



TERT Promoter Mutation C228T Increases Risk for Tumor Recurrence and Death in Head and Neck Cancer Patients

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Arantes LMRB, Cruvinel-Carloni A, de Carvalho AC, Sorroche BP, Carvalho AL, Scapulatempo-Neto C and Reis RM (2020) TERT Promoter Mutation C228T Increases Risk for Tumor Recurrence and Death in Head and Neck Cancer Patients. Front. Oncol. 10:1275. doi: 10.3389/fonc.2020.01275 **Background:** Head and neck squamous cell carcinoma (HNSCC) is usually associated to tobacco and alcohol consumption. Increased telomerase activity has been consistently detected in 80–90% of malignant tumors, including HNSCC. Mutations within the promoter region of telomerase reverse transcriptase (*TERT*) that confer enhanced *TERT* promoter activity have been reported in two major hotspots, designated C228T and C250T.

Objectives: To evaluate *TERT* promoter mutations C228T and C250T in HNSCC patients from Brazil and correlate with patients' outcome.

Materials and Methods: Formalin-fixed paraffin-embedded tissues were obtained from 88 HNSCC patients and analyzed for *TERT* promoter mutations C228T and C250T by pyrosequencing.

Results: The overall prevalence of hotspot *TERT* mutations in HNSCC samples was of 27.3%, with 6.8% at locus C228T and 20.5% at C250T. The majority (92%) of mutated cases were located in oral cavity, mainly at the tongue. We observed that 94.4% of the patients harboring *TERT* promoter mutation C250T were alcohol consumers (p = 0.032) and 66.7% of the patients harboring *TERT* promoter mutation C228T mutation C228T were not alcohol consumers (p = 0.035). The presence of C228T mutation impacted patient outcome, with a significant decrease in disease-free survival (20.0 vs. 63.0%, p = 0.017) and in overall survival (16.7 vs. 45.1%, p = 0.017).

Conclusion: This is the first report of a *TERT* promoter mutations in HNSCC patients from South America. The high prevalence of *TERT* mutation, as well as its association with poor disease-free survival and overall survival, particular at C228T locus might serve as a prognostic biomarker in HNSCC to help clinicians in the management of treatment.

Keywords: HNSCC, TERT promoter mutations C228T and C250T, prognostic biomarker, disease-free survival, overall survival

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INTRODUCTION

Approximately 834,860 new cases of head and neck cancer are diagnosed each year in the world that encompasses tumors of the oral cavity, pharynx, and larynx (1). The most common type is squamous cell carcinoma (HNSCC) which accounts for over 90% of all head and neck cancers (2). Usually associated to tobacco and alcohol consumption (3), over the past decades, human papillomaviruses (HPV) have emerged as an important etiological factor for a subset of HNSCC from the oropharynx (4, 5). Despite significant progress in all therapeutic modalities, the 5-year overall survival (OS) rate for HNSCC patients is \sim 50% and the main reason for treatment failure is the frequent development of loco-regional recurrences (6).

Most HNSCC treatments are associated with high morbidity and toxicity, where recurrent and metastatic disease is usually incurable, highlighting the need for more effective therapies for these patients (7). No new targeted therapies have been approved for HNSCC for decades, other than cetuximab in 2006, which affords only modest response rates (10–15%) as monotherapy (8, 9). The landscape of HNSCC explains the limited response rates of targeted therapies, as most tumors have multiple genetic factors of oncogenesis and are constantly evolving when it comes to therapy (7, 10, 11). In an era of personalized cancer therapy, several investigations are currently examining new biological markers as prognostic and predictive factors in HNSCC (12).

Cancer cells, including HNSCC, are characterized by increased telomerase activity (13). This enzymatic complex is active in ~80–90% of all cancer types and is responsible for the lengthening of telomeres (13, 14). One cancer hallmark is to avoid senescence and unrestricted proliferation, a process called immortalization, and one way to achieve this is by reactivating telomerase in somatic cells (15, 16). Telomerase activity has been consistently detected in 80–90% of malignant tumors (16). Mutations within the promoter region of telomerase reverse transcriptase (*TERT*) that confer enhanced *TERT* promoter activity, have been reported in two major hotspots, which are located at -124 and -146 base pairs upstream of the transcriptional start site (also designated C228T and C250T, respectively) (17–19).

TERT promoter mutation has been extensively evaluated in different tumors: thyroid, glioblastoma, urothelial, melanoma, among others (20). Literature reports the use of *TERT* promoter mutation screening programs in thyroid tumors in order to select patients who would benefit from adjuvant treatment and closer follow-up (21), since many studies related the presence of these mutations with poor prognosis (22–25). Glioblastomas also are reported to present a poor prognosis in patients harboring *TERT* mutations, which were commonly evaluated in combination with *IDH* and *MGMT* methylation (26–29). Urothelial carcinoma (30, 31) and melanoma also showed worse prognosis in patients harboring *TERT* mutation (32, 33).

TERT promoter mutations resulting in increased telomerase expression have been detected in a significant proportion of HNSCC patients (13, 18, 19, 34–39). It may vary from 16 to 70% of all head and neck subsites, being frequently reported as highly mutated in the oral cavity (37–40). Studies evaluating

TERT promoter mutation in head and neck patients were only performed in a few countries (United States, China, India, Taiwan, Italy, and Poland). To date, no studies have evaluated these mutations in the Brazilian population, therefore the aim of the present study was to evaluate the prevalence of *TERT* promoter mutations in head and neck cancer patients in Brazil and evaluate for associations with outcome.

MATERIALS AND METHODS

Patient Samples and DNA Isolation

This retrospective study included formalin-fixed paraffinembedded (FFPE) HNSCC samples from 88 patients surgically treated between 2006 and 2011 at the Department of Head and Neck Surgery of the Barretos Cancer Hospital, Barretos, SP, Brazil. The inclusion criteria were as follows: previously untreated patients with primary HNSCC, submitted to surgery

TABLE 1 | Clinical and pathological data of the patients enrolled in the study.

