutathione peroxidases [6]. Manganese superoxide dismutase (MnSOD2) catalyzes the conversion of superoxide radicals into oxygen and H_2O_2. Glutathione peroxidase 1 (GPX1) protects cells against oxidative damage by reducing a wide range of organic peroxides [7]. Besides antioxidant enzyme systems, DNA repair proteins are crucial in the recovery from ROS-mediated DNA damage. The xeroderma pigmentosum group D (XPD) of proteins is one of the main components of the nucleotide excision repair (NER) pathway, a major repair mechanism, and is responsible for removing bulky DNA adducts [8].

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Since there is large individual variability in ROS detoxification and DNA repair ability, polymorphisms in *SOD2*, *GPX1* and *XPD* are important for understanding the role of genetic susceptibility in the development of HNSCC. Several functional single nucleotide polymorphisms (SNPs) have been identified in these genes, among them *SOD2* Val16Ala (C>T, rs4880), *GPX1* Pro198Leu (C>T, rs1050450) and *XPD* Lys751Gln (A>C, rs13181) polymorphisms, which could come to the forefront as their functional effects are essential for cellular detoxification and the protection of macromolecules from attack by reactive species. Based on their critical role, the present study aimed to evaluate the association between the *GPX1*, *SOD2* and *XPD* gene polymorphisms and the risk of HNSCC, and the potential modifying influences of tobacco smoking, alcohol consumption and gender.

MATERIALS AND METHODS

Study subjects and inclusion-exclusion criteria

The study included 139 unrelated patients with HNSCC and 265 healthy individuals. All subjects were Caucasian. The diagnosis of HNSCC was confirmed by qualified pathological reviews of all histological slides. Blood samples used in this study were obtained from the Department of Otolaryngology, Head and Neck Surgery, Ankara Oncology Education and Research Hospital, Ankara, Turkey. The controls were healthy individuals without any known history of cancer. All participants provided informed consent. Peripheral blood samples were collected from all study subjects. Cases and controls were personally interviewed with detailed questionnaires and data on demographic features, and smoking and alcohol histories were also collected. The information required in the questionnaire included demographic factors: age, gender, ethnicity, smoking status and alcohol use of the participants, which were classified as ever and never (ever groups include current and previous smokers). Detailed characteristics of the study populations are presented in Table 1. Medical records were reviewed to obtain clinical information about the final histopathologic diagnosis, the date of diagnosis and the primary tumor site for the cases. Patients with primaries outside the upper aerodigestive tract, metastases of unknown primary origin and a histopathological diagnosis other than squamous cell carcinoma were excluded. The study protocol was accepted by Dr. Abdurrahman Yurtaslan Ankara Oncology Education and Research Hospital Ethics Committee and conducted in accordance with Good Clinical Practice and the Helsinki declaration.

Genomic DNA isolation and genotyping

Genomic DNA was extracted from peripheral blood samples with the use of the Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA). All samples were stored at -80°C until analysis. The *XPD* Lys751Gln, *SOD2* Ala16Val and *GPX1* Pro198Leu genotypes were determined using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). Details of the primers, cycling conditions and restriction fragment analysis for *XPD* (Lys751Gln, rs13181), *GPX1* (Pro198Leu, rs1050450) and *SOD2* (Val16Ala, rs4880) were described previously [9-11]. RFLP products were visualized on ethidium bromide-stained 2% agarose gels or silver-stained 6% polyacrylamide gels. For quality control, a random 10% of the samples were repeated to evaluate the reproducibility of the results. All results were identical to the first test.

Statistical analysis

All statistical analyses were performed using SPSS (version 26 for Microsoft Windows, Armonk, NY: IBM Corp.). The normality of the continuous variables was tested with the Shapiro-Wilk test. Parametric and

Table 1. Demographic characteristics of HNSCC and control individuals.

