Molecular drivers of oral cavity squamous cell carcinoma in non-smoking and non-drinking patients: what do we know so far?

Sophia Uddin,[1] Alka Singh,[2] Vasudha Mishra,[2] Nishant Agrawal,[3] Zhen Gooi,[1] Evgeny Izumchenko[2]

[1]Department of Neurology, [2]Department of Medicine, Section of Hematology and Oncology, [3]Department of Surgery, Section of Otolaryngology-Head and Neck Surgery, University of Chicago, USA

Abstract

Oral cavity squamous cell carcinoma (OCSCC) is one of the most common head and neck cancers worldwide. It is well known that risk factors for OCSCC include tobacco and excess alcohol consumption. However, in recent years, OCSCC incidence has been increasing in patients without these traditional risk factors. The cause of this increase is unclear and various genetic, environmental, and infectious factors have been hypothesized to play a role. Additionally, there are expert opinions that oral cancer in non-smoking, non-drinking (NSND) patients have a distinct phenotype resulting in more aggressive disease presentation and poorer prognosis. In this review, we summarize the current state of knowledge for oral cavity cancer in patients without traditional risk factors.

Correspondence: Sophia Uddin, Department of Neurology, University of Chicago, USA.
E-mail: sophiauddin@gmail.com.

Key words: oral squamous cell carcinoma; non-smoking; non-drinking; tobacco; alcohol.

Contributions: SU performed literature search, analyzed the data, and wrote the original draft; SU, AS, VM, EI, NA, ZG developed study concepts, performed data interpretation, manuscript writing, editing and review. EI, NA acquired funding. All authors reviewed and edited the final manuscript, approved the final version of the manuscript and agreed to be accountable for all aspects of the work. SU, AS and VM have made an equal contribution to this article. NA, ZG and EI share senior authorship of this article.

Funding: The present study was supported in part by U.S. National Institutes of Health (NIH) grants R01DE027809 to E. I., and R01DE028674 to N. A.

Conflict of interest: The authors declare no potential conflict of interest.

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Received for publication: 27 June 2021.
Accepted for publication: 9 November 2021.

Oncology Reviews 2022; 16:549
doi:10.4081/oncol.2022.549

Introduction

Oral cavity squamous cell carcinoma (OCSCC) is the most common head and neck malignancy.[1] With annual domestic projected incidence of 35,310 new cases and 7,110 deaths in 2020 alone,[2] OCSCC has a significant impact on populations in the United States and around the world. According to data collected by the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program between 2010 and 2016, the 5-year survival rates are estimated at 85.1%, 66.8%, and 40.1% for localized, regional, and distant metastatic OCSCC, respectively.[3]

Current treatment regimens for OCSCC include surgical resection, followed by adjuvant radiation, chemotherapy, or chemo-radiotherapy, depending on the disease stage.[4] Cetuximab (anti-EGFR monoclonal antibody), is the only approved standard of care targeted therapy for OCSCC.[5] However, the response rate to cetuximab is low, and majority of patients develop resistance or relapse even after an initial response. Given the limited success of targeted treatment and cytotoxic chemotherapy, the current clinical focus has turned to immunotherapy with antibodies targeting T cell inhibitory receptors that function as immune checkpoints, such as programmed death 1 (PD-1).[6,7] However, despite recent therapeutic advancements and numerous clinical trials underway, the overall survival of OCSCC is still hovering around 50%.[8]

Consumption of alcohol and tobacco products, poor dental hygiene, and chewing betel quid and areca nut increase risk of OCSCC.[9-14] Although human papillomavirus (HPV) is a prominent risk factor in oropharyngeal cancer, the rate of HPV infection in OCSCC is low, and its significance remains debatable.[12,13,15,16] According to the Centers for Disease Control and Prevention (CDC), cigarette smoking among US adults has reached an all-time low of 13.7% in 2018, a decline of approximately two-thirds over the last 50 years.[17] While alcohol consumption has not experienced such a drastic decline, it has also decreased sharply over the past couple of decades. Surprisingly, however, epidemiological studies have shown a steady rise in the incidence of head and neck squamous cell carcinomas (HNSCCs) in non-smoking, non-drinking (NSND) patients, especially young adults.[9,18,19]

While several large sequencing studies provided a comprehensive landscape of molecular alterations that may serve as prognostic and therapeutic biomarkers in OCSCC,[20-22] most of these studies included heterogeneous patients' population with different risk factors. As such, molecular changes that drive the tumorigenesis in NSND patients remain scarce. Although more recently several studies have attempted to evaluate the clinical characteristics of OCSCC in non-smoking (NS) patients,[10,23-25] the clinicopathologic data regarding the frequency of such tumors and age of tumor onset are conflicting,[10,23,25,26] and relatively few molecular drivers of the progression of OCSCC in NSND patients are currently recognized. In this overview, we summarize the current state of knowledge regarding the clinicopathologic, survival, and molecu-

lar characteristics of OCSCC patients without traditional risk factors. Better understanding of complex biological processes underlying this unique subset of patients would allow for improved diagnosis, risk assessment, management, and ultimately prevention.

Methods

Relevant literature was identified using the following PubMed search strings:

1. "oral squamous cell carcinoma" OR "oral cancer" OR "tongue cancer" OR "gum cancer" OR "oral cavity cancer" OR "floor of mouth cancer" AND nonsmok* AND nondrink* returned 41 results (including hyphenated terms "non-smok*" and "non-drink*). Asterisks indicate wildcard endings (*e.g.*, nonsmok* includes "nonsmoking" and "nonsmoke").
2. "HNSCC" AND nonsmok* AND nondrink*_returned 69 results, 28 of which were non-overlapping with search #1, for a total of 97 publications. HNSCC denotes "head and neck squamous cell cancer" or "head and neck squamous cell carcinoma".

Articles were excluded if only tangentially related to the subject matter, not available in English, dealt primarily with anatomic sites outside the oral cavity, or were primarily studies about tobacco and alcohol as risk factors for disease (*e.g.*, using healthy NSND controls). After exclusion, 48 articles remained. To provide necessary context and background information, additional literature was included at the discretion of the authors.