Variable	Categories	n (%)
Age	≤60 years	54 (61.4)
	>60 years	34 (38.6)
Gender	Male	74 (84.1)
	Female	14 (15.9)
Tobacco use	Yes	73 (85.9)
	No	12 (14.1)
Alcohol use	Yes	61 (74.4)
	No	21 (25.6)
Anatomic site	Tongue	35 (39.8)
	Floor of mouth	19 (21.6)
	Pharynx	8 (9.1)
	Larynx	11 (12.5)
	Gingiva	11 (12.5)
	Hard palate and jugal mucosa	4 (4.5)
Т	cT1-cT2	27 (30.7)
	cT3–cT4	61 (69.3)
Ν	cN0	41 (46.6)
	cN+	47 (53.4)
Clinical stage	1/11	15 (17.0)
	III/IV	73 (83.0)
Radiotherapy	Yes	71 (68.3)
	No	33 (31.7)
Chemotherapy	Yes	33 (33.0)
	No	67 (67.0)
Surgical margins	Negative	75 (86.2)
	Positive	12 (13.8)
Extranodal extension	Negative (N0)	33 (46.5)
	No (N+)	15 (21.1)
	Yes (N+)	23 (32.4)
Perineural invasion	Yes	27 (36.0)
	No	48 (64.0)
Vascular invasion	Yes	19 (26.8)
	No	52 (73.2)

as the first therapeutic modality with curative intent. The use of these samples was approved by the Barretos Cancer Hospital Institutional Review Board. Hematoxylin and eosin sections corresponding to paraffin blocks containing the samples of interest were reviewed by an expert pathologist to confirm the diagnosis and for characterization of the cellular components present in the samples. Scrapings from the region of tissue identified as having at least 80% of tumor cells were processed using QIAamp DNA FFPE Tissue Kit (*Qiagen*, Germany). DNA was quantified in the NanoDrop 2000C (*Thermo Scientific*TM) and stored at -20° C until use.

TERT Promoter Mutational Analysis

A pyrosequencing assay was performed to examine these two *TERT* promoter mutations. The primer for pyrosequencing was designed immediately upstream of C250T so that these two mutations are analyzed in the same assay by producing a 162

bp amplicon, which contained the sites of C228T and C250T mutations, as previously described (41). Pyrosequencing assays were performed on a PyroMark Q96ID system using PyroMark Gold reagents (*Qiagen*).

Sanger sequencing was then performed to confirm the results of pyrosequencing. A fragment of the TERT promoter region was amplified by PCR using the 5'-AGTGGATTCGCGGGCACAGA-3' and primers 5'-CAGCGCTGCCTGAAACTC-3', resulting in a PCR product of 235 bp, which contained the sites of the c.-124 C>T and c.-146 C>T mutations as previously described (26, 42, 43). PCR was performed with initial denaturation at 95°C for 15 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 64°C for 90 s, elongation at 72°C for 30 s, and final elongation at 72°C for 7 min. The quality of PCR products was confirmed by gel electrophoresis. DNA sequencing of the PCR product was performed using the BigDye Terminator version



3.1 Cycle Sequencing Kit (*Applied Biosystems*, USA) and ABI PRISM 3500xL Genetic Analyzer (*Applied Biosystems*, USA). The sequencing reaction was performed in forward direction. An independent PCR amplification/sequencing, in forward direction, was performed in positive samples or samples that were inconclusive.

Statistical Analysis

Statistical analysis was performed using the software IBM SPSS Statistics 23 for Windows. Categorical variables were compared using Fisher's exact test. Survival curves were calculated by Kaplan–Meier method and differences between groups were compared using the log-rank test. For all analysis, we considered statistical significance when $p \leq 0.05$.

RESULTS

Patient Characteristics

Clinical and histopathological data of the 88 HNSCC patients enrolled in this study are presented in Table 1. Most of the patients profiled in this cohort were male (84.1%) with age ranging from 32 to 82 years (median = 58 years). Tobacco and alcohol consumption were self-reported by 85.9 and 74.4% of the cases, respectively. Tumor sites were subdivided into oral cavity (78.4%), larynx (12.5%), and pharynx (9.1%). Regarding tumor sub-sites within the oral cavity were as follows: 39.8% (35/88) in the oral tongue, 21.6% (19/88) in the floor of the mouth, 12.5% (11/88) in the gums and 4.5% (4/88) in the hard palate and jugal mucosa. Clinical stage was T1/T2 in 27 cases (30.7%) and T3/T4 in 61 (69.3%); 47 (53.4%) of the cases had clinically positive cervical lymph nodes and 41 cases (46.6%) were N0; collectively, 73 (83.0) had advanced disease at diagnosis. Perineural invasion, vascular invasion, and extranodal extension were present in 27 (36.0%), 19 (26.8%), and 23 (32.4%) cases, respectively (Table 1). It was described a self-reported measure of tobacco and alcohol consumption in three categories: yes, no, and former, which were acquired from the patient's medical records. Analysis was performed considering "yes" vs. "no," where "yes" comprehended smokers or alcohol consumers added to former.

TERT Promoter Mutation in Head and Neck Squamous Cell Carcinoma

To determine the prevalence of *TERT* promoter mutations in this cohort of Brazilian patients with HNSCC, genomic DNA was extracted and pyrosequenced. Primers were used to amplify and sequenced a region containing two previously described recurrent *TERT* promoter mutations (C228T and C250T, **Figure 1**). The results showed a frequency of 27.3% (24/88) *TERT* mutations in HNSCC, being 20.5% at C250T and 6.8% at C228T hotspot regions (**Table 2**). The mutations occurred in a mutually exclusive manner with a heterozygous genotype. The mutation frequency of C228T in tongue was 50.0%, in the gums, hard palate and jugal mucosa and pharynx was 16.67%, while the floor of the mouth and larynx did not present this mutation (**Table 2**). Regarding C250T mutation, the frequency was 55.56% for tongue, 33.33% for the floor of the mouth, 5.56% for hard palate and jugal mucosa, and for larynx,

 TABLE 2 | TERT promoter mutation in HNSCC samples according to tumor sub-sites.

Tumor sub-site	C228T n (%)	C250T n (%)	WT n (%)
Tongue	3 (50.0)	10 (55.56)	22 (34.38)
Floor of the mouth	O (0.0)	6 (33.33)	13 (20.13)
Gums	1 (16.67)	0 (0.0)	10 (15.63)
Hard palate and jugal mucosa	1 (16.67)	1 (5.56)	2 (3.13)
Larynx	O (0.0)	1 (5.56)	10 (15.63)
Hypopharynx	1 (16.67)	0 (0.0)	1 (1.56)
Oropharynx	O (0.0)	0 (0.0)	6 (9.37)
Total	6 (6.8)	18 (20.5)	64 (72.7)



while the gums and the pharynx did not present this mutation (**Table 2**). When HNSCC sub-sites were grouped in oral cavity, pharynx and larynx, *TERT* mutations were present in 31.9, 12.5, and 9.1% of the cases, respectively.