Variables	Controls (N=265)	Cases (N=139)	OR (95% CI)	P value
Age (years)	47.092 ± 13.39	59.5 ± 20.15		**<0.001**[a]
Gender				
Male	179 (67.54)	121 (87.05)	3.23(1.85-5.64)	
Female	86 (32.45)	18 (12.94)		**<0.001**[b]
Smoking status				
Ever	173 (65.28)	117 (84.17)	2.82(1.68-4.76)	
Never	92 (34.71)	22 (15.82)		**<0.001**[b]
Alcohol use				
Never	212 (80.00)	107 (76.97)		
Ever	53 (20.00)	32 (23.02)	1.19(0.73-1.96)	0.479

N: number of samples, level of significance P≤0.05 calculated by one-sample t-test[a] and χ^2-test[b].

nonparametric tests were applied when appropriate. Concordance of genotype distribution with the Hardy-Weinberg equilibrium was assessed with the use of the χ^2 test. The categorical variables were summarized as counts and percentages, and continuous variables were summarized as medians with means±standard deviation where appropriate. The differences in demographic characteristics were compared between the HNSCC cases and controls using the Student's t-test and the χ^2 test. The Student's t-test was used to compare the difference in age, and the χ^2 test was used to compare the differences in gender, smoking habit and alcohol consumption. The statistical difference in genotype distributions of *GPX1* Pro198Leu, *SOD2* Val16Ala and *XPD* Gln751Lys polymorphisms was calculated by χ^2; the odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using an unconditional logistic regression model. Three different genetic models were run under the assumption of additive (AA vs. Aa vs. aa), dominant (AA vs. Aa/aa), or recessive (AA/Aa vs. aa) inheritance. For SNP-SNP interactions, an adjusted logistic regression model was used to estimate the multiplicative interaction effect of the three SNPs by two-way and three-way ANOVA. P<0.05 was considered statistically significant.

RESULTS

Demographic characteristics of the cases and controls are summarized in Table 1 as counts and percentages for categorical variables, and continuous variables as means±standard deviation. The potentially confounding effects of age, gender, alcohol use and tobacco smoking were considered for adjustment in the logistic regression analysis. Although there was no significant difference between the cases and controls in terms of alcohol use, smoking, gender, and age were associated with an elevated risk of HNSCC (P<0.001 for each). A history of smoking was associated with a 2.82-fold increased risk in HNSCC (95%CI=1.68-4.76). Males had an increased risk of HNSCC with OR 3.23 (95%CI=1.82-5.64). Genotype distributions among controls and cases were consistent with the Hardy-Weinberg equilibrium in the study (P>0.05).

Table 2 presents the comparison of the genotype frequencies and the analysis of the *GPX1, SOD2* and *XPD* polymorphisms among cases and controls based on three genetic models along with ORs. There was an increased risk for HNSCC for the CT genotype of *SOD2* (OR=1.902, 95%CI=1.10-3.28, P=0.021). In addition to correlation analysis of the general population, genotype frequencies were further analyzed among subgroups of smokers, males and females by three genetic models.

The results showed that the incidence of the TT genotype of *GPX1* was higher in the patients than in the control subjects in the additive model (OR=2.549 95%Cl=1.14-5.71, P=0.023), and in a recessive model (OR=2.54, 95%Cl=1.18-5.46, P=0.017) among smokers. In addition, for *SOD2* rs4880 an increased HNSCC risk was found in the additive model (CT genotype

Table 2. Comparison of genotype frequencies and the analysis of the *GPX1*, *SOD2* and *XPD* polymorphisms between patients and controls based on three genetic models.

Gene Model	Genotype /Allele	Control N=265	Cancer N=139	OR (95%Cl)	*p*-value
GPX1					
Additive	CC (Pro)	129	65	1	
	CT	104	50	1.095 (0.64-1.81)	0.722
	TT (Leu)	32	24	1.854 (0.94-3.67)	0.076
Dominant	CC (Pro)	129	65		
	CT+TT	136	74	1.214 (0.77-1.91)	0.405
Recessive	CC+CT	233	115		
	TT (Leu)	32	24	1.776 (0.94-3.34)	0.080
SOD2					
Additive	CC (Ala)	83	34	1	
	CT	**123**	**79**	**1.902 (1.10-3.28)**	**0.021**
	TT (Val)	59	26	1.045 (0.53-2.05)	0.899
Dominant	CC (Ala)	83	34		
	CT+TT	182	105	1.590 (0.96-2.63)	0.070
Recessive	CC+CT	206	113		
	TT (Val)	59	26	1.482 (0.85-2.59)	0.168
XPD					
Additive	AA	88	58	1	
	AC	133	56	0.688 (0.42-1.14)	0.146
	CC	44	25	0.784 (0.40-1.51)	0.468
Dominant	AA (Lys)	88	58		
	AC+CC	177	81	1.503 (0.95-2.39)	0.09
Recessive	AA+AC	221	114		
	CC (Gln)	44	25	1.056 (0.59-1.90)	0.857

N: number of samples, OR: odds ratio, CI: confidence interval; P≤0.05 considered as statistically significant. ORs were adjusted for age, sex and smoking status of the study cohort in a logistic regression model.