Results

Epidemiology of oral cavity squamous cell carcinoma in non-smoking, non-drinking populations

Common OCSCC risk factors include tobacco, excess alcohol consumption, and in many regions of the world, betel quid chewing.[27] Prior US data indicate that most patients are male, between 55 and 64 years of age, with higher incidence among Black and Hispanic populations.[3] Over the past few decades, however, the incidence of OCSCC is rising globally among NSND patients.[18] Evidence suggests a bimodal age distribution, with most NSND OCSCC found in distinct 'younger' and 'older' age cohorts when compared to traditional smoking/drinking (SD) patients.[28-31] For example, in a study of 128 SD and 41 NSND patients with new or recurrent OCSCC, Koo *et al*. noted a bimodal age distribution with peaks at 50-59 and 70-79 years in NSND, but a single peak at 60-69 years in SD patients.[24] Further, in a retrospective cohort study of 172 NSND and 1131 ever-smoking ever-drinking (ESED) patients newly diagnosed with HNSCC, Dahlstrom *et al*.[28] found that 41% of NSND were under 50 years old, compared with only 22% of ESED. For those over 70, these numbers were 18% and 15% respectively. In another study that reported perineural invasion (PNI) as an independent prognostic factor for disease specific survival in young patients with OCSCC, presence of PNI was not associated with either tobacco or alcohol consumption.[32] Additionally, OCSCC incidence is rising among females.[19,24,29,33] Patel *et al*. examined SEER data for OCSCC from 1975 to 2007, and found that disease incidence decreased across this time period except in patients aged 18-44.[19] Among these patients, white females experienced the steepest rise in OCSCC, with an annual percentage increase of 2.2% during this time period. Notably, among young white women within the 18-44 age group, the annual percentage increase was even more prominent at 4.0%.[19] Evidence for a similar pattern in elderly white women comes from a cross-sectional study of 1633 HNSCC patients: elderly white NSND women had higher rates of OCSCC than their SD counterparts, who tended to have cancers at other sites.[34] These observations were further supported by a study of 195 NSND HNSCC patients in the Netherlands.[35] In summary, in the past several decades, OCSCC incidence has been rising among individuals who do not fit the expected demographic or risk factor profiles.

Genetic aberrations

Various studies have explored potential genetic contributors to OCSCC in atypical patients. While the conclusions of these reports have been varied, some studies reported unique genetic or genomic characteristics of OCSCC in the NSND population. Unsurprisingly, some of the mutated genes are related to apoptosis and/or cell cycle progression signaling networks. In a study of 505 OCSCC patients (including 201 NS 230 ND patients), Tang *et al*.[36] identified a polymorphism in caspase-8 (a gene regulating apoptosis) that appears to be a risk factor for OCSCC in NS and ND patients. Specifically, a *CASP8* genotype containing a single nucleotide polymorphism (SNP) rs1306963 conferred an increased risk of OCSCC in NS (odds ratio (OR) 1.6) and ND (OR 2.2) patients.

Another study of 282 patients found an association between a *CCND1* gene polymorphism at codon 242 (exon 4) in ND patients only (OR 7.5).[37] While mutations in *CCND1* (which encodes the cyclin D1 protein) were reported to play role in oncogenesis of several types of cancer including HNSCC,[38] this study examined all squamous cell cancers of the upper aerodigestive tract, so the applicability of this polymorphism to OCSCC specifically is yet to be determined.

DEC1, a gene of unknown function that is often down-regulated in esophageal cancer,[39] has also been implicated in OCSCC. In a case-control study of 1111 HNSCC patients, Huang *et al*. (2010)[40] examined four SNPs in *DEC1*, with a particular focus on a c.606 T>C mutation. They found a protective effect of the c.606CC homozygous genotype in NS, ND, and younger patients, although notably the study included large numbers of both OCSCC and OPSCC. While *DEC1* aberrations may pose as potential OCSCC biomarkers for NSND individuals, the molecular mechanism of this protective effect remains to be elucidated.[40]

A recent study of 448 HNSCCs from The Cancer Genome Atlas (TCGA) dataset found acquired uniparental disomy (aUPD) regions that were associated with cancer in NSND patients.[41] Specifically, aUPD regions on chromosome 5q occurred more frequently in ND than alcohol-using patients, and in NS than smoking patients. This association was observed in the entire HNSCC cohort as well as in the subset of oral cavity cancers.[41] 5q deletions have been associated with myelodysplastic syndromes and are thought to involve deletions of multiple tumor suppressor genes.[42,43]

Adding another twist to this already complicated picture, a large study of over 2000 HNSCC cases identified 10 E2F transcription factors 1 and 2 (E2F1 and E2F2) "risk genotypes" that did not correlate with cancer risk alone, but when 5 or more were combined, increased the risk of cancer among NSND and young patients.[44] While these observations suggest that E2F1 and E2F2 genetic variants may jointly play roles in NSND head and neck carcinogenesis, it should be noted that this study included tumors from different histological sites, and the role of these "risk genotypes" in OCSCC requires further clarification.

In contrast, Pickering *et al*. found no meaningful differences between OCSCC in SD and NSND populations.[45] The authors per-

formed an analysis of whole-exome sequencing profiles of patients from TCGA-HNSC dataset, as well as oral tongue cancers collected from young (<46) and older patients treated at the MD Anderson cancer center. While in both datasets more mutations were seen in older patients, known cancer driver genes (*e.g.*, *TP53, FAT1,* and *CASP8*) were proportionately mutated in both groups, and no specific aberrations defining the NSND population were identified. Similarly, whole-genome copy number analyses did not identify any differences between the two cohorts.[45]

In summary, genetic changes potentially associated with OCSCC in NSND include aberrations in *CCND1, CASP8, DEC1,* and 5q region. Notably, tumor suppressor genes such as *TP53* are mutated at similar rates in both NSND and SD cancer patients. While there is no single predominant pattern of cancer progression in NSND, it may be necessary for multiple aberrations to be present concurrently to promote carcinogenesis.

