Promoter region mutations of the telomerase reverse transcriptase *(TERT)* gene in head and neck squamous cell carcinoma

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Objective. The aim of the present study was to assess the prevalence of *TERT* promoter region mutations in tumor samples of patients with squamous cell carcinoma at different sites of the head and neck region and correlate it with patients' clinicopathologic data.

Study Design. Mutations in promoter region of the *TERT* gene were analyzed with polymerase chain reaction–based direct sequencing method using formalin-fixed, paraffin-embedded tumor samples of 189 HNSCCs. *TERT* promoter region mutations were assessed in terms of age, gender, location, smoking, alcohol consumption, and overall survival.

Results. *TERT* promoter region mutations were detected in the oral cavity (75%); larynx (8.4%), hypopharynx (16.6%), and oropharynx (0%). *TERT* promoter region mutations are associated with younger age and female gender and have a reverse relationship with smoking and alcohol consumption.

Conclusions. We found statistically significant higher rates of *TERT* promoter region mutations in tumor samples of patients with squamous cell carcinoma in the oral cavity compared with other locations in the head and neck region. (Oral Surg Oral Med Oral Pathol Oral Radiol 2020;130:63–70)

Squamous cell carcinoma (SCC) is the most common type of head and neck cancer and is the sixth most common malignant tumor worldwide. Tobacco and its derivatives and alcohol consumption are the main etiologic factors. In the last 2 decades, it has been shown that human papillomavirus (HPV) infection plays a major role in development of oropharyngeal SCC.[1-3] Surgery, chemotherapy, and radiotherapy are used alone or in combination in the treatment of head and neck squamous cell carcinomas (HNSCCs). Unlike other cancer types, there are only very few predictive biomarkers and targeted therapies for HNSCCs.[3-5]

It is well known that HNSCCs are characterized by genetic alterations, genomic instability, and different immune defects, and studies to find new mutations and its effective treatment modalities are still ongoing. A gene mutation, an overexpressed protein, or a protein that reflects the activation status of a signaling pathway may be useful as a biomarker and assist in monitoring of the response to treatment or may provide information regarding prognosis and outcome.[4] The Cancer Genome Atlas study represents the most comprehensive integrative genomic analysis of HNSCC.[5] In this study, multiple different somatic genomic alterations in HPV-related and HPV-nonrelated HNSCC were detected. The results of our analysis offer new hope for individualized immunotherapy and targeted therapy for patients.[5] But the genetic events in the noncoding regions of human genomes in HNSCCs have not been comprehensively studied.

Telomerase activation is the hallmark of cancer and is detected in greater than 90% of malignant tumors. The telomerase reverse transcriptase *(TERT)* gene, which encodes the catalytic subunit of telomerase, is a determinant of telomerase activity. *TERT* promoter region mutations have been demonstrated to confer increased transcriptional activity by creating binding motifs for E-twenty-six (ETS)/ternary complex factor (TCF) transcription factors, thereby allowing cancer cell immortality.[6] *TERT* promoter region mutations have been

Statement of Clinical Relevance

Our study indicates that promoter region mutations of the telomerase reverse transcriptase *(TERT)* gene in head and neck squamous cell carcinomas are associated with younger age, female gender, and have a reverse relationship with smoking and alcohol consumption.

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reported in a variety of tumors and often shown to be associated with aggressive behavior. *TERT* promoter region mutations are common in melanoma, glioma, hepatocellular carcinoma, thyroid, bladder, and skin cancers, and it occurs infrequently in esophageal, lung, and gastric cancers.[6-11] Two different mutations (C228 T and C250 T) have been described in *TERT* promoter region in most of the cancers mentioned above.[6,7,9,12]

The literature has few studies on *TERT* promoter region mutations in patients with HNSCCs, and there are not enough data to designate its role in etiopathogenesis and prognosis.[13-19] The aim of the present study was to assess the prevalence of *TERT* promoter region mutations in patients with SCC at different sites of the head and neck region and correlate it with patients' clinicopathologic data.

MATERIALS AND METHODS

Patient selection

Data on 212 patients with HNSCCs were collected from the archives of the Department of Pathology, Sultan Abdulhamid Han Training Hospital, University of Health Sciences and Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey. The study was approved by the medical ethics committee of the Istanbul Faculty of Medicine, Istanbul University (Approval No: 16.06.2017/707) and was conducted according to the tenets of the Declaration of Helsinki. All patients had undergone appropriate diagnosis and clinical follow-up.