TERT Promoter Mutation Correlation With Clinical and Pathological Features

In a univariate analysis, 94.4% of the patients harboring *TERT* promoter mutation C250T were alcohol consumers (p = 0.032). Moreover, 66.7% of the patients harboring *TERT* promoter mutation C228T were non-alcohol consumers (p = 0.035). When considering cases with either one of the mutations tested, no statistically significant association between the presence of mutation (C228T or C250T vs. wild-type) and clinical-pathological features was observed.

Kaplan-Meier survival curves were used to estimate survival according to *TERT* promoter mutation status. No statistically significant association between the presence of mutation (C228T or C250T vs. wild-type) and survival was observed. Also, no statistically significant association between the presence of mutation C250T and survival was observed. Importantly, we
 TABLE 3 | Results of univariate analysis of selected prognostic factors for disease-free survival.

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C228T or C250T mutation Second S	Yes	18	6	62.8		1.005 (0.410–2.461)		
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Yes 24 10 51.2 1.557 (0.728–3.331)	No	64	20	63.3	0.250	Reference	0.254	
	Yes	24	10	51.2		1.557 (0.728–3.331)		

*Clinical stage according to TNM Classification of Malignant Tumors-7th ed. Bold values indicates the statistical significance ($p \le 0.05$).

observed that the 5-year disease-free survival (DFS) for patients harboring mutation C228T was 20.0 vs. 63.0% for patients without this mutation (p = 0.017; Figure 2).

Univariate analysis of the effect of this mutation on DFS of patients showed that C228T *TERT* promoter mutation was significantly associated with an increased risk of tumor relapse (HR = 3.372; 95% CI: 1.17–9.73; p = 0.025; **Table 3**). Lower disease-free survival

was associated, as expected, with the following clinical characteristics: N-stage (log-rank p = 0.04), extranodal extension (log-rank p = 0.031) and surgical margins (log-rank p = 0.038; **Table 3**).

The same negative impact of C228T *TERT* promoter mutation was observed in the 5-year overall survival (OS) with only 16.7% of the cases found alive after 5 years, in comparison to 45.1% of patients without this mutation (p = 0.017; **Figure 3**).

Also, a statistically significant increased risk of death was also observed for the cases harboring this C228T mutation (HR = 2.708; 95% CI: 1.15–6.374; p = 0.023; **Table 4**). A decrease in overall survival was also associated with important clinical factors such as: N-stage (log-rank p = 0.020), T-stage (log-rank p = 0.031), clinical stage (log-rank p = 0.018), surgical margins (log-rank p = 0.031) and perineural invasion (log-rank p = 0.032; **Table 4**).

Finally, a multivariable Cox regression model including alcohol consumption, N-stage, surgical margins and the status of C228T for disease-free survival was performed and, only the status of C228T remained significant (HR = 3.372; 95% CI: 1.169–9.730; p = 0.025). In the multivariable Cox regression model including T-stage, N-stage, clinical stage, surgical margins, and the status of C228T for overall survival, clinical stage remained significant (HR = 2.373; 95% CI: 1.072–5.256; p = 0.033) and the status of C228T was marginally significant (HR = 2.352; 95% CI: 0.995–5.558; p = 0.051).

DISCUSSION

TERT expression is downregulated as a normal cell divides, resulting in telomere shortening and replicative senescence (36, 44). Telomere length is important for cell cycle regulation, cell senescence, and genetic instability regulation (45). In most cancers, *TERT* expression is reactivated and overexpressed during tumorigenesis leading to replicative immortality (35, 44).

TERT promoter mutation has been heavily reported in melanoma, glioma, urothelial, thyroid, hepatocellular, and nonsmall cell lung cancer (17, 19). For head and neck tumors, the frequency of those mutations varies significantly among studies (Table 5). These differences could be explained by tumor subsite, sample size, methodological sensitivity, risk factors, and population ethnicity. In our cohort, 27.5 of cases showed TERT promoter mutation, being higher in the C250T than in the C228T locus, 20.5 and 6.8%, respectively. Inversely to those results, when all sites in the head and neck were considered, TERT promoter mutations of C250T and C228T were observed in 2.8 and 14.8% in Killela et al. (19) study, 0 and 16.6% in Cheng et al. (35) study and 6.3 and 30.2% in Morris et al. (39) study, respectively (Table 5). When only the oral cavity is considered, TERT promoter mutations of C250T was also more frequent than C228T in our study, 24.6 and 7.2%, respectively, corroborating to Barczak et al. (13) report of 40.0% of C250T mutation being in the mouth. Conversely to our results, frequency of C250T and C228T were 9.7 and 22.0% in Vinothkumar et al. (36) study, 12.9 and 51.7% in Chang et al. (37) study, 13.3 and 15% in Annunziata et al. (38) study and 20 and 55% in Morris et al. (39) study, respectively (Table 5). In Boscolo-Rizzo et al. (40) study, both mutations were evaluated without distinction, showing 83.3% frequency in oral cavity. For larynx, our results showed 5.5% of C250T and 0% of C228T mutation. Similarly, Qu et al. (34) reported 23.8% frequency for mutation C250T and 3.4% for C228T, which also differs from Morris et al. (39) laryngeal cohort, reported to be 0% in C250T and 14.3% in C228T (Table 5). Those differences could be explained due to the different proportion of



head and neck sub-sites, the number of samples analyzed, and the ethnicity of the patient population (USA, China, India, Taiwan, Poland, and Italy). Brazilian head and neck cancer patients are more likely to be heavy tobacco and alcohol consumers (3) than HPV^+ (46–48), which can explain the mutagenic effect on the mucosal epithelia of the upper aerodigestive tract. At Barretos Cancer Hospital, we use p16-immunohistochemistry (p16-IHC) as a surrogate marker for HPV infection, as recommended by the 8th edition of AJCC TNM staging system specifically for oropharyngeal squamous cell carcinomas. For the 6 oropharynx SCC evaluated in this study (all WT *TERT*), p16-IHC was only available for four of them: 1/4 was classified as p16-positive and 3/4 as HPV-negative. Further studies are required in order to assess the correlation between *TERT* and HPV.

Our data showed a trend in significance with 91.7% of all mutations occurring in the oral cavity (p = 0.068), which was similar to the results reported by Barczak et al. (13) and Killela et al. (19) stating that *TERT* mutation was correlated to oral cavity, most specifically tongue sub-site.

Interesting, a comparison study evaluating *TERT* mutation in a head and neck metastatic cohort, the mutation was significantly more frequent in the recurrence then in the primary HNSCC tumors (39). Unfortunately, we did not have the metastatic tumor to compare its mutation profile with the primary tumor in our cohort. Moreover, Chang et al. (37) reported that *TERT* mutation C228T in oral cavity was also correlated to betel nut chewing, while our study did not find association with tobacco consumption. In contrast, this report found that 94.4% of the patients harboring *TERT* promoter mutation C250T were alcohol consumers while 66.7% of the patients harboring C228T were non-alcohol consumers, in a cohort with 74.4% of alcohol consumers patients.