Transcriptomic changes

In addition to the aberrations on DNA level, differences in gene expression patterns have also been reported in association with NSND cases. Soares *et al*. examined HNSCC tumor tissue from 47 NSND and 37 SD patients matched for stage, grade, and site.[46] They assessed the expression of glutathione S-transferase π *(GTSP1*), a detoxification enzyme upregulated in the presence of carcinogens. They found that while *GTSP1* was expressed in tumors from both NSND and SD patients, its expression level was substantially higher in tumor margins from SD individuals. As *GTSP1* plays a protective role in patients exposed to tobacco and alcohol carcinogens, the authors suggest that low *GTSP1* expression in NSND individuals might make them susceptible to other carcinogens. This may be a mechanism of carcinogenesis in the absence of tobacco/alcohol. Nevertheless, studies in larger cohorts are required to support or refute this observation.

A different study that analyzed 55 cancer tissue samples showed that positive staining for PD-L1 protein was more prominent in OCSCC samples collected from NSND patients.[47] Although sample sizes were small, this observation suggests that NSND may represent a promising candidate group for immunotherapy with PD-1/PD-L1 axis blockade.

Another group analyzed gene expression profiles derived from a mixed cohort of 89 SD and 15 NSND patients, and found 49 genes that were differentially dysregulated (28 overexpressed and 21 downregulated) between the two groups of patients.[48] The genes involved had a wide range of functions; notable examples include upregulation of IFN-g related genes and downregulation of genes involved in the NFKB pathway in NSND patients.

Interestingly, though dysregulation of the tumor suppressor gene p53 is associated with many types of cancer, a study including 33 NSND and 29 SD HNSCC patients reported no differences in p53 expression (assessed by immunohistochemical staining) between the two cohorts,[49] with another small study of 11 NSND,[50] further supporting the suggestion that p53 does not play a major role in NSND tumorigenesis.

While elevated expression of a gene does not necessarily correspond with an increase in the activity of its product, transcriptomic changes play crucial role in driving OCSCC evolution.[51,52] Due to the limited number of currently available NSND datasets it is challenging to dissect the molecular pathways underlying cancer progression in NSND patients, but as more data sets become available, a comprehensive transcriptomic analysis may aid in identifying functional categories or pathways that may be relevant as therapeutic targets.

Epigenetic changes

As mutations alone are insufficient to explain pervasive transcriptomic changes seen in OCSCC malignancies, it was suggested that epigenetic modifications may be central to gene expression dysregulation during oral carcinogenesis.[53] One study, utilizing a cohort of HNSCC tumors collected from 136 patients, found 8 microRNAs differentially regulated between drinking and ND patients.[54] However, other studies have failed to find such differences. For example, a study of 90 SD and NSND participants (in both cancer and control groups) found higher levels of p15 methylation in cancer patients, regardless of tobacco/alcohol use. Notably, a higher prevalence of p15 methylation was found in histologically-normal surgical margin epithelia of SD compared with NSND, suggesting that p15 methylation is not a major driver of tumorigenesis in the NSND population.[55] While epigenetic alterations are common in tobacco-associated oral carcinogenesis, epigenetic changes specifically associated with NSND OCSCC patients are less understood. Further studies with larger sample sizes are needed to define specific epigenetic alterations that have vital importance in NSND patients.

Infectious factors

HPV infection is hypothesized to be a contributing factor to the increasing rates of OCSCC in NSND patients, analogous to HPV-associated OPSCC.[56-58] It is acknowledged that patients with HPV-associated OPSCC have a distinct epidemiologic profile, with younger age at presentation, absence of strong smoking history, and higher socioeconomic status. The etiologic contribution of HPV in OCSCC is less understood.[12,13,15,16,56,59] One study of 53 patients with oral tongue cancer showed an association between HPV positivity and NSND status, however sample sizes were small as only 4 of the 53 tumors tested positive for high-risk HPV.[60]

In an international study of 3680 samples, Castellsagué *et al*. estimated HPV positivity rates to be 22.4% for OPSCC but only 4.4% for OCSCC, suggesting that HPV plays a more important role in OPSCC than OCSCC.[61] Another study examined samples from 45 HNSCC patients younger than 40. While the association between HPV and cancer was found when the entire cohort was analyzed together, only 2 of 18 OCSCCs were positive for HPV.[62] In a larger study, Belobrov *et al*. performed immunohistochemical staining for biomarkers such as p53, p16, cyclin D1, and EGFR using samples from 129 OCSCC patients.[58] While overexpression of these biomarkers was seen in many of the analyzed specimens, p16 overexpression was significantly stronger in NSND cases under the age of 70. Expression of p16 has been used as a proxy for HPV-associated oncogenesis, as inactivation of Rb by the HPV viral protein E7 leads to p16 upregulation.[63] In a more recent study, Dediol *et al*. (2016) found that p16 overexpression was more frequent in NSND than in SD with OCSCC.[11] Although these findings support the role of HPV in a subset of younger NSND patients, the accuracy of p16 as a marker for HPV-associated oncogenesis in OCSCC remains debatable. In a study of 409 OCSCCs, Lingen *et al*. found a positive predictive value of only 41.3% for p16 expression when compared with PCR detection of HPV E6/E7 mRNA, with only 5.9% of OCSCCs found to be related to HPV infections.[13] Mirghani *et al*. further supported the notion that p16 immunohistochemistry is an unreliable surrogate marker for HPV oncogenesis in OCSCC, given its low specificity,[12] whereas Tomo *et al*. demonstrated that p16 levels may be high even in the absence of HPV infection.[64] Moreover, presence of HPV DNA fails to accurately represent a transcriptionally active viral process in OCSCC.[14,65] Taken together, while evidence suggests that HPV may play a significant etiological role in NSND for OPSCC, HPV

does not appear to be a main driver of OCSCC.