Mutation analysis

Mutations in the promoter region of the *TERT* gene (chr5, 1,295,228 C>T and 1,295,250 C>T) were examined on representative formalin-fixed, paraffin-embedded (FFPE) tumor samples by using our previously described[20] polymerase chain reaction (PCR)−based direct sequencing (analytical sensitivity 25%) method. Tumor targets (> 75% viable tumor) were manually microdissected from 5-μm thick unstained histologic sections for enrichment of tumor cellularity. Deparaffinization of the sections was performed. Then, DNA was isolated by using QIAamp DNA FFPE Tissue Kit (50) (catalog #: 56404) (QIAGEN, Hilden, Germany). The amplification process was carried out in a Thermal Cycler (Veriti 96−Well Thermal Cycler; Applied Biosystems, Thermo Fisher Scientific Inc., Foster City, CA). HotStarTaq DNA Polymerase kit (catalog #: 203205) (QIAGEN, Hilden, Germany), forward (5'CAGCGCTGCCTGAAACTC'3), and reverse (5'GTCCTGCCCCTTCACCTT'3) primers were used in the preparation of the PCR master mix. The final volume of PCR reactions were 50 μL. The mixture of PCR comprised 50 ng of each tumor DNA, 10 μL Q solution, 7 μL of each primer (4 pmol/μL), 5 μL 10 × PCR buffer, 1.5 μL 10 mM dNTP mix (Applied Biosystems, Thermo Fisher Scientific Inc., Foster City, CA), and 0.25 μL of HotStarTaq DNA polymerase and nuclease free water (QIAGEN, Hilden, Germany). The PCR condition protocol consisted of an initial denaturation at 95°C for 15 minutes, followed by 42 cycles of 30 seconds denaturation at 95°C, 30 seconds optimized annealing at 55°C, and 45 seconds extension at 72°C. The PCR run ended with a final extension at 72°C for 10 minutes. The intensities of PCR products and reagent contamination control were analyzed by using gel electrophoresis with an ultraviolet transilluminator (Gel Logic 200 Imaging System, Eastman Kodak Company, Rochester, NY). Then, all resultant PCR products were cleaned to remove the unincorporated primers and dNTPs for Sanger sequencing by using QIAquick PCR Purification Kit (catalog #: 28106) (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. The purified amplicons were sequenced in both the forward and reverse directions by using reagents from the Big Dye Terminator v3.1 Cycle Sequencing kit (catalog #: 4337455) (Applied Biosystems, Thermo Fisher Scientific Inc., Foster City, CA) in accordance with the manufacturer's protocol. After ethanol precipitation, the reaction products were run on the ABI-3730 (48 capillary) automatic sequencer (Applied Biosystems, Thermo Fisher Scientific Inc., Foster City, CA). Bidirectional sequence traces were analyzed with SeqScape Software v3.0 (Applied Biosystems, Thermo Fisher Scientific Inc., Foster City, CA) and manually reviewed.

Clinical data

Tumors were classified according to their localization as oral cavity (tongue, buccal mucosa, hard palate, retromolar trigone, alveolar arch, floor of mouth); oropharyngeal (base of tongue, soft palate, and tonsil); laryngeal and hypopharyngeal tumors. Clinical information, including age, gender, localization, smoking and alcohol consumption history, and overall survival (OS) were collected from patients' files (Table I).

Statistical analysis

Statistical analysis was performed by using SPSS 15.0 software (SPSS Inc., Chicago, IL). Mean and standard deviations were used for numeric variables, whereas median was used for categorical ones. Survival function was analyzed by using the Kaplan-Meier method. Risk assessment was studied by using the χ^2 test. Age difference was examined by using the Mann-Whitney U test. Confidence level for statistical significance was determined as 95%. Post hoc power analysis was studied by using G*Power 3.1 for parameters with a significant difference, and type II error rates were greater than 0.001 except for alcohol and age (0.45 and 0.21, respectively).

Table I. Demographic data of patients with head and neck squamous cell carcinomas (HNSCCs)

Data	*Number of patients with HNSCCs*
Gender	
Female	50
Male	139
Age (mean ± standard deviation [SD])	63.35 ± 12.82
Smoking	
Smokers	125
Nonsmokers	57
Unknown	7
Alcohol consumption	
Drinkers	45
Nondrinkers	137
Unknown	7
Location	
Oral cavity	
Tongue	59
Hard palate	6
Alveolar arch	5
Floor of mouth	12
Buccal	16
Retromolar trigone	4
Oropharynx	
Base of tongue	5
Soft palate	4
Tonsil	13
Larynx	59
Hypopharynx	6
Total	189

RESULTS

Twenty-three test samples were found to have inadequate DNA quality and were excluded from the study, and 189 patients' tumor tissue samples were analyzed. Patients were classified according to 4 subsites of the head and neck region: oral cavity (102 patients); larynx (59 patients); hypopharynx (6 patients); and oropharynx (22 patients).