Importantly, the present data was able to show the effect of *TERT* mutation C228T on the 5-year disease-free survival, TABLE 4 | Results of univariate analysis of selected prognostic factors for overall survival.

Tobacco use No. 12 8 50.0 9.24 Reference 0.924 Yeis 73 51 41.1 1.037 (0.482-186) 0.345 Alcohol use 0.924 1.037 (0.482-186) 0.345 Yeis 61 40 45.9 0.766 (0.428-1.551) 0.766 (0.428-1.551) Clinical tumor status 1.858 (1.049-3.291) 0.034 1.858 (1.049-3.291) 0.034 Ti-72 2 16 63.0 0.031 Reference 0.034 Ti-72 2 16 63.7 0.020 Reference 0.034 Ti-72 2 16 7 0.020 Reference 0.022 N+ 47 38 31.9 1.819 (1.089-3.038) 0.016 Reference 0.022 Net and Milly 75 7 80.0 0.018 Reference 0.905 Advancedi Milly 19 14 31.6 1.037 (0.570-1.886) 0.034 Strigatin margin 12	Characteristics	Number of cases	Number of deaths	5-year overall survival	<i>P</i> -value (long-rank)	Hazard ratio for death (95% CI)	P-value (Cox)	
No.12850.00.324Reference0.327Vacon Use1.0370.492-2.1801.0370.492-2.1801.0370.492-2.1801.0370.492-2.1801.0370.492-2.1801.0370.492-2.1800.335 <t< td=""><td>Tobacco use</td><td></td><td></td><td></td><td></td><td></td><td></td></t<>	Tobacco use							
Yea735141.11.037 (0.492-2.186)ActoniusNo611638.10.343Reference0.756 (0.423-1.351)Clinical turor statusT1-7277160.031Reference0.031T1-7277160.031Reference0.020Clinical statusWith Control and statusNo412437.00.020Reference0.020Clinical roll735334.22.513 (1.140-5.537)0.020Reference0.021Clinical roll735534.22.513 (1.140-5.537)0.021Reference0.021Clinical roll735534.22.513 (1.140-5.537)0.021Reference0.021Cal cavity on dilyrix15780.00.018Reference0.026Cal cavity on dilyrix161631.61.037 (0.570-1.880)0.014Surgical margins754946.70.031Reference0.032Vagative (Ni)hi151133.31.380 (0.660-2.821)0.032Polariyon161133.31.380 (0.660-2.821)0.032Vagative Ni)hi151133.31.380 (0.660-2.821)0.032Vagative Nihhi151133.31.380 (0.660-2.821)0.032Vagative Nihhi161616.310.032Reference0.032Vagative Nihhi151133.31.380 (0.660-2.821)0.032<	No	12	8	50.0	0.924	Reference	0.924	
AbcoluseNa61636.3436.3486.3487.5686.439.Vision616.306.3686.3687.5687.6587.658Childmor status6.306.307.688	Yes	73	51	41.1		1.037 (0.492-2.186)		
No211638.10.343Reference0.345Yes610.00.766 (0.42-1.51)7T1-T2271663.00.031Reference0.034T3-T4671663.00.031Reference0.034T3-T471663.00.031Reference0.022Chincat nodal status12453.70.020Reference0.022N4412453.70.020Reference0.022Chincat TMatage*780.00.018Reference0.022Chincat TMatage*780.00.018Reference0.022Chincat TMatage*780.00.018Reference0.022Chincat TMatage*780.00.018Reference0.022Chincat TMatage*780.00.018Reference0.022Chincat TMatage*780.00.018Reference0.021Chincat TMatage*780.00.018Reference0.021Chincat TMatage*780.00.018Reference0.021Chincat TMatage*780.00.018Reference0.021Chincat TMatage*780.00.018Reference0.021Chincat TMatage*780.00.021Reference0.021Chincat TMatage*780.00.021Reference0.021Chincat TMatage*786.10.021Reference <td>Alcohol use</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>	Alcohol use							
Yaa614045.90.758 (0.423-1.351)Clinicaturos statusC11-7271663.00.031Reference0.031T3-74614632.81.858 (1.049-3.291)1Clinicat nodel statusUUUUClinicat nodel statusUUUUUUClinicat nodel statusUUUUUUUUAdvanced ul/VReference0.020Advanced ul/VReferenceUUUAdvanced ul/VReferenceUAdvanced ul/VReference0.020Pariad statusUUUData Not and to the statusPariad statusUPariad statusUPariad statusPariad statusUPariad statusPariad statusPariad statusPariad status <td colsp<="" td=""><td>No</td><td>21</td><td>16</td><td>38.1</td><td>0.343</td><td>Reference</td><td>0.345</td></td>	<td>No</td> <td>21</td> <td>16</td> <td>38.1</td> <td>0.343</td> <td>Reference</td> <td>0.345</td>	No	21	16	38.1	0.343	Reference	0.345
Clincial tumor statusJJJIII <th< td=""><td>Yes</td><td>61</td><td>40</td><td>45.9</td><td></td><td>0.756 (0.423-1.351)</td><td></td></th<>	Yes	61	40	45.9		0.756 (0.423-1.351)		
T1-T2271663.00.031Reference0.034T3-T46163.00.031Reference0.034T3-T4612453.70.020Reference0.022N+472831.91.819 (1.089-3.038)1Clinical TMN stage*780.00.018Reference0.022Clinical TMN stage*780.00.018Reference0.022Clinical TMN stage*780.00.018Reference0.022Concord (III/V)736534.22.513 (1.140-5.537)0.021Concord (III/V)736534.22.513 (1.140-5.537)0.031Concord (III/V)736534.22.513 (1.140-5.537)0.031Phatomic site731.61.037 (0.570-1.886)0.031Reference0.032Concord (III/V)731431.61.037 (0.570-1.886)0.031Reference0.031Positive121216.72.001 (1.064-3.799)0.031Reference0.032Positive13131.380 (0.660-2.882)0.3920.3920.3920.392Positive NO151133.31.380 (0.660-2.882)0.3920.031Positive NO151233.31.381 (1.043-3.189)0.072Positive NO162652.91.818 (1.043-3.189)0.021Positive NO121636.81.610 (1.919-3.073)0.073Positive NO </td <td>Clinical tumor status</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>	Clinical tumor status							