Foy *et al*. have recently postulated that other viruses, such as HSV-2, may cause OCSCC via changes in chronic inflammatory gene expression (*e.g*., *JAK2*) and epigenetic deregulation.[66] Theoretically, such changes could persist after clearance of the virus. While another study detected no significant viral RNA transcripts in a set of 68 oral cancers from NSND patients,[67] this may be a fruitful avenue for investigation into mechanisms of OCSCC in NSND populations.

In summary, although the role of HPV in OPSCC is well-demonstrated, evidence for HPV-dependent carcinogenesis in OCSCC is equivocal. In the future, other oncogenic viruses might be explored as contributors to OCSCC oncogenesis.

Other factors

Environmental factors have also been explored as potential contributors to OCSCC in NSND patients. One such factor is metallic dental hardware. In a retrospective analysis of 54 NSND patients with OCSCC, Yesensky *et al*. administered a dental health questionnaire to identify a history of metallic hardware and orthodontic procedures.[30] Forty of the 54 patients (74%) had a history of metal-containing dental hardware, including braces in younger patients, and crowns, implants, or dentures in older ones. A retrospective analysis of 31 OCSCC patients found that lesions occurring next to dental implants were more common in female and NSND patients.[68] The mechanism for hardware-associated OCSCC is unclear, but DNA damage from cytotoxic metal ions,[69] and chronic mucosal irritation from adjacent hardware[30] have been proposed. Poor oral hygiene and occupational exposures may also be involved.[70]

Yan *et al*. conducted a case-control study of 319 NSND OCSCC patients and 994 NSND controls using an "environmental exposure index" consisting of nine variables, including aspects of diet, dental hygiene, hardware, and exposure to other potential carcinogens.[71] They found a linear relationship between the index score and the risk of OCSCC, in particular identifying "recurrent oral ulceration" as the strongest risk factor. Interestingly, these authors noted that the effects of these exposures appeared to be more prominent in patients with a family history of cancer, suggesting that genetic predisposition may also contribute to OCSCC in NSND cases.[71] Several other studies have found compelling evidence for dietary effects. A case-control study of 421 NSND OCSCC patients and 1398 NSND controls reported protective effects of tea and milk consumption.[72] Another case-control study of 236 oral cancer patients and 300 controls reported a higher level of erythrocyte membrane fatty acids (a proxy for dietary fatty acid intake) in the control group, and found that the association between fatty acid levels and cancer was stronger in NSND patients, further supporting that dietary factors can affect cancer risk in NSND populations.[73]

In summary, while environmental factors certainly play a role in the development of OCSCC in NSND, more work is needed to determine the contributions of these various factors to oral tumorigenesis and their interactions with smoking and alcohol.

Clinical behavior

It has been suggested that oral cancers in NSND patients are more aggressive, but this has not been conclusively determined. Evidence in favor of this suggestion includes a study of 76 oral leukoplakia patients, which showed faster and more frequent progression in NSND and female patients.[10] A meta-analysis of 26 articles with a total of 2532 oral cancer patients (not limited to the oral cavity) found that those with PD-L1 overexpression had lower disease-specific and disease-free survival; these patients also tended to be females, NS, and/or ND.[74] Others have found a higher rate of recurrent/persistent disease and poorer 5-year survival in elderly NSND women *vs* SD male and female age-matched controls,[23] and proposed that elderly NSND females,[24] or young NSND patients[75] constitute a clinically distinct subset of OCSCC. Interestingly, NSND patients tend to develop malignancies at different histological sites than SD individuals, with more tumors located in the cheek mucosa and alveolar ridge, which also suggests different clinical behavior.[76,77]

The true prognostic impact of OCSCC in NSND patients is likely more complicated. Bachar *et al*. studied a group of 175 SD and 116 NSND patients with oral tongue cancer, and showed similar rates of overall, local, and regional recurrence in both cohorts. Disease-specific and disease-free survival were also similar. When the cases in the cohort were limited to patients under 40, however, NSND patients had significantly worse disease-free and overall survival.[25] The authors suggest that, because of these differences in prognosis, there may be a distinct mechanism of carcinogenesis in this younger subset of NSND patients with more aggressive disease. On the other hand, some studies have found no association between tobacco/alcohol use and prognosis.[26,78] For example, a historical cohort study of 120 patients (66 ever-smoking (ES)) by Durr *et al*. found no significant differences in overall survival or recurrence-free survival between NS and ES patients with oral tongue cancer, although worse overall survival in NSND patients trended towards significance.[79] Finally, a series of studies suggest that NSND patients with OCSCC may have better survival than their SD counterparts. A study of 218 HNSCC patients (24 NS, 50 ND) found worse survival among smoking and/or drinking patients after chemoradiation, although it should be noted that this study included cancers from various histological locations.[80] In study of 1165 oral cancer patients, NSND had better overall and disease-specific survival than SD patients.[81] Similarly, a retrospective comparative study of patients with oral lichen planus associated OCSCC (OLP-OCSCC) and non-OLP-OCSCC reported that patients with OLP-OCSCC were more likely to be NSND and female, and had better overall and disease-specific survival. Interestingly, however, these OLP-OCSCC patients had an increased risk of recurrence and second primary tumors.[82]

Assuming true differences in prognosis and disease progression between these patient subsets, it is reasonable to suggest that distinct mechanisms could underlie carcinogenesis in these subsets. Indeed, it was reported that changes in copy number variation burden predicted prognosis in young oral cancer patients but not older ones, suggesting that copy number variation may uniquely contribute to carcinogenesis and/or disease progression in NSND cases.[83] Another hypothesis is that loss of heterozygosity (LOH) at tumor suppressor loci in premalignant lesions may contribute to malignant transformation. This is supported by the finding that particular "high risk" LOH profiles may predict the risk of progression in a subset of premalignant oral lesions.[84] While LOH of 3p and/or 9p (containing p16INK4a and p14ARF) was reported to predict progression of oral leukoplakia in both NSND and SD patients, there is some evidence that the contribution of LOH to malignant transformation is greater in NSND subset. For example, Rock *et al*. (2018) found that NS patients with oral epithelial dysplasia had a higher risk of progression to cancer than those who smoked (38-fold higher for floor-of-mouth lesions).[84] Based on these observations, the authors conclude that LOH may be a more important factor for progression in NS than in smoking patients. While these studies provide early evidence for different molecular drivers in NSND OCSCC patients, the mechanisms underlying cancer initiation and progression in this group of patients remain elusive.