TERT promoter region mutations in the tumor samples of patients with oral squamous cell carcinoma (OSCC) (77 of 102) were significantly higher than those from the other locations (oropharynx (0 of 22), larynx (5 of 59) and hypo-pharynx (1 of 6) ($P < .001$) (Table II). There was no statistically significant difference among the oral cavity subsites with regard to mutation rates (Table III).

We identified 3 different *TERT* promoter region mutations (Figure 1). These mutations were C228T (56 of 83; 67.5%), C250T (22 of 83; 26.5%), and C228A (5 of 83; 6%). C228T mutation was almost 2 times more frequent than C250T and C228A mutations in total (Table IV).

Of the 189 patients with HNSCCs, 50 were women (26.4%) and 139 were men (73.6%), and median age was 63.3 years. The clinical features of the patients according to *TERT* mutation status are shown in Table V.

TERT mutation was observed significantly higher in tumor samples of female patients among all HNSCCs. Of the cases harboring *TERT* mutation, 72% (36 of 50) were females, and 33.8% (47 of 139) were males ($P < .001$) (see Table V). Among the 102 oral cavity tumors, there was no statistically significant association between *TERT* mutation status and gender ($P = .196$). *TERT* mutation was detected in the tumor samples of 36 (81.8%) and 41 (70.7%) of the 44 female and 58 male patients with OSCCs, respectively (Table VI).

In the overall cohort, a statistically significant difference in age was found between patients with *TERT* promoter region mutation−positive HNSCCs (median age 60.39 years) and those with *TERT*-negative HNSCCs (median age 65.68 years) ($P = .041$) (see Table V). *TERT* promoter region mutations were also found to be significantly higher in the OSCC tissue samples of younger patients ($P = .004$) (see Table VI).

TERT mutations were observed significantly lower in smokers ($P < .001$) and in patients with a history of alcohol consumption ($P = .044$) among all HNSCC tumor samples (see Table V). Of the 102 OSCC tumor samples, *TERT* mutation was observed to be significantly lower in patients with a history of alcohol consumption ($P = .033$) but did not reach statistical significance in smokers ($P = .177$) (see Table VI).

Median OS after surgery for the entire cohort was 106 months (95% confidence interval [CI] 90.62−121.99 months). Patients with HNSCC with *TERT* mutation−negative tumor samples had slightly shorter median OS (97 months; 95% CI 80.24−113.76 months), but this was not statistically significant ($P = .364$) compared with patients harboring *TERT* mutation in their tumors (median 110 months; 95% CI 98.01−121.99 months) (Figure 2A). In the oral cavity−specific cohort, the median OS for patients with and those without *TERT* mutation in their tumors was similar (median 110−109 months; 95% CI 101.84−118.16 months and 96.62−121.38 months, respectively; $P = .499$) (Figure 2B).

DISCUSSION

Although tobacco, alcohol, and HPV are the main risk factors for HNSCC, epigenetic alterations have been associated with the carcinogenesis of SCC. Telomeres are important for chromosomal integrity, and their dysfunction may result in carcinogenic activity.[15,16] *TERT* promoter region mutations have been found in a variety of cancers, and it may also be associated with some prognostic factors and survival.[9,12,13,16] A few studies have evaluated *TERT* promoter region mutations in HNSCC, but most of these studies did not evaluate the

Table II. Telomerase reverse transcriptase *(TERT)* mutations according to locations of head and neck squamous cell carcinomas (HNSCCs)

Location	*TERT wt #*	*TERT mutated #*	*Odds ratio (OR)*	*95% confidence interval (CI)*		*P*
Oral cavity	25 (24.51%)	77 (75.49%)	41.58	16.177	106.875	< .001
Other locations	81 (93.10%)	6 (6.90%)				
Larynx	54 (91.53%)	5 (8.47%)				
Hypopharynx	5 (83.33%)	1 (16.67%)				
Oropharynx	22 (100%)	0 (0%)				

Table III. Telomerase reverse transcriptase *(TERT)* mutations according to subsites of the oral squamous cell carcinoma (OSCC)

Location	#	*TERT mutated*	*TERT wt #*	*P*
Tongue	59	47 (79.7%)	12 (20.3%)	0.251
Hard palate	6	4 (66.6%)	2 (33.4%)	0.633
Alveolar arch	5	3 (60%)	2 (40%)	0.594
Floor of mouth	12	8 (66.7%)	4 (33.3%)	0.482
Buccal	16	13 (81.3%)	3 (17.7%)	0.755
Retromolar trigone	4	2 (50%)	2 (50%)	0.251
Total	102	77 (75.5%)	25 (24.5%)	

relationship between the subsites of HNSCCs and the clinicopathologic features of the patients.[7,11-13,15-19] In our study, we sought to assess the presence of *TERT* promoter region mutations in patients with SCC at different sites of the head and neck region and to compare it with patients' clinicopathologic data.