Table 3. Comparison of genotype frequencies and analysis of the *GPX1*, *SOD2* and *XPD* polymorphisms between patients and controls based on three genetic models among smokers.

Gene Model	Genotype /Allele	Control N=173	Cancer N=117	OR (95%Cl)	*p*-value
GPX1					
Additive	CC (Pro)	88	57	1	
	CT	68	38	1.010 (0.57-1.79)	0.972
	TT (Leu)	**17**	**22**	**2.549 (1.14-5.71)**	**0.023**
Dominant	CC (Pro)	88	57		
	CT+TT	85	60	1.308 (0.76-2.24)	0.328
Recessive	CC+CT	156	95		
	TT (Leu)	**17**	**22**	**2.540 (1.18-5.46)**	**0.017**
SOD2					
Additive	CC (Ala)	58	29	1	
	CT	**76**	**65**	**2.148 (1.16-3.99)**	**0.016**
	TT (Val)	39	23	1.178 (0.56-2.48)	0.666
Dominant	CC (Ala)	58	29		
	CT+TT	**115**	**88**	**1.78 (0.99-3.17)**	**0.052**
Recessive	CC+CT	134	94		
	TT (Val)	39	23	1.36 (0.72-2.56)	0.341
XPD					
Additive	AA	58	50	1	
	AC	79	45	1.454 (0.70-3.04)	0.321
	CC	36	22	0.831 (0.40-1.73)	0.620
Dominant	AA (Lys)	58	60		
	AC+CC	115	67	1.648 (0.95-2.86)	0.075
Recessive	AA+AC	137	95		
	CC (Gln)	36	22	1.0.87 (0.56-2.12)	0.807

N: number of samples, OR: odds ratio, CI: confidence interval; P≤0.05 considered as statistically significant. ORs were adjusted for the age and sex status of the study cohort in a logistic regression model.

Table 4. Comparison of genotype frequencies and analysis of the *GPX1*, *SOD2* and *XPD* polymorphisms between patients and controls based on three genetic models among males.

Gene Model	Genotype /Allele	Control N=179	Cancer N=121	OR (95%Cl)	*p*-value
GPX1					
Additive	CC (Pro)	89	55	1	
	CT	69	44	1.209 (0.69-2.09)	0.500
	TT (Leu)	**21**	**22**	**2.016 (0.95-4.26)**	**0.067**
Dominant	CC (Pro)	89	55		
	CT+TT	90	66	1.396 (0.84-2.31)	0.196
Recessive	CC+CT	158	99		
	TT (Leu)	**21**	**22**	**1.876 (0.93-3.78)**	**0.079**
SOD2					
Additive	CC (Ala)	64	30	1	
	CT	**77**	**66**	**2.049 (1.13-3.71)**	**0.018**
	TT (Val)	38	25	1.253 (0.61-2.56)	0.539
Dominant	CC (Ala)	64	30		
	CT+TT	**115**	**91**	**1.754 (1.01-3.05)**	**0.047**
Recessive	CC+CT	141	96		
	TT (Val)	38	25	1.231 (0.67-2.28)	0.508
XPD					
Additive	AA	57	50	1	
	AC	91	48	1.275 (0.63-2.60)	0.504
	CC	31	23	0.746 (0.37-1.49)	0.409
Dominant	AA (Lys)	57	50		
	AC+CC	122	71	1.624 (0.97-2.73)	0.067
Recessive	AA+AC	148	98		
	CC (Gln)	31	23	1.074 (0.56-2.05)	0.829

N: number of samples, OR: odds ratio, CI: confidence interval; P≤0.05 considered as statistically significant. ORs were adjusted for the age and sex status of the study cohort in a logistic regression model.