Table 1. Clinical/preclinical studies highlighting molecular profile and treatment outcomes of patients with non-smoking, non-drinking oral cavity squamous cell carcinoma

Authors (Year)	Study design/objective	Treatment modality	Sample size (n)	NSND cases (n)	NSND prevalence (%)	Clinical significance	Ref
Yang et al. 2021	Retrospective cohort; treatment response and disease-specific prognosis	Surgery and adjuvant CRT	353	86	24.4	* NSND OCSCC unrelated to HPV status. * NSND patients showed better locoregional control and disease-specific survival than SD patients. * Higher expression of p53, p63, Ki-67 in SD compared to NSND population.	(85)
Adeoy et al. 2021	Retrospective cohort; treatment response and disease-specific prognosis	Surgery and adjuvant CRT	313	171	54.6	* No significant difference between recurrence free survival and disease-specific survival in NSND compared to SD patients. * Better overall survival in NSND patients compared to SD.	(86)
Koo et al. 2021	Molecular profiling	Not specifically indicated	176	59	33.5	* Higher prevalence of somatic CDKN2A mutations, EGFR amplifications and BRCA2 deletion in NSND OCSCC patients. * Unrelated to HPV infection. * No significant difference in overall survival between NSND and SD patients.	(87)
Bao et al. 2020	Clinicopathological characteristics and disease-specific prognosis	Surgery and adjuvant CRT	1165	646	55.5	* NSND patients associated with better overall survival and disease-specific survival than SD patients	(81)
Lenouvel *et al.* 2020	Retrospective cohort; molecular profiling	Not specifically indicated	55	8	14.5	* PD-L1 overexpression in NSND patients, potentially contributing to early tumorigenesis.	(74)
Brennan et al. 2017	Retrospective cohort; clinicopathological characteristics and molecular profiling	Not specifically indicated	528	184	34.8	* NSND OCSCC associated with distinct molecular profile, low overall somatic mutation rates in these patients compared to SD patients. * NSND group presents CIMP high and low phenotypes. * Significant differential hypermethylation profile in NSND group compared to SD. * Overexpression of PD-L1, PD-L2 and CD8+ T cells in NSND group.	(88)
De Angelis et al. 2018	Retrospective cohort; clinicopathological characteristics and disease-specific prognosis	Surgery and adjuvant CRT	287	70	24.4	* Elderly NSND females are associated with poor disease-specific survival compared to elderly SD patients. * No significant difference between disease-specific survival in NSND compared to SD patients.	(23)
Soares et al. 2017	Retrospective cohort; molecular profiling	Not specifically indicated	1633	47	2.87	* No significant difference in expression of GTSP1 in tumor of NSND and SD patients. * Lack of carcinogen detoxification could be associated with carcinogenesis in NSND patients.	(46)
Foy et al. 2017	Retrospective cohort; molecular profiling	Not specifically indicated	213	93	43.7	* Increased expression of PD-L1, IDO-1, CD8+ T cells in NSND patients. * Associated with low somatic mutation, copy number variation and incidence of chr 11q13 amplification.	(89)
Dediol et al. 2016	Retrospective cohort; clinicopathological characteristics and disease-specific prognosis	Surgery	1074	103	9.6	* No significant difference in disease specific survival, locoregional recurrence and metastasis between NSND and SD patients.	(11)
Fan et al. 2014	Retrospective cohort; clinicopathological characteristics and disease-specific prognosis	Surgery and adjunctive CRT	100	54	54	* No significant difference in recurrence free survival or overall survival between NSND and SD patients.	(26)
Farshadpour *et al.* 2012	Retrospective cohort; clinicopathological characteristics and molecular profiling	Not specifically indicated	104	15	14.4	* Differential gene expression signatures in NSND patients. * Increased expression Major histocompatibility complex (MHC) and downregulation of NF B related genes in NSND patients.	(48)
Bachar et al. 2011	Retrospective cohort; clinicopathological characteristics and disease-specific prognosis	Surgery and adjuvant CRT	291	116	39.9	* No significant difference in disease-free survival or overall survival between NSND and SD patients. * Young patients associated with poor overall survival.	(25)
Albuquerque et al. 2011	Retrospective cohort; clinicopathological characteristics	Not specifically indicated	354	146	41.2	* NSND patients associated with a distinct clinicopathological profile compared to SD patients.	(90)

NSND: non-smoking, non-drinking; OCSCC: oral cavity squamous cell carcinoma; CRT: chemoradiotherapy; PD-L1: programmed death-ligand 1; EGFR: epidermal growth factor receptor; MHC: major histocompatibility complex; HPV: human papillomavirus; PD-L2: programmed death-ligand 2; IDO-1: indoleamine 2,3-dioxygenase.

Comparing the aggressiveness of cancers in NSND and SD patients may be difficult due to the heterogeneity of the NSND population. The study that reported worse survival in NSND patients indicated the greatest effects in patients under 40 years old.[25] In contrast, both studies describing better survival among NSND patients had a NSND cohort that was older, on average, than the SD cohort. Consequently, it is tempting to rationalize that there are distinct populations of NSND OCSCC patients. A younger population may be genetically predisposed to OCSCC, with a distinct mechanism of carcinogenesis and more aggressive disease. In contrast, an older group might develop cancer via mechanisms similar to the SD patients, in which case their NSND status may be protective, as it confers a lower cumulative exposure to carcinogens that would worsen the disease. In this group, factors such as dental hardware and environmental exposures may play a more prominent role. This hypothesis would be in accordance with data showing a bimodal age distribution among NSND OCSCC patients (see "Epidemiology of OCSCC in NSND populations").

Conclusions

In this overview, we have discussed the recent rising incidence of OCSCC in patients who lack traditional risk factors such as smoking and drinking, and who skew towards a younger, female demographic. Attempts to explain this phenomenon have not been conclusive, in part due to the relatively small number of NSND cases and high heterogeneity of the disease (Table 1).