Vinothkumar et al. reported *TERT* promoter region mutation in 31.7% (13 of 41) of HPV-negative OSCCs, and C228T mutation was 2 times higher than C250T mutation.[14] In our study, we found *TERT* promoter region mutation in 75.49% of OSCCs, and in accordance with this finding, we found C228T mutation to be 2.5 times higher compared with C250T mutations. In addition, we also performed a subsite analysis of the oral cavity and found no significant difference among *TERT* mutation rates; however, a subsite analysis was not mentioned in the report by Vinothkumar et al.[14] Chang et al. reported the largest *TERT* promoter region mutation analysis of OSCCs (201 patients).[15] They found *TERT* promoter region mutation in 64.7% in the oral cavity, and the prevalence rates of C228T and C250T in OSCCs were 51.7% and 12.9%, respectively.[15] In our study, we found a higher *TERT* mutation rate (77 of 102; 75.49%). Chang et al. did not report the mutation rates; however, they reported on the number of patients categorized on the basis of different subsites. We evaluated the mutations according to the subsites in the oral cavity and found out that the buccal mucosa (81.3%) and the tongue (79.7%) had the highest mutation rates among the subsites. The prevalence of C228T, C250T, and C228A in OSCCs were 50%, 20.59%, and 4.90%, respectively.

Morris et al. reported *TERT* mutations in 16 of 30 HPV-negative recurrent and metastatic HNSCCs (53%) and in none of the 23 HPV-positive recurrent and metastatic HNSCCs. They found 11 C228T, 3 C250T and 2 C228A mutations.[17] Annunziata et al. reported *TERT* promoter region mutation in 9 of 15 patients with OSCCs (60%), and 2 of these patients were HPV positive.[18] Barczak et al. reported C250T *TERT* promoter region mutations in 22 of 61 patients with HNSCCs (36%).[19] We found a higher rate of *TERT* promoter region mutations (75.49%) compared with the above-mentioned studies, and in accordance with previous studies, C228T mutations were more frequent than C250T and C228A mutations in total.

Killela et al. evaluated 70 cases of HNSCCs for *TERT* promoter region mutations, and they found 12 mutated tumors.[16] Eleven of the 12 mutated cancers occurred in the anterior tongue and 1 in the alveolar ridge. Killela et al. did not report *TERT* promoter region mutations in any cancers of the floor of the mouth (n = 8); mandible (n = 1); hard palate (n = 2); larynx (n = 7); oropharynx/hypopharynx (n= 3); tonsil (n = 18); and base of the tongue (n = 7). We also did not find *TERT* promoter region mutations in any cancers of the oropharynx (tonsil and base of the tongue) and hypopharynx. We found *TERT* mutations in 5 (8.47%) of the 59 patients with laryngeal SCC. In contrast, we found *TERT* promoter region mutations in cancers of the floor of the mouth, hard palate, alveolar arcus, and buccal and retromolar trigone, so our results did not support the self-renewing hypothesis of *TERT* promoter region mutations in the oral cavity as reported by the Killela et al. study. As those authors mentioned, the squamous epithelia of the tongue certainly would not be expected to self-renew any less than other squamous epithelia of the oral cavity.[16]

In accordance with previous studies, we found a low frequency of *TERT* promoter region mutations in patients with SCC of the oropharynx (0%) and hypopharynx (6.6%). Only one 1 had *TERT* C228T mutation among the 6 hypopharyngeal SCCs and none in the 22 oropharyngeal SCCs. Annunziata et al. also did not find *TERT* promoter region mutations in the 9 oropharyngeal SCCs in their study, and 5 of these were HPV positive.[18] Killela et al. studied *TERT* promoter region