T3-T4614632.81.588 (1.049-3.291)Chicat statusVision of the statusN4473831.9Neterence0.020Other of the statusUnited (10)16780.00.802Reference0.021Advanced (10/10)736382.02.513 (1.140-5.57)0.021Advanced (10/10)736382.082.00.055Reference0.905Advanced (10/10)736344.90.905Reference0.905Advanced (10/10)736344.90.905Reference0.905Station of the status7363636363636363Station of the status73 <td>T1–T2</td> <td>27</td> <td>16</td> <td>63.0</td> <td>0.031</td> <td>Reference</td> <td>0.034</td>	T1–T2	27	16	63.0	0.031	Reference	0.034	
Olive Series of Series	T3–T4	61	46	32.8		1.858 (1.049–3.291)		
N0412453.70.020Reference0.021N+473831.91.819 (1.089-3.039)5Clinical TMM stage*534.92.513 (1.140-5.537)Inikal (//)735534.22.513 (1.140-5.537)Advanced (ll/V)735534.22.513 (1.140-5.537)Anatomic site2.513 (1.140-5.537)5Anatomic site3.633.422.513 (1.140-5.537)Prayrix and layrix191431.61.037 (0.570-1.866)Prayrix and layrix191431.61.037 (0.570-1.866)Prayrix and layrix191431.62.001 (1.604-3.799)Prayrix and layrix121216.72.001 (1.604-3.790)Positive13201.5150.022ReferencePositive (N/)33201.5150.023ReferenceNo (N+)13103.041.777 (0.398-3.367)0.078Preneural Invasion133.041.777 (0.398-3.367)0.078Yes (N+)23183.041.603Reference0.092Yes (N-)23123.040.073Reference0.092Yes (N+)1313.00.6602.8020.0710.777Yes (N+)23180.023Reference0.092Yes (N+)1313.00.6602.8020.021Yes (N+)1413.60.032Reference0.092<	Clinical nodal status							
N÷473831.91.819 (1.089-3.038)Clinical TMM stage"Unitial (M)15780.00.018Reference0.20Advanced (II/M)733234.22.513 (1.140-5.537)Anatomi site </td <td>NO</td> <td>41</td> <td>24</td> <td>53.7</td> <td>0.020</td> <td>Reference</td> <td>0.022</td>	NO	41	24	53.7	0.020	Reference	0.022	
Clinical TNM stages"Initial (M)15780.00.018Reference0.022Advanced (M/V)15780.00.018Reference0.021Atatomistic1231.61.037 0.570 -1.8800.005Pharyns and laynx191431.60.001Reference0.001Negative754946.70.031Reference0.001Positive121216.72.001 (1.054 -3.799)1Positive121216.72.001 (1.054 -3.799).0167Positive (NO)1510.2Reference0.032ReferenceNo (N+1)1513.13.030 0.660 -2.8820.392.0321Yes (N+1)23183.041.777 (0.938 -3.6710.078Princurs invession1.380 0.660 -2.8820.392.0321No482952.10.602Reference0.021Ves (M+1)151.013.030.602.0161.0161Princurs invession1.818 (1.043 - 3.189).0162.0161.0162Ves (M+1)1216.70.003Reference0.022.0161Ves (M+1)1616.70.003Reference0.021Ves (M+2)1616.70.003Reference0.021Ves (M+2)1616.70.003Reference0.021Ves (M+2)1616.70.003Reference0.021	N+	47	38	31.9		1.819 (1.089–3.038)		
Initial (III)15780.00.018Reference0.022Advanced (III/IV)735534.22.513 (1.140-5.537)Anatomis site4431.60.905Reference0.905Oral cavity694843.60.905Reference0.905Pharynx and laynx191431.61.037 (0.570-1.886)Surgical margins1.032 (0.570-1.886)0.034Negative754946.70.031Reference0.034Positive754946.70.031Reference0.034Positive151.133.32.001 (1.54-3.799)3.29No (N+)151.133.31.380 (0.660-2.882)0.392Perineural invasion133.31.380 (0.660-2.882)0.392Prineural invasion233.31.818 (1.043-3.169)3.29Vas2333.31.818 (1.043-3.169)3.29Vas191636.81.681 (0.919-3.073)3.29Vas191636.92.708 (1.50-6.374)3.20Vas4216.70.003Reference0.023Vas662.092.708 (1.50-6.374)3.20Vas83.683.680.883 (0.465-1.715)Vas1883.680.883 (0.465-1.715)3.33Vas1883.680.883 (0.465-1.715)3.33Vas188	Clinical TNM stage*							
Advanced (III/V)735534.22.513 (1.140-5.537)Atatomic siteOral cavity694844.90.905Reference0.905Phaynx and laynx194844.90.905Reference0.905Surgical margins </td <td>Initial (I/II)</td> <td>15</td> <td>7</td> <td>80.0</td> <td>0.018</td> <td>Reference</td> <td>0.022</td>	Initial (I/II)	15	7	80.0	0.018	Reference	0.022	
Anatomic siteOral cavity694844.90.905Reference0.905Pharynx and larynx191431.61.037 (0.570-1.886)Surgical margins1.021.02Negative754946.70.031Reference0.034Positive1216.72.001 (1.054-3.799)5.00Extrancial extension332051.50.202Reference0.392No (N+f)332051.50.202Reference0.3920.392Ves (N+f)231830.41.777 (0.393-3.67)0.078Perineural invasion3262.10.032Reference0.035Ves (N+f)622852.10.032Reference0.035Ves (N+f)191636.81.811 (0.43-3.169)0.078Ves (N+f)191636.81.681 (0.919-3.073)0.778Ves (N+f)191636.81.681 (0.919-3.073)0.778Ves (N+f)191636.82.708 (1.150-6.374)0.003Ves (N+f)1016.10.003Reference0.733Ves (N+f)1016.116.70.003Reference0.021Ves (N+f)101636.81.681 (0.919-3.073)0.778Ves (N+f)101636.82.708 (1.150-6.374)0.778Ves (N+f)101616.70.003Refer	Advanced (III/IV)	73	55	34.2		2.513 (1.140–5.537)		