Among the factors explored as potential contributors to OCSCC in atypical patients are: genetic factors, infectious factors (particularly HPV), and environmental factors (*e.g.*, chemical exposure and dental hardware). Studies examining genetic underpinnings of OCSCC in atypical patients have identified mutations, gene expression changes, and epigenetic factors. Due to variation in the study populations, these studies may not be directly comparable, and more work in larger cohorts is needed to elucidate genetic contributions to OCSCC in atypical patients. Additionally, while there is strong evidence that HPV is an etiologic factor for a large proportion of OPSCC in NSND patients, this is unlikely to be the case for OCSCC (Table 1).

Finally, it has been suggested that oral cancers in atypical and typical patients differ by clinical course, with more aggressive disease being prevalent among atypical cases. While reports are controversial and this evidence is not yet conclusive, cumulative data suggest that OCSCC in atypical patients may indeed be more aggressive, and that this may be associated with genetic mechanisms (*e.g.*, copy number variation, LOH, and other genomic aberrations) that set OCSCC in atypical patients apart. Nevertheless, a comprehensive analysis of genetic alterations in a large cohort of NSND cases is required to identify the genetic drivers of cancer progression in this unique group of patients.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209-49.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020;70:7-30.
3. Howlader NA, Krapcho M, Miller D, et al. (eds.). SEER Cancer Statistics Review, 1975-2017, National Cancer Institute. Bethesda, USA.
4. Bonner JA, Harari PM, Giralt J, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med 2006;354:567-78.
5. Taberna M, Oliva M, Mesia R. Cetuximab-containing combinations in locally advanced and recurrent or metastatic head and neck squamous cell carcinoma. Front Oncol 2019;9:383.
6. Ferris RL, Blumenschein G Jr., Fayette J, et al. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. N Engl J Med 2016;375:1856-67.
7. Shin DS, Ribas A. The evolution of checkpoint blockade as a cancer therapy: what's here, what's next? Curr Opin Immunol 2015;33:23-35.
8. Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. Nat Rev Cancer 2011;11:9-22.
9. Shiboski CH, Schmidt BL, Jordan RC. Tongue and tonsil carcinoma: increasing trends in the U.S. population ages 20-44 years. Cancer 2005;103:1843-9.
10. Wu W, Wang Z, Zhou Z. Role of the human papillomavirus in malignant transformation of oral leukoplakia distinct from oropharyngeal squamous cell carcinoma: A study of 76 patients with internal-control specimens. Oral Surg Oral Med Oral Pathol Oral Radiol 2019;128:273-9.
11. Dediol E, Sabol I, Virag M, et al. HPV prevalence and p16INKa over-expression in non-smoking non-drinking oral cavity cancer patients. Oral Dis 2016;22:517-22.
12. Mirghani H, Amen F, Moreau F, St Guily JL. Do high-risk human papillomaviruses cause oral cavity squamous cell carcinoma? Oral Oncology 2015;51:229-36.
13. Lingen MW, Xiao W, Schmitt A, et al. Low etiologic fraction for high-risk human papillomavirus in oral cavity squamous cell carcinomas. Oral Oncol 2013;49:1-8.
14. Zafereo ME, Xu L, Dahlstrom KR, et al. Squamous cell carcinoma of the oral cavity often overexpresses p16 but is rarely driven by human papillomavirus. Oral Oncol 2016;56:47-53.
15. Cardesa A, Nadal A. Carcinoma of the head and neck in the HPV era. Acta Dermatovenerol Alp Pannonica Adriat 2011;20:161-73.
16. Boscolo-Rizzo P, Del Mistro A, Bussu F, et al. New insights into human papillomavirus-associated head and neck squamous cell carcinoma. Acta Otorhinolaryngol Ital 2013;33:77-87.
17. Creamer MR, Wang TW, Babb S, et al. Tobacco Product Use and Cessation Indicators Among Adults - United States, 2018. Centers for Disease Control and Prevention, Morbidity and Mortality Weekly Report. 2019;68:1013-19.
18. Myers JN ET, Roberts D, Byers RM. Squamous cell carcinoma of the tongue in young adults: increasing incidence and factors that predict treatment outcomes. Otolaryngol Head Neck Surg 2000;122:44-51
19. Patel SC, Carpenter WR, Tyree S, et al. Increasing incidence of oral tongue squamous cell carcinoma in young white women, age 18 to 44 years. J Clin Oncol 2011;29:1488-94.
20. Pickering CR, Zhang J, Yoo SY, et al. Integrative genomic characterization of oral squamous cell carcinoma identifies frequent somatic drivers. Cancer Discov 2013;3:770-81.
21. India Project Team of the International Cancer Genome C. Mutational landscape of gingivo-buccal oral squamous cell carcinoma reveals new recurrently-mutated genes and molecular subgroups. Nat Commun 2013;4:2873.
22. Cancer Genome Atlas N. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature 2015;517:576-82.