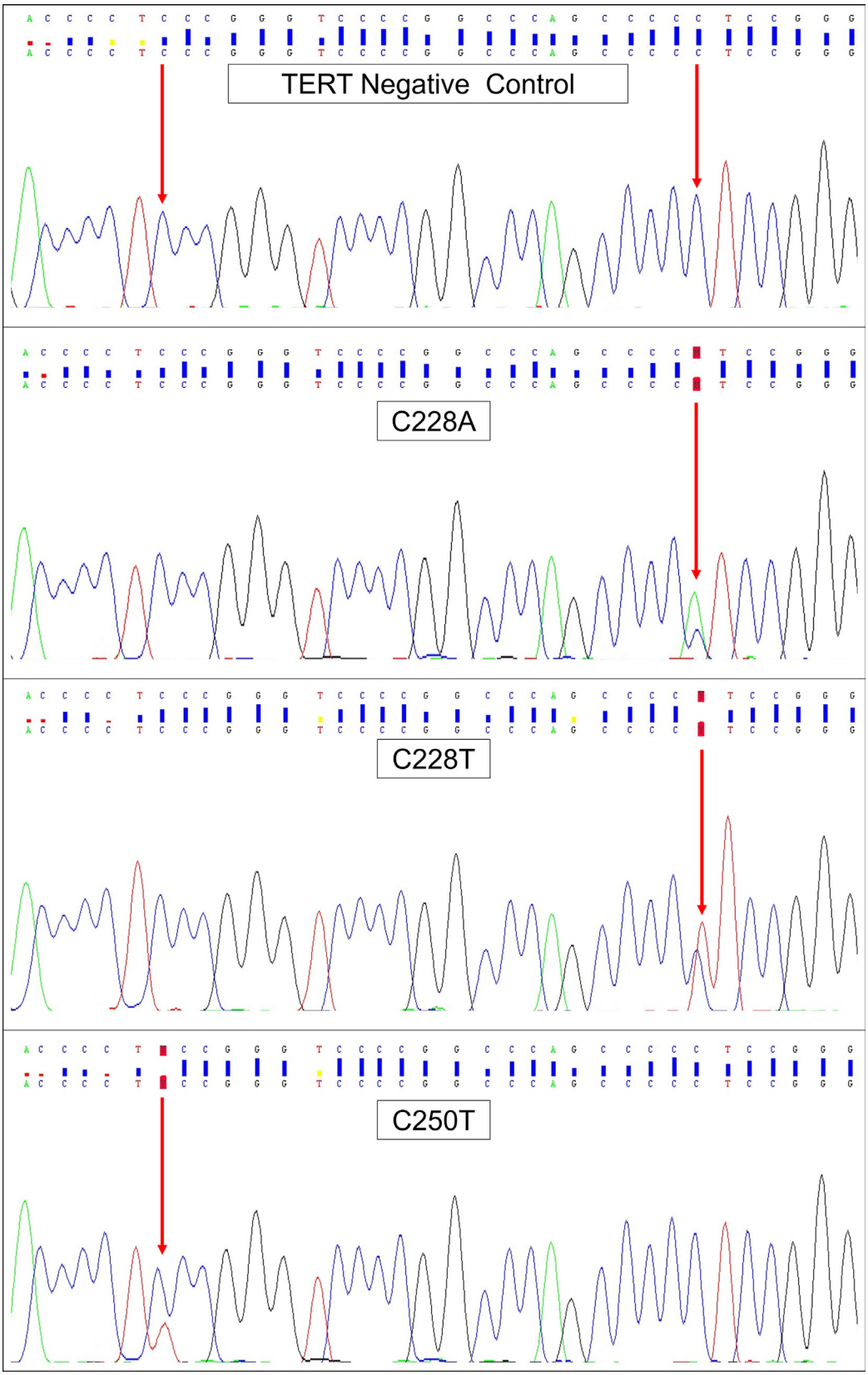

Fig. 1. Samples of sequencing electropherograms of mutated tumor cases for the telomerase reverse transcriptase *(TERT)* gene. Mutated tumor cases are shown in the lower lanes, whereas corresponding regions of the IVS-000 polyclonal control DNA's reference sequence are shown in the upper lane.

Table IV. Telomerase reverse transcriptase *(TERT)* mutation types according to locations of head and neck squamous cell carcinomas (HNSCCs)

TERT mutation	*Oral cavity (n =102)*	*Larynx (n = 59)*	*Hypopharynx (n = 6)*	*Oropharynx (n = 22)*
C228T	51 (50.00%)	4 (6.78%)	1 (16.66%)	0 (0%)
C250T	21 (20.59%)	1 (1.70%)	0 (0%)	0 (0%)
C228A	5 (4.90%)	0 (0%)	0 (0%)	0 (0%)
Total	77 (75.49%)	5 (8.48%)	1 (16.66%)	0 (0%)

n = Number of positive samples/numbers of total samples.

Table V. The clinical features of the patients with head and neck squamous cell carcinomas (HNSCCs) according to telomerase reverse transcriptase *(TERT)* mutation status in their tumor samples

	TERT wt (N (%))	*TERT mutated (N [%])*	*Odds ratio (OR)*	*95% confidence interval (CI)*		*P*
Gender						
Female	14 (28)	36 (72)	5.033	2.474	10.241	< .001
Male	92 (66.2)	47 (33.8)				
Smoking*						
Smokers	88 (70.4)	37 (29.6)	0.194	0.099	0.382	< .001
Nonsmokers	18 (31.6)	39 (68.4)				
Alcohol consumption*						
Drinkers	32 (71.1)	13 (28.9)	0.477	0.231	0.987	.044
Nondrinkers	74 (54)	63 (46)				
Age (mean ± standard deviation [SD])	65.68 ± 10.18	60.39 ± 15.17				.041

*In 7 patients, a history of smoking and alcohol consumption was not available, so these cases were excluded from statistical analyses.