Oral cavity694844.90.905Reference0.905Phaynx and laynx191431.61.037 (0.570-1.886)Surgical marginsWegative754946.70.031Reference0.034Positive12122.001 (1.50-4.379)2.001 (1.50-4.379)0.031Destive12123.32.001 (1.50-4.379)0.031Extranolal extension13.31.380 (0.660-2.882)0.392No (N+)15113.31.380 (0.660-2.882)0.392Yes (N+)23183.0.41.777 (0.383-3.367)0.078Perieural invasionNo482852.10.032ReferenceVes (N-)20163.31.818 (1.043-3.169)1.618 (0.919-3.073)Vascular invasion13.61.6181 (0.919-3.073)1.618 (0.919-3.073)Vas (Na523248.10.088Reference0.092Yes (N-)6652.92.708 (1.150-6.374)1.628C250T mutation152.92.708 (1.150-6.374)1.718No704048.10.606Reference0.733Yes (Na704046.90.4646.053 (0.465-1.715)C250T mutation11.710.243 (0.710-2.175)1.243 (0.710-2.177)No6445.10.605Reference0.733Yes (Na704046.90.463Reference	Anatomic site							
Phaynx and laynx191431.61.037 (0.570-1.886)Surgical marginsNegative754946.70.031Reference0.034Positive1212120.031Reference0.034Destative </td <td>Oral cavity</td> <td>69</td> <td>48</td> <td>44.9</td> <td>0.905</td> <td>Reference</td> <td>0.905</td>	Oral cavity	69	48	44.9	0.905	Reference	0.905	
Starical margins Negative 75 49 46.7 0.031 Reference 0.034 Positive 12 12 16.7 2.001 (1.054-3.799) Image: constraint of the second	Pharynx and larynx	19	14	31.6		1.037 (0.570–1.886)		
Negative754946.70.031Reference0.034Positive121216.72.001 (1.054-3.79)Extranodal extension332051.50.202ReferenceNo (N+)151133.31.380 (0.660-2.882)0.392Yes (N+)231830.41.777 (0.938-3.677)0.392Perimural invasion72333.31.818 (1.043-3.169)0.003Ves (N+)292333.31.818 (1.043-3.169)0.092Yes272333.31.818 (1.043-3.169)0.092Vescular invasion2336.81.818 (0.43-3.169)0.092Yes293248.10.088Reference0.092Yes191636.81.681 (0.919-3.073)1.618Yes6652.92.708 (1.150-6.374)1.621Yes6652.92.708 (1.150-6.374)1.621Yes6652.92.708 (1.150-6.374)1.621Yes74353.60.606Reference0.733Yes6652.92.708 (1.150-6.374)1.773Yes74043.10.606Reference0.733Yes8665.92.708 (1.150-6.374)1.775Yes1810.606Reference0.7331.733Yes194043.10.606Reference0.733Yes1010<	Surgical margins							
Positive121216.72.001 (1.054–3.799)Extranodal extensionNegative (NO)332051.50.202ReferenceNo (N+)151133.31.380 (0.660–2.822)0.392Yes (N+)23180.041.777 (0.938–3.67)0.078Perimeral invasionuNo282852.10.032Reference0.032Yes292333.31.818 (1.043–3.169)1.8180.035NoVacular invasion2332.333.31.818 (1.043–3.169)2.323Yes191636.8Reference0.092Yes191636.8Reference0.092Yes6652.92.708 (1.150–6.374)7.713C287 mutation116.70.003Reference0.023Yes3066.322.708 (1.150–6.374)7.733C287 mutation116.70.003Reference0.733Yes316.606Reference0.7337.733Yes316.606Reference0.7337.733Yes316.606Reference0.7337.733Yes316.606Reference0.7337.733Yes32323232.7337.733Yes323233.73333.73333.733Yes323233.73333.733Yes3233	Negative	75	49	46.7	0.031	Reference	0.034	
Extranodal extensionNegative (NO)332051.50.202ReferenceNo (N+)151133.31.380 (0.660-2.882)0.392Yes (N+)231830.41.777 (0.938-3.67)0.078Perineural invasionNo482852.10.032Reference0.035Yes27233.31.818 (1.043-3.169)1Vacular invasionVacular invasion<td colspan="</td> <td>Positive</td> <td>12</td> <td>12</td> <td>16.7</td> <td></td> <td>2.001 (1.054-3.799)</td> <td></td>	Positive	12	12	16.7		2.001 (1.054-3.799)		
Negative (N0) 33 20 51.5 0.202 ReferenceNo (N+) 15 11 33.3 1.380 (0.660-2.882) 0.392 Yes (N+) 23 18 30.4 1.777 (0.938-3.367) 0.078 Perineural invasion V V V V V No 48 28 52.1 0.032 Reference 0.035 Yes (N +) 27 23 33.3 1.818 (1.043-3.169) V Vacular invasion V V 36.8 Reference 0.092 Yes (N +) 19 16 36.8 1.681 (0.919- 3.073) V C223T mutation V V 1.681 0.023 0.023 0.023 Yes (N +) 62 42 16.7 0.003 Reference 0.023 Yes (N +) 12 12 1.681 0.023 0.023 0.023 Yes (N +) 62 42 16.7 0.003 Reference 0.023 Yes (N +) 12 12 1.681 0.023 0.023 0.023 Yes (N +) 12 12 12 0.023 0.023 0.023 0.023 Yes (N +) 12 12 12 0.023 0.023 0.023 0.023 0.023 Yes (N +) 12 12 12 12 0.023 0.023 0.023 0.023 0.023 0.023 Yes (N +) 12 12 12 12 12 0.023 0.023 0.02	Extranodal extension	ı						
No (N+) 15 11 33.3 1.380 (0.660-2.82) 0.392 Yes (N+) 23 18 30.4 1.777 (0.938-3.37) 0.078 Perineural invasion V V V No 48 28 52.1 0.032 Reference 0.035 Yes (N+) 27 23 33.3 1.818 (1.043-3.169) V Vacular invasion 27 23 33.3 1.818 (1.043-3.169) V Vacular invasion 52 32 48.1 0.088 Reference 0.092 Yes 19 16 36.8 1.681 (0.919-3.073) V 0.092 Yes 19 16 36.8 1.681 (0.919-3.073) V 0.092 Yes 6 6 52.9 2.708 (1.150-6.374) V 0.092 Yes 70 40 48.1 0.606 Reference 0.733 Yes 18 8 53.6 0.893 (0.465-1.715) V 0.446 Yes <td>Negative (N0)</td> <td>33</td> <td>20</td> <td>51.5</td> <td>0.202</td> <td>Reference</td> <td></td>	Negative (N0)	33	20	51.5	0.202	Reference		