23. DeAngelis A, Breik O, Koo K, et al. Non-smoking, non-drinking elderly females, a 5-year follow-up of a clinically distinct cohort of oral squamous cell carcinoma patients. Oral Oncol 2018;86:113-20.
24. Koo K, Barrowman R, McCullough M, et al. Non-smoking non-drinking elderly females: a clinically distinct subgroup of oral squamous cell carcinoma patients. Int J Oral Maxillofac Surg 2013;42:929-33.
25. Bachar G, Hod R, Goldstein DP, et al. Outcome of oral tongue squamous cell carcinoma in patients with and without known risk factors. Oral Oncol 2011;47:45-50.
26. Fan Y, Zheng L, Mao MH, et al. Survival analysis of oral squamous cell carcinoma in a subgroup of young patients. Asian Pac J Cancer Prev 2014;15:8887-91.
27. Rivera C. Essentials of oral cancer. Int J Clin Exp Pathol 2015;8:11884-94.
28. Dahlstrom KR, Little JA, Zafereo ME, et al. Squamous cell carcinoma of the head and neck in never smoker-never drinkers: a descriptive epidemiologic study. Head Neck 2008;30:75-84.
29. Kruse AL, Bredell M, Gratz KW. Oral squamous cell carcinoma in non-smoking and non-drinking patients. Head Neck Oncol 2010;2:24.
30. Yesensky JA, Hasina R, Wroblewski KE, et al. Role of dental hardware in oral cavity squamous cell carcinoma in the low-risk nonsmoker nondrinker population. Head Neck 2018;40:784-92.
31. Paderno A, Morello R, Piazza C. Tongue carcinoma in young adults: a review of the literature. Acta Otorhinolaryngol Ital 2018;38:175-80.
32. Mascitti M, Tempesta A, Togni L, et al. Histological features and survival in young patients with HPV-negative oral squamous cell carcinoma. Oral Dis 2020;26:1640-8.
33. Wiseman SM, Swede H, Stoler DL, et al. Squamous cell carcinoma of the head and neck in nonsmokers and nondrinkers: an analysis of clinicopathologic characteristics and treatment outcomes. Ann Surg Oncol 2003;10:551-7.
34. Moyses RA, Lopez RV, Cury PM, et al. Significant differences in demographic, clinical, and pathological features in relation to smoking and alcohol consumption among 1,633 head and neck cancer patients. Clinics (Sao Paulo) 2013;68:738-44.
35. Farshadpour F, Hordijk GJ, Koole R, Slootweg PJ. Non-smoking and non-drinking patients with head and neck squamous cell carcinoma: a distinct population. Oral Dis 2007;13:239-43.
36. Tang YI, Liu Y, Zhao W, et al. Caspase-8 polymorphisms and risk of oral squamous cell carcinoma. Exp Ther Med 2015;10:2267-76.
37. Nishimoto IN, Pinheiro NA, Rogatto SR, et al. Cyclin D1 gene polymorphism as a risk factor for squamous cell carcinoma of the upper aerodigestive system in non-alcoholics. Oral Oncol 2004;40:604-10.
38. Qie S, Diehl JA. Cyclin D1, cancer progression, and opportunities in cancer treatment. J Mol Med (Berl) 2016;94:1313-26.
39. Nishiwaki T, Daigo Y, Kawasoe T, Nakamura Y. Isolation and mutational analysis of a novel human cDNA, DEC1 (deleted in esophageal cancer 1), derived from the tumor suppressor locus in 9q32. Genes Chromosomes Cancer 2000;27:169-76.
40. Huang YJ, Niu J, Wei S, et al. A novel functional DEC1 promoter polymorphism -249T>C reduces risk of squamous cell carcinoma of the head and neck. Carcinogenesis 2010;31:2082-90.
41. Tuna M, Amos CI, Mills GB. Genome-wide analysis of head and neck squamous cell carcinomas reveals HPV, TP53, smoking and alcohol-related allele-based acquired uniparental disomy genomic alterations. Neoplasia 2019;21:197-205.
42. Ebert BL. Deletion 5q in myelodysplastic syndrome: a paradigm for the study of hemizygous deletions in cancer. Leukemia 2009;23:1252-6.
43. Eisenmann KM, Dykema KJ, Matheson SF, et al. 5q-myelodysplastic syndromes: chromosome 5q genes direct a tumor-suppression network sensing actin dynamics. Oncogene 2009;28:3429-41.
44. Lu M, Liu Z, Yu H, et al. Combined effects of E2F1 and E2F2 polymorphisms on risk and early onset of squamous cell carcinoma of the head and neck. Mol Carcinog 2012;51:E132-41.
45. 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;20:3842-8.
46. Soares PO, Maluf Cury P, Mendoza Lopez RV, et al. GTSP1 expression in non-smoker and non-drinker patients with squamous cell carcinoma of the head and neck. PLoS One 2017;12:e0182600.
47. Lenouvel D, Gonzalez-Moles MA, Ruiz-Avila I, et al. Clinicopathological and prognostic significance of PD-L1 in oral cancer: A preliminary retrospective immunohistochemistry study. Oral Dis 2021;27:173-82.
48. Farshadpour F, Roepman P, Hordijk GJ, et al. A gene expression profile for non-smoking and non-drinking patients with head and neck cancer. Oral Dis 2012;18:178-83.
49. Farshadpour F, Hordijk GJ, Koole R, Slootweg PJ. Head and neck squamous cell carcinoma in non-smoking and non-drinking patients with multiple tumors: etiologic significance of p53 and Ki-67 in non-tumorous epithelium. J Oral Pathol Med 2008;37:549-54.
50. Sorensen DM, Lewark TM, Haney JL,et al. Absence of p53 mutations in squamous carcinomas of the tongue in nonsmoking and nondrinking patients younger than 40 years. Arch Otolaryngol Head Neck Surg 1997;123:503-6.
51. Makarev E, Schubert AD, Kanherkar RR, et al. In silico analysis of pathways activation landscape in oral squamous cell carcinoma and oral leukoplakia. Cell Death Discov 2017;3:17022.
52. Zhang Q, Zhang J, Jin H, Sheng S. Whole transcriptome sequencing identifies tumor-specific mutations in human oral squamous cell carcinoma. BMC Med Genomics 2013;6:28.
53. Hsu PJ, Yan K, Shi H, et al. Molecular biology of oral cavity squamous cell carcinoma. Oral Oncol 2020;102:104552.
54. Saad MA, Kuo SZ, Rahimy E, et al. Alcohol-dysregulated miR-30a and miR-934 in head and neck squamous cell carcinoma. Mol Cancer 2015;14:181.
55. Wong TS, Man MW, Lam AK, et al The study of p16 and p15 gene methylation in head and neck squamous cell carcinoma and their quantitative evaluation in plasma by real-time PCR. Eur J Cancer 2003;39:1881-7.
56. Deschler DG, Richmon JD, Khariwala SS,et al. The "new" head and neck cancer patient-young, nonsmoker, nondrinker, and HPV positive: evaluation. Otolaryngol Head Neck Surg 2014;151:375-80.
57. Martin-Hernan F, Sanchez-Hernandez JG, Cano J, et alOral cancer, HPV infection and evidence of sexual transmission. Med Oral Patol Oral Cir Bucal 2013;18:e439-44.
58. Belobrov S, Angel C, Wiesenfeld D, McCullough M. Histopathological analysis of oral squamous cell carcinoma in nonsmokers and nondrinkers. Translational Research in Oral Oncology 2016.
59. Jelihovschi I, Bidescu AC, Tucaliuc SE, Iancu LS. Detection of human papilloma virus in head and neck squamous cell carci-