Table VI. The clinical features of the patients with oral squamous cell carcinoma (OSCC) according to telomerase reverse transcriptase *(TERT)* mutation status in their tumor samples

	TERT wt (N [%])	*TERT mutated (N [%])*	*Odds ratio (OR)*	*95% confidence interval (CI)*		*P*
Gender						
Female	8 (18.2)	36 (81.8)	0.536	0.207	1.389	.196
Male	17 (29.3)	41 (70.7)				
Smoking*						
Smokers	15 (32.6)	31 (67.4)	0.530	0.209	1.341	.177
Nonsmokers	10 (20.4)	39 (79.6)				
Alcohol consumption*						
Drinkers	9 (45)	11 (55)	0.331	0.177	0.938	.033
Nondrinkers	16 (21.3)	59 (78.7)				
Age (mean ± standard deviation [SD])	67.28 ± 8.81	59.89 ± 15.50				.004

*In 7 patients, a history of smoking and alcohol consumption was not available, so these cases were excluded from statistical analyses.

mutation in 70 tumor specimens of different head and neck locations, and no mutation was found in the 2 hypopharyngeal SCCs.[16] Zhao et al. studied 313 esophageal SCCs to show *TERT* promoter region mutations, and they only identified 4 C228T and 1 C250T somatic mutations (1.6%).[10] Although the number of studied patients was less than those with other types of cancers, these results suggest that *TERT* promoter region mutations may have no relationship to the etiopathogenesis of oropharyngeal, hypopharyngeal, and esophageal SCCs.

In previous studies,[14,15] no statistical association was found between *TERT* mutations and gender or age in HNSCCs. In a meta-analysis,[9] *TERT* promoter region mutations were reported at a higher age at diagnosis in patients with glioma and lung and thyroid cancers, whereas patients with melanoma displayed an opposite pattern.[9] In that report, it was mentioned that male patients with thyroid cancer, melanoma, and hepatocellular carcinoma exhibited a significantly elevated risk of having *TERT* mutations.[9] In our study, *TERT* mutations were observed to be significantly higher in females and younger patients with SCCs at the head and neck and oral cavity locations ($P < .001$/$P = .196$ and $P = .041$/$P = .004$, respectively).

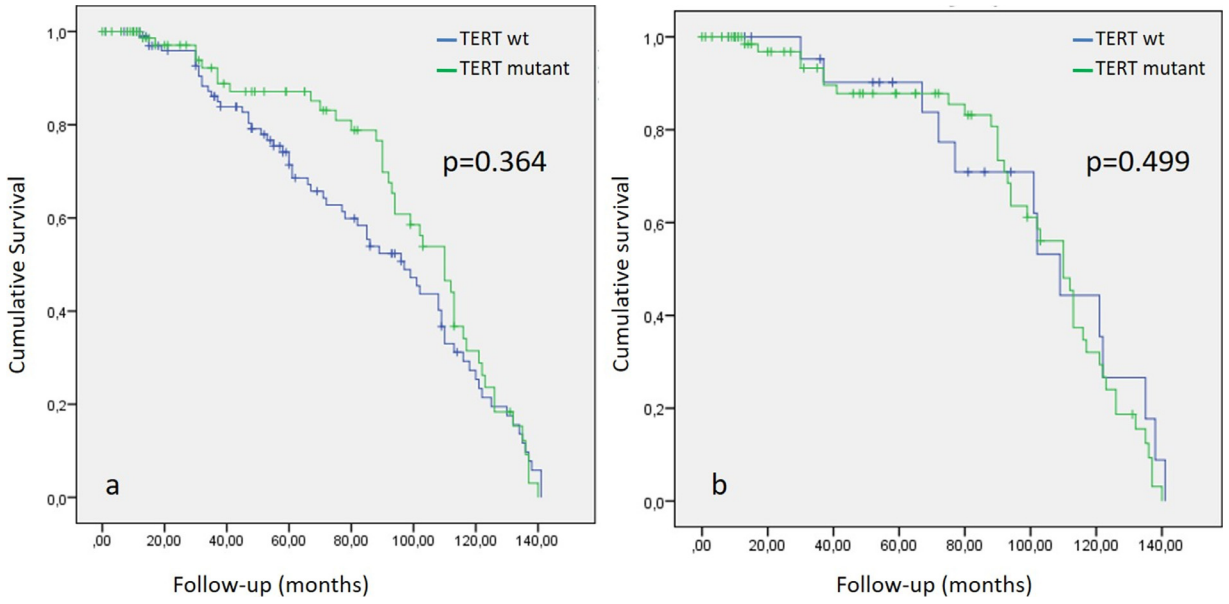

Fig. 2. Survival curve following surgery by presence of telomerase reverse transcriptase *(TERT)* promoter region mutation in the overall cohort **(A)** and the oral cavity specific cohort **(B)**.