Yes (N+)231830.41.777 (0.938-3.367)0.078Perineural invasion482852.10.032Reference0.035Yes272333.31.818 (1.043-3.169)7Vacular invasion723248.10.088Reference0.092Vacular invasion191636.81.681 (0.919-3.073)7C228T mutationUNo824216.70.003Reference0.023Ves652.92.708 (1.150-6.374)77C250T mutationUNo704048.10.606Reference0.733Ves704083.60.606Reference0.733C250T mutation18853.60.893 (0.465-1.715)0.733Ves644546.90.445Reference0.446	No (N+)	15	11	33.3		1.380 (0.660-2.882)	0.392	
Perineural invasion 48 28 52.1 0.032 Reference 0.035 Yes 27 23 33.3 1.818 (1.043–3.169) 1 Vascular invasion 52 32 48.1 0.088 Reference 0.092 Yes 19 16 36.8 1.681 (0.919–3.073) 1 C287 mutation No 82 42 16.7 0.003 Reference 0.023 Yes 6 6 52.9 2.708 (1.150–6.374) 1 C250T mutation No 70 40 48.1 0.606 Reference 0.733 C250T mutation No 70 40 48.1 0.606 Reference 0.733 C250T mutation No 8 63.6 0.893 (0.465–1.715) C250T mutation No 64 46.9 0.445 Reference 0.446 S24 17 29.2	Yes (N+)	23	18	30.4		1.777 (0.938–3.367)	0.078	
No482852.10.032Reference0.035Yes272333.3 $1.818 (1.043-3.169)$ Vacular invasionVascular invasion523248.10.088Reference0.092Yes191636.8 $1.681 (0.919-3.073)$ C228T mutation1.681 (0.919-3.073)C228T mutationNo824216.70.003Reference0.023Yes6652.9 $2.708 (1.150-6.374)$ C250T mutationC250T mutationC250T mutationC250T mutation0.606Reference0.733No704048.10.606Reference0.7330.735C250T mutationC250T muta	Perineural invasion							
Yes272333.31.818 (1.043–3.169)Vascular invasion523248.10.088Reference0.092Yes191636.81.681 (0.919–3.073)700C228T mutationNo824216.7 0.003 Reference 0.023 Yes6652.92.708 (1.150–6.374)700C250T mutationNo704048.10.606Reference0.733Yes18853.60.893 (0.465–1.715)721C228T or C250T mutationNo644546.90.445Reference0.446Yes241729.21.243 (0.710–2.177)	No	48	28	52.1	0.032	Reference	0.035	
Vascular invasionNo523248.10.088Reference0.092Yes191636.81.681 (0.919–3.073)C2287 mutationNo824216.70.003Reference0.023Yes6652.92.708 (1.150–6.374)0.023C250T mutationNo704048.10.606Reference0.733Yes18853.60.893 (0.465–1.715)0.228C228T or C250T mutationNo644546.90.445Reference0.446Yes241729.21.243 (0.710–2.177)0.446	Yes	27	23	33.3		1.818 (1.043–3.169)		
No523248.10.088Reference0.092Yes191636.81.681 (0.919–3.073)C228T mutationNo824216.70.003Reference0.023Yes652.92.708 (1.150–6.374)C250T mutationC250T mutationC250T mutationNo704048.10.606Reference0.733Yes18853.60.893 (0.465–1.715)C228T or C250T mutationNo644546.90.445Reference0.446Yes241729.21.243 (0.710–2.177)C109	Vascular invasion							
Yes191636.81.681 (0.919–3.073)C228T mutationNo824216.70.003Reference0.023Yes652.92.708 (1.150–6.374)C250T mutationC250T mutationVVNo704048.10.606Reference0.733Yes18853.60.893 (0.465–1.715)C228T or C250T mutationNo644546.90.445Reference0.446Yes241729.21.243 (0.710–2.177)C243 (0.710–2.177)	No	52	32	48.1	0.088	Reference	0.092	
C228T mutation No 82 42 16.7 0.003 Reference 0.023 Yes 6 6 52.9 2.708 (1.150–6.374) C250T mutation No 70 40 48.1 0.606 Reference 0.733 Yes 18 8 53.6 0.893 (0.465–1.715) 70 No 64 45 46.9 0.445 Reference 0.446 Yes 24 17 29.2 1.243 (0.710–2.177)	Yes	19	16	36.8		1.681 (0.919–3.073)		
No 82 42 16.7 0.003 Reference 0.023 Yes 6 6 52.9 2.708 (1.150–6.374) C250T mutation V V </td <td>C228T mutation</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>	C228T mutation							
Yes6652.92.708 (1.150–6.374)C250T mutationNo704048.10.606Reference0.733Yes18853.60.893 (0.465–1.715)C228T or C250T mutationNo644546.90.445Reference0.446Yes241729.21.243 (0.710–2.177)	No	82	42	16.7	0.003	Reference	0.023	
C250T mutation No 70 40 48.1 0.606 Reference 0.733 Yes 18 8 53.6 0.893 (0.465–1.715) C228T or C250T mutation No 64 45 46.9 0.445 Reference 0.446 Yes 24 17 29.2 1.243 (0.710–2.177) 1.243 (0.710–2.177)	Yes	6	6	52.9		2.708 (1.150-6.374)		
No 70 40 48.1 0.606 Reference 0.733 Yes 18 8 53.6 0.893 (0.465–1.715) C228T or C250T mutation No 64 45 46.9 0.445 Reference 0.446 Yes 24 17 29.2 1.243 (0.710–2.177) 1.243 (0.710–2.177)	C250T mutation							
Yes 18 8 53.6 0.893 (0.465-1.715) C228T or C250T mutation V No 64 45 46.9 0.445 Reference 0.446 Yes 24 17 29.2 1.243 (0.710-2.177)	No	70	40	48.1	0.606	Reference	0.733	
C228T or C250T mutation K <thk< th=""> K K K</thk<>	Yes	18	8	53.6		0.893 (0.465–1.715)		
No 64 45 46.9 0.445 Reference 0.446 Yes 24 17 29.2 1.243 (0.710-2.177) 1.243 (0.710-2.177)	C228T or C250T muta	ation						
Yes 24 17 29.2 1.243 (0.710–2.177)	No	64	45	46.9	0.445	Reference	0.446	
	Yes	24	17	29.2		1.243 (0.710–2.177)		