OR=2.15, 95%Cl=1.16-3.99, P=0.016) and in the dominant model (CT+TT genotype OR=1.78, 95%Cl=0.99-3.17, P=0.052) among smokers (Table 3)).

Positive correlations between *SOD2* rs4880 and HNSCC risk were also identified in additive (CT genotype OR=2.049, 95%Cl=1.13-3.71, P=0.018) and dominant (OR=1.754, 95%Cl=1.01-3.05, P=0.047) models among males as shown in Table 4. The risk of HNSCC and *GPX1* gene additive and recessive models tend towards significance for TT carriers (OR=2.016, 95%Cl=0.95-4.26, P=0.067 and OR=1.876, 95%Cl=0.93-3.78, P=0.079).

Table 5 summarizes the interaction analysis of the three SNPs and overall risk for HNSCC using a conditional logistic regression model. The analysis revealed that individuals with *GPX1* TT and *SOD2* CT genotypes had a 3.84-fold increased risk of HNSCC (OR=3.839, 95%Cl=1.585-9.296, P=0.003). In terms of the subgroup analyses, similar results were observed among smokers and males since *GPX1* TT and *SOD2* CT allele carriers had 5.75- and 4.72-fold increased risk for HNSCC, respectively. The overall combined genotype analysis of the three SNPs showed that individuals with *GPX1* TT, *SOD2* CT and *XPD* CC alleles had a 12.471-fold increased risk for HNSCC (OR=12.471, 95%Cl=1.342-115.873, P=0.027).

DISCUSSION

Accumulating evidence suggests that oxidative stress and ROS play an important role in cancer development [12]. It is widely accepted that HNSCC is generally associated with tobacco consumption, alcohol

Table 5. Interaction analysis of *GPX1*, *SOD2* and *XPD* polymorphisms.

SNP-SNP interactions	*B*	SE	Wald statistics	OR	95%Cl	*p*-value
Overall						
GPX1 – SOD2	0.283	0.128	4.889	**1.327**	1.033-1.705	**0.027**
GPX1 TT and SOD2 CT carriers	1.345	0.451	8.887	**3.839**	1.585-9.296	**0.003**
GPX1 – XPD	-0.016	0.136	0.014	0.984	0.753-1.285	0.906
SOD2 – XPD	0.067	0.115	0.343	1.070	0.854-1.341	0.558
In smokers						
GPX1 – SOD2	0.441	0.162	7.392	**1.554**	1.131-2.135	**0.007**
GPX1 TT and SOD2 CT carriers	1.749	0.581	9.055	**5.748**	1.840-17.956	**0.003**
GPX1 – XPD	-0.006	0.157	0.002	0.968	0.730-1.352	0.968
SOD2 – XPD	0.062	0.127	0.237	1.064	0.829-1.366	0.626
In males						
GPX1 – SOD2	0.494	0.158	9.723	**1.638**	1.201-2.235	**0.002**
GPX1 TT and SOD2 CT carriers	1.551	0.539	8.293	**4.716**	1.641-13.551	**0.004**
GPX1 – XPD	-0.007	0.150	0.002	0.993	0.741-1.332	0.965
SOD2 – XPD	0.085	0.126	0.449	1.088	0.850-1.393	0.503
Overall						
GPX1-XPD-SOD	0.128	0.117	1.203	1.137	0.904-1.429	0.273
GPX1 TT, *SOD2* CT and *XPD* CC carriers	2.523	1.137	4.923	**12.471**	1.342-115.873	**0.027**

B: partial logistic regression coefficient, SE: standard error of partial slope coefficient, OR: odds ratio, CI: confidence interval; P≤0.05 considered as statistically significant.

Table 6. Summary of selected relevant case-control studies focused on *SOD2*, *GPX1* and *XPD* polymorphisms associated with HNSCC