Vinothkumar et al. reported that among the *TERT* mutation−positive cases (13 of 41), 12 had a tobacco habit and 3 had a history of alcohol consumption in addition to tobacco abuse.[14] Chang et al. reported a higher rate of *TERT* mutations in patients with OSCCs who had a betel nut chewing habit, but Chang et al. did not report any association among smoking, alcohol consumption, and *TERT* promoter region mutation.[15] Qu et al. also did not find a statistically significant association between *TERT* promoter region mutations in laryngeal SCCs and smoking.[7] In our study, *TERT* mutations were significantly lower in patients who were smokers and in patients with an alcohol consumption habit in the overall cohort and in the oral cavity−specific cohort ($P < .001$/ $P = .177$ and $P = .044$/ $P = .033$, respectively).

The incidence of tongue SCC in the young adult population is increasing in the United States, and it has been shown that the genomic alterations in young (age < 45 years) nonsmokers are similar to those in older smokers.[21] Interestingly, we found significantly less *TERT* mutations in smokers among HNSCC cases and that patients harboring *TERT* mutations in their tumors at oral cavity locations were likely to be female and younger.

In previous studies, *TERT* promoter region mutations were associated mostly with poor prognosis in many human cancers, such as melanoma, urothelial carcinoma, thyroid cancer, gynecologic cancers, gliomas, and laryngeal carcinomas.[7,9] Few studies have investigated the association of *TERT* promoter region mutation status and OS in HNSCCs. Qu et al. reported TERT promoter region mutation in 235 patients with laryngeal SCC; found C250T (56 of 235) and C228T (8/235) mutations with a rate of 27%[7] and a significant worse survival in patients with C250T mutation. In contrast to the Qu et al. study, our *TERT* mutation rate in laryngeal SCCs was lower (4 C228T and 1 C250T in 59 patients (8.47%). Our results determined that *TERT* promoter region mutation is not frequent and cannot be used as a prognostic biomarker in patients with laryngeal SCC. Although patients with HNSCCs harboring *TERT* mutation had slightly shorter median OS, we did not find a significant association between *TERT* promoter region mutation and OS in patients with HNSCC and also in patients with OSCC ($P = .364$ and $P = .499$, respectively). Chang et al. also did not report a statistically significant association between *TERT* promoter region mutation and OS.[15] Future studies will be required to determine whether *TERT* promoter region mutations have prognostic implications in HNSCCs.

The limitation of the present study is the absence of data regarding the HPV status of our patients. We discussed in detail above 3 studies in the literature that investigated both HPV status and *TERT* promoter region mutations in HNSCCs. Two of them identified *TERT* promoter region mutations in only HPV-negative OSCCs. In our study, the HPV status of the tumors could not be evaluated, but the absence of *TERT* promoter region mutations in oropharyngeal SCCs, which is the typical location for HPV-related SCC, and a higher rate of *TERT* promoter region mutations in usually HPV-unrelated OSCCs are remarkable. *TERT* promoter region mutation and HPV infection may represent parallel mechanisms of telomerase activation in HNSCCs as noted in these studies. The other study mentioned that the frequency of *TERT* promoter region mutations was independent of the HPV infection status

in OSCC and cervical SCC. But, the limited number of the investigated cases with conflicting results does not allow the same assumption for HNSCCs.

CONCLUSIONS

We found statistically significant higher rates of *TERT* promoter region mutations in the tumor samples of patients with OSCCs when we compared them with samples from other locations in the head and neck region. The highest rate was related to the buccal location and the lowest to the floor of the mouth (82.35% and 61.53%, respectively). Anterior tongue tumors were the highest among OSCCs (n = 62), and *TERT* promoter region mutation was found with a rate of 77.42% (48 of 62) in the anterior tongue location. These results may suggest an important role of *TERT* promoter region mutations in OSCCs.

Our study is the first to investigate *TERT* promoter region mutations in different subsites of the oral cavity and other parts of the head and neck region. Our study indicates that *TERT* promoter region mutations in HNSCCs are associated with younger age and female gender and have an inverse relationship to smoking and alcohol consumption. Prospective and well-designed studies are needed to validate our findings.