*Clinical stage according to TNM Classification of Malignant Tumors-7th ed. Bold values indicates the statistical significance ($p \le 0.05$).

which was 20.0% for patients harboring this mutation vs. 63.0% for patients without this mutation, being therefore associated with an increased risk of tumor relapse. The same negative impact of *TERT* promoter mutation C228T was observed in the 5-year overall survival with only 16.7% of the cases found alive after 5 years, in comparison to 45.1% of patients without this mutation, also associated with a statistically significant increased risk of death. Corroborating our results, Qu et al. (34)

found *TERT* mutation associated with poor overall survival in laryngeal tumor patients (cases with *TERT* promoter mutations had 72.2 vs. 78.2 months for wild-type patients, p = 0.04). The mechanism by which *TERT* promoter mutations ultimately facilitate cancer progression and can constitute prognostic factors are not fully elucidated. It has been reported that C228T and C250T mutations are functionally distinct, with C228T leading to GABPA recruitment, whereas C250T generate both an ETS site

TABLE 5 | Summary of studies evaluating the association between head and neck tumors with TERT promoter mutations.

Study	Tumor Site	Total <i>TERT</i> promoter mutation % (n)	C228T % (n)	C250T % (n)	Results	Method	Country
(19)	Head and neck	17.1 (12/70)	14.8 (10/70)	2.8 (21/70)	<i>TERT</i> mutations were correlated to tongue sub-site ($p < 0.0001$)	PCR and Sanger sequencing	USA
(34)	Larynx	27.0 (64/235)	3.4 (8/235)	23.8 (56/235)	Laryngeal <i>TERT</i> mutations were significantly associated with poor survival ($\rho = 0.03$). The cases with <i>TERT</i> promoter mutations had significantly shorter survival than those with wild-type <i>TERT</i> (72.2 vs. 78.2 months, $\rho = 0.04$). Also, <i>TERT</i> C250T mutation was significantly associated with worse survival (69.2 vs. 78.2 months, $\rho = 0.01$)	Pyrosequencing	China
(35)	Head and neck	16.6 (2/12)	16.6 (2/12)	0.0 (0/12)	No significant correlation was observed	PCR and Sanger sequencing	USA
(36)	Oral cavity	31.7 (13/41)	22.0 (9/13)	9.7 (4/13)	No significant correlation was observed	PCR and Sanger sequencing	India
(37)	Oral cavity	64.7 (130/201)	51.7 (104/201)	12.9 (26/201)	The C228T mutation in oral cavity was associated with betel nut chewing ($\rho=0.05$)	PCR and Sanger sequencing	Taiwan
(13)	Head and neck	63.93 (39/61)		63.93 (39/61)	The C250T mutation in HNSCC indicated an association between the frequency of the homozygous mutation (T/T) and the grade of the tumor (T1 = 27%; T2 = 36%; T3 = 35%; T4 = 46%; $P \le 0.0001$). Also, C250T mutation was identified in 40% of patients with mouth cancer ($P = 0.001$)	High resolution melting using qPCR	Poland
(18)	Head and neck	28.6 (8/28)			No significant correlation was observed	Next-generation sequencing	USA
(38)	Oral Cavity	60.0 (9/15)	20.0 (3/15)	13.3 (2/15)	No significant correlation was observed	PCR and Sanger sequencing	Italy
	Oropharynx	0.0 (0/15)	0.0 (0/15)	0.0 (0/15)			
(39)	Oral Cavity	70.0 (14/20)	55.0 (11/20)	15.0 (3/20)	Frequency of TERT promoter	MSK-IMPACT	USA
	Oropharynx	5.5 (1/18)	5.5 (1/18)	0.0 (0/18)	mutation was higher in the metastasis	assay	
	Hypopharynx	0.0 (0/2)	0.0 (0/2)	0.0 (0/2)	0.0001)	sequencing	
	Larynx	14.3 (1/7)	14.3 (1/7)	0.0 (0/7)	,		
(40)	Oral Cavity Pharynx Larynx	9.90 (10/101) 0.99 (1/101) 0.99 (1/101)			No significant difference in OS emerged for mutational status, although 5-year OS was higher in patients with TERT mutations than in those without mutations. TERT levels were not associated with TERT mutations	PCR and Sanger sequencing	Italy
Present study	Oral Cavity Larynx Pharynx	31.9 (22/69) 5.6 (1/11) 16.7 (1/8)	7.2 (5/69) 0 (0/11) 16.7 (1/8)	24.6 (17/69) 5.6 (1/11) 0 (0/8)	C250T TERT promoter mutation was associated with alcohol consumption ($p = 0.032$), whereas C228T was not associated with alcohol consumption ($p = 0.035$). The presence of C228T mutation impacted patient outcome, with a significant decrease in disease-free survival (20.0 vs. 63.0%, p = 0.017) and in overall survival (16.7 vs. 45.1%, $p = 0.017$)	Pyrosequencing	Brazil

and a functional p52 site requiring ETS1/2 (49). In the present study, we identified only C228T mutations association of worse outcome. This could be due to small size of patients C228T mutated and further studies with a large number should be done to extend and validate this finding.

Chiba et al. (50) recently discovered that *TERT* promoter mutations may occur in two phases: (1) early stages of tumorigenesis resulting in telomerase activity, which is insufficient to prevent telomere shortening and (2) during subsequent divisions, when the number of short telomeres increases, and telomerase activity becomes rate-limiting, causing telomere-driven genomic instability. Therefore, those mutations promote a dual role in tumorigenesis: cancer cell immortalization and genome instability (50), which seem to be responsible for the increased risk of tumor relapse and increased risk of death in patients harboring the mutation C228T.

Recent studies have reported the feasibility of TERT promoter mutation detection and its applicability in screening programs for patients with thyroid cancer to predict patient's outcome (21, 23). Our study suggests that in head and neck tumors, TERT promoter mutations could be performed in a screening setting to help clinicians decide for a more aggressive initial treatment accompanied by a closer follow-up, in order to give these patients a better chance to survive. Studies validating these results should be performed in order to confirm the predictive value of this marker for prognosis. In addition, minimally invasive approaches to detect TERT promoter mutations could be performed in head and neck samples in order to evaluate the feasibility of this marker, and since oral cavity is the most affected site, saliva could possibly be a good source, taking into consideration previous data in other tumors: FNA (Fine-Needle Aspiration) in thyroid tumors (51-53) and cell-free DNA (cfDNA) from urine in urothelial tumors (54-56), which were able to detect the mutation in fluids.

In conclusion, to the best of our knowledge, this is the first study evaluating Brazilian population reporting high prevalence of *TERT* promoter mutations in HNSCC and associating with poor disease-free survival and overall survival. Thus, *TERT* promoter mutation C228T might serve as a prognostic biomarker in head and neck squamous cell carcinoma to help clinicians in the management of treatment.

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DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants and use of these samples were reviewed and approved by the Barretos Cancer Hospital Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RR, CS-N, and ALC designed the study. LA conducted the study, performed the data analysis and interpretation, statistical analysis, and wrote the manuscript. AC-C conducted the study and performed the data analysis. ACC and BS performed the data interpretation and statistical analysis. BS compiled the clinical data. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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