Gene	Country	Ethnicity	Cancer type	Sample size		Results	Reference
				Case	Control		
***SOD2* rs4880**	USA	Caucasian	Esophageal adenocarcinoma	144	94	Not associated	[31]
	Ireland	Caucasian	Esophageal adenocarcinoma	207	223	Not associated	[32]
	China	Han Chinese	Oral squamous cell carcinoma (OSCC)	362	358	CT genotype associated with higher risk for OSCC	[23]
***GPX1* rs1050450**	USA	Caucasian and African American	Head and neck cancer (HNC)	133	517	TT genotype associated with higher risk for HNC	[27]
	Taiwan	Taiwanese	Oral cavity cancer	122	122	Not associated	[28]
***XPD* rs13181**	Malaysia	Malaysian	Nasopharyngeal carcinoma	157	136	Not associated	[33]
	India	Caucasian	HNSCC	278	278	CC genotype associated with 2-fold increased risk for the HNC	[17]
	Korea	Asian	HNSCC	290	358	Not associated	[34]
	Poland	Caucasian	HNC	105	110	CC genotype was associated with a higher risk of HNC	[35]
	North India	Caucasian	HNSCC	275	385	CC, AC and dominant model AC+CC genotypes associated with increased risk	[10]
	USA	Caucasian	HNSCC	829	854	Not associated	[36]
	Poland	Caucasian	HNC	172	143	Not associated	[37]
	USA	Caucasian	HNC	425	683	Not associated	[38]
	USA	Caucasian	HNC	189	496	Not associated	[39]

abuse, or both. However, even though they are high-risk factors for HNSCC, not every individual who smokes and consumes alcohol gets cancer since genetic differences and exposure to the human papilloma virus (HPV), Epstein-Barr virus (EBV) infection, radiation exposure and epigenetic hypermethylation might also contribute to development of HNSCC [1, 13]. Based on this information, the antioxidant defense system and DNA repair ability against ROS-induced lesions could be potential indicators for susceptibility to HNSCC. Identification of appropriate molecular biomarkers contributes to early-stage detection of HNSCC; PET imaging, HPV16 DNA for the determination of HPV status and programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) are validated diagnostic and predictive biomarkers currently used in clinical practice. In the future, other molecular markers such as genetic and epigenetic markers alone or in combination with imaging markers, could be used for early-stage detection of HNSCC.

To the best of our knowledge, we present the first study that demonstrates the role of *SOD2* Val16Ala, *GPX1* Pro198Leu and *XPD* Lys751Gln gene polymorphisms, together and in combination in three different genetic models in the development of HNSCC. The head and neck area is readily exposed to potential carcinogens that can influence carcinogenesis by the formation of bulky DNA adducts. XPD protein is a member of the NER pathway, a major repair mechanism, and is responsible for removing bulky DNA adducts [8]. Since there is a link between an individual's ability to repair DNA damage, it is reasonable to expect that variations in the DNA repair gene can play a vital role in the development and progression of HNSCC [14]. It was previously reported that *XPD* Lys751Gln polymorphism results in a lower DNA repair capacity or higher risk for carcinogenesis [15], and that the relationship between cancers of the upper respiratory tract and *XPD* Lys751Gln polymorphism indicates that the CC genotype of *XPD* causes a 2-fold increased risk in upper aerodigestive tract cancer [16]. Similarly, it was found that *XPD* Lys751Gln variants enhanced the risk of HNSCC 2-fold in the north India population [17]; however, studies carried out on several populations revealed no correlation between *XPD* Lys751Gln polymorphism and HNSCC [18, 19].

In our study, there was no significant difference in Lys751Gln polymorphism between HNSCC cases and controls and in subgroups of smokers or males. However, although below statistical significance, under dominant model analysis there was a slightly increased risk for individuals who were carriers of the T allele. The apparent discrepancy of results concerning *XPD* Lys751Gln polymorphism and cancer could be attributed to many factors, including ethnic differences, organ region, control groups comprised of patients' relatives, and other DNA repair genes involved in the repair mechanism.

Among antioxidant SOD enzymes, SOD2 is located in the mitochondria, which is a major site for ROS production [20]. The rs4880 polymorphism of the *SOD2* gene results in an amino acid change of Ala to Val, and this substitution decreases SOD2 antioxidant activity by 30-40% [21]. Moreover, the Val variant has been known to impair cotranslational import, which leads to slower mitochondrial import and lowers SOD2 activity, consequently leading to different pathologies including cancer [22]. There are a few studies on head and neck area cancers and the *SOD2* Val16Ala relationship. In the current study, it was established that the CT genotype of the *SOD2* gene was associated with a higher risk of HNSCC in smoker and male subgroups, and further analysis of genotype dominance indicated a significant association between the dominant genotype (CT+TT) and HNSCC in smokers and males, which is in accordance with a previously published study conducted on oral squamous cell carcinoma patients [23]. Since we found an association between *SOD2* CT genotype and HNSCC in males but not females, we suggest that reproductive hormones might have a protective role against oxidative stress, which is supported by previous studies [24, 25].