PRESENTATION

This work was presented previously as an oral presentation at the 29th European Congress of Pathology, September 2−6, 2017, in Amsterdam, The Netherlands.

REFERENCES

1. Gooi Z, Chan JY, Fakhry C. The epidemiology of the human papillomavirus related to oropharyngeal head and neck cancer. *Laryngoscope*. 2016;126:894-900.
2. Mallen-St Clair J, Alani M, Wang MB, Srivatsan ES. Human papillomavirus in oropharyngeal cancer: the changing face of a disease. *Biochim Biophys Acta*. 2016;1866:141-150.
3. Hess J. Predictive Factors for outcome and quality of life in HPV-positive and HPV-negative HNSCC. *Recent Results Cancer Res*. 2017;206:233-242.
4. Juodzbalys G, Kasradze D, Cicciù M, et al. Modern molecular biomarkers of head and neck cancer. Part I. Epigenetic diagnostics and prognostics: systematic review. *Cancer Biomark*. 2016;17:487-502.
5. Cancer Genome Atlas Network. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature*. 2015;517:576-582.
6. Miao Y, Wang R, Ju H, Ren G, Guo W, Lyu J. TERT promoter mutation is absent in oral mucosal melanoma. *Oral Oncol*. 2015;51:e65-e66.
7. Qu Y, Dang S, Wu K, et al. TERT promoter mutations predict worse survival in laryngeal cancer patients. *Int J Cancer*. 2014;135:1008-1010.
8. Ma X, Gong R, Wang R, et al. Recurrent TERT promoter mutations in non-small cell lung cancers. *Lung Cancer*. 2014;86:369-373.
9. Yuan P, Cao JL, Abuduwufuer A, et al. Clinical characteristics and prognostic significance of TERT promoter mutations in cancer: a cohort study and a meta-analysis. *PLoS One*. 2016;11:e0146803.
10. Zhao Y, Gao Y, Chen Z, Hu X, Zhou F, He J. Low frequency of TERT promoter somatic mutation in 313 sporadic esophageal squamous cell carcinomas. *Int J Cancer*. 2014;134:493-494.
11. Griewank KG, Murali R, Schilling B, et al. TERT promoter mutations are frequent in cutaneous basal cell carcinoma and squamous cell carcinoma. *PLoS One*. 2013;8:e80354.
12. Cheng KA, Kurtis B, Babayeva S, et al. Heterogeneity of TERT promoter mutations status in squamous cell carcinomas of different anatomical sites. *Ann Diagn Pathol*. 2015;19:146-148.
13. Boscolo-Rizzo P, Da Mosto MC, Rampazzo E, et al. Telomeres and telomerase in head and neck squamous cell carcinoma: from pathogenesis to clinical implications. *Cancer Metastasis Rev*. 2016;35:457-474.
14. Vinothkumar V, Arunkumar G, Revathidevi S, et al. TERT promoter hot spot mutations are frequent in Indian cervical and oral squamous cell carcinomas. *Tumour Biol*. 2016;37:7907-7913.
15. Chang KP, Wang CI, Pickering CR, et al. Prevalence of promoter mutations in the TERT gene in oral cavity squamous cell carcinoma. *Head Neck*. 2017;39:1131-1137.
16. Killela PJ, Reitman ZJ, Jiao Y, et al. TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. *Proc Natl Acad Sci U S A*. 2013;110:6021-6026.
17. Morris LG, Chandramohan R, West L, et al. The molecular landscape of recurrent and metastatic head and neck cancers: insights from a precision oncology sequencing platform. *JAMA Oncol*. 2017;3:244-255.
18. Annunziata C, Pezzuto F, Greggi S, et al. Distinct profiles of TERT promoter mutations and telomerase expression in head and neck cancer and cervical carcinoma. *Int J Cancer*. 2018;143:1153-1161.
19. Barczak W, Suchorska WM, Sobecka A, et al. hTERT C250 T promoter mutation and telomere length as a molecular markers of cancer progression in patients with head and neck cancer. *Mol Med Rep*. 2017;16:441-446.
20. Onder S, Ozturk Sari S, Yegen G, et al. Classic architecture with multicentricity and local recurrence, and absence of TERT promoter mutations are correlates of BRAF (V600 E) harboring pediatric papillary thyroid carcinomas. *Endocr Pathol*. 2016;27:153-161.
21. Pickering CR, Zhang J, Neskey DM, et al. Squamous cell carcinoma of the oral tongue in young non-smokers is genomically similar to tumors in older smokers. *Clin Cancer Res*. 2014;20:3842-3848.

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