GPX1 is the main glutathione peroxidase in the mammalian liver, therefore genetic variations in *GPX1* have been a focus for susceptibility to cancer. The transition of C to T in exon 2 of the *GPX1* gene corresponds to an amino acid change from proline (Pro) to leucine (Leu) that leads to a lower GPX1 activity, which might be associated with cancer [26]. The association between head and neck cancer and *GPX1* Pro198Leu polymorphism was investigated, with the authors showing that individuals who carried the TT

allele had a 1.8-fold increased risk of cancer compared with controls [27]. On the other hand, a relationship between oral cavity cancer and *GPX1* polymorphism was not found in a smoker-male population [28]. In our study, we did not find any association between *GPX1* gene Pro198Leu polymorphism and HNSCC. However, in smokers, we observed that individuals with the TT genotype had 2.55- and 2.54-fold increased risks for HNSCC in the additive and recessive models, respectively. From this aspect, this is the first study to examine the potential role of *GPX1* Pro198Leu polymorphism in the association of smoking status and male gender between HNSCC cases and controls.

The efficiency of the GPX1 enzyme decreases in T allele carriers and it has been previously reported that smoking results in lower GPX activity, and that T allele carriers who are also smokers have decreased GPX1 enzyme activity [29]. Because H_2O_2 is inefficiently expelled from the medium, ROS that are caused by the surplus production of hydroxyl radicals interact with DNA and other molecules. The resulting mutations might lead to the initiation of cancer development, and a higher risk for cancer can be found in individuals who smoke and have a mutant *GPX1* genotype.

Recent studies demonstrated that reduced expression of *SOD2* increased the incidence of cancer, and it has been known that reduced expression levels of SOD2 might also result in increased DNA damage due to lower protection against ROS, and that the *XPD* mutant genotype had a lower capacity for repair than the wild type [23, 30]. Consequently, these genotypes together can lead to an increase in cancer risk.

A summary of selected relevant case-control studies focusing on *SOD2*, *GPX1* and *XPD* polymorphisms in association with HNSCC is presented in Table 6, which confirms the inconclusive results in the field, and provides support that this is the first study evaluating these three polymorphisms together. Our results indicate that homozygosity for *GPX1* (TT), heterozygosity for *SOD2* and homozygosity for the *XPD* (CC) are associated with a 12.47-fold increased risk of HNSCC, which supports the hypothesis that oxidative stress and DNA damage together contribute to the development of HNSCC. In addition, in smokers and males, individuals carrying *GPX1* TT and *SOD* CT belong to a higher risk group for the development of HNSCC. Antioxidant supplementation might be helpful for these groups since they are prone to the development of HNSCC. Moreover, regarding genotype-based personalized antioxidant supplementation, ROS elimination should also be considered to overcome the therapeutic challenges of chemo-/radiotherapy. As previously shown and revealed in our study, exposure to tobacco smoke is a risk factor for HNSCC, as well as the genetic background.

CONCLUSION

Several studies have assessed the independent relationship between *SOD2*, *XPD* and *GPX1* genetic variations and cancer susceptibility; however, their conclusions remain inconsistent since a single genetic variant is insufficient to predict the risk of such a complex disease. To draw a more comprehensive estimation of this possible association, we conducted an analysis under three different genetic models and the SNP-SNP interaction model for *SOD2* rs4880*, GPX1* rs1050450 and *XPD* rs13181 genes. For the first time, we show that in the overall genotype analysis of the *SOD2*, *XPD* and *GPX1* genes, individuals with *GPX1* TT, *SOD2* CT and *XPD* CC alleles had 12.471-fold increased risk for HNSCC. As this is the first report regarding the correlation between these polymorphisms and HNSCC, further studies with larger sample sizes and different ethnic groups should be performed to confirm this correlation.

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Data availability: Data underlying the reported findings have been provided as part of the submitted article and are available at: https://www.serbiosoc.org.rs/NewUploads/Uploads/Kose%20et%20al_7770_Data%20Report.pdf

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