nomas: A literature review. Rev Med Chir Soc Med Nat Iasi 2015;119:502-9.
60. Tsimplaki E, Argyri E, Xesfyngi D, et al. Prevalence and expression of human papillomavirus in 53 patients with oral tongue squamous cell carcinoma. Anticancer Res 2014;34:1021-5.
61. Castellsague X, Alemany L, Quer M, et al. HPV involvement in head and neck cancers: Comprehensive assessment of biomarkers in 3680 patients. J Natl Cancer Inst 2016;108:djv403.
62. van Monsjou HS, van Velthuysen ML, van den Brekel MW, et al. Human papillomavirus status in young patients with head and neck squamous cell carcinoma. Int J Cancer 2012;130:1806-12.
63. Sano T, Oyama T, Kashiwabara K, et al. Expression status of p16 protein is associated with human papillomavirus oncogenic potential in cervical and genital lesions. Am J Pathol 1998;153:1741-8.
64. Tomo S, Biss SP, Crivelini MM, et al. High p16(INK4a) immunoexpression is not HPV dependent in oral leukoplakia. Arch Oral Biol 2020;115:104738.
65. Poling JS, Ma XJ, Bui S, et al. Human papillomavirus (HPV) status of non-tobacco related squamous cell carcinomas of the lateral tongue. Oral Oncol 2014;50:306-10.
66. Foy JP, Bertolus C, Boutolleau D, et al. Arguments to support a viral origin of oral squamous cell carcinoma in non-smoker and non-drinker patients. Front Oncol 2020;10:822.
67. Perot P, Falguieres M, Arowas L, et al. Investigation of viral etiology in potentially malignant disorders and oral squamous cell carcinomas in non-smoking, non-drinking patients. PLoS One 2020;15:e0232138.
68. Ramos JC, Alves FA, Kowalski LP, et al. Epidemiological profile and clinical implications of oral squamous cell carcinoma adjacent to dental implants. Oral Dis 2021;27:1687-98.
69. Faccioni F, Franceschetti P, Cerpelloni M, Fracasso ME. In vivo study on metal release from fixed orthodontic appliances and DNA damage in oral mucosa cells. Am J Orthod Dentofacial Orthop 2003;124:687-93.
70. Vargas-Ferreira F, Nedel F, Etges A, et al. Etiologic factors associated with oral squamous cell carcinoma in non-smokers and non-alcoholic drinkers: a brief approach. Braz Dent J 2012;23:586-90.
71. Yan L, Chen F, He B, et al. A novel environmental exposure index and its interaction with familial susceptibility on oral cancer in non-smokers and non-drinkers: a case-control study. Eur Arch Otorhinolaryngol 2017;274:1945-50.
72. Chen F, Yan L, Lin L, et al. Independent and joint effects of tea and milk consumption on oral cancer among non-smokers and non-drinkers: a case-control study in China. Oncotarget 2017;8:50091-7.
73. Chen Q, Wang J, Wang J, et al. Erythrocyte omega-3 polyunsaturated fatty acids are inversely associated with the risk of oral cancer: a case-control study. Nutr Diabetes 2020;10:35.
74. Lenouvel D, Gonzalez-Moles MA, Ruiz-Avila I, et al. Prognostic and clinicopathological significance of PD-L1 overexpression in oral squamous cell carcinoma: A systematic review and comprehensive meta-analysis. Oral Oncol 2020;106:104722.
75. Santos-Silva AR, Carvalho Andrade MA, Jorge J, et al. Tongue squamous cell carcinoma in young nonsmoking and nondrinking patients: 3 clinical cases of orthodontic interest. Am J Orthod Dentofacial Orthop 2014;145:103-7.
76. Jovanovic A, Schulten EA, Kostense PJ, et al. Tobacco and alcohol related to the anatomical site of oral squamous cell carcinoma. J Oral Pathol Med 1993;22:459-62.
77. Campbell BH, Mark DH, Soneson EA, et al. The role of dental prostheses in alveolar ridge squamous carcinomas. Arch Otolaryngol Head Neck Surg 1997;123:1112-5.
78. Sim YC, Hwang JH, Ahn KM. Overall and disease-specific survival outcomes following primary surgery for oral squamous cell carcinoma: analysis of consecutive 67 patients. J Korean Assoc Oral Maxillofac Surg 2019;45:83-90.
79. Durr ML, van Zante A, Li D, et al. Oral tongue squamous cell carcinoma in never-smokers: analysis of clinicopathologic characteristics and survival. Otolaryngol Head Neck Surg 2013;149:89-96.
80. Descamps G, Karaca Y, Lechien JR, et al. Classical risk factors, but not HPV status, predict survival after chemoradiotherapy in advanced head and neck cancer patients. J Cancer Res Clin Oncol 2016;142:2185-96.
81. Bao X, Liu F, Chen Q, et al. Propensity score analysis exploring the impact of smoking and drinking on the prognosis of patients with oral cancer. Head Neck 2020;42:1837-47.
82. Best DL, Herzog C, Powell C, et al. Oral lichen planus-associated oral cavity squamous cell carcinoma is associated with improved survival and increased risk of recurrence. J Oral Maxillofac Surg 2020;78:1193-202.
83. Gu X, Coates PJ, Boldrup L, et al. Copy number variation: A prognostic marker for young patients with squamous cell carcinoma of the oral tongue. J Oral Pathol Med 2019;48:24-30.
84. Rock LD, Rosin MP, Zhang L, et al. Characterization of epithelial oral dysplasia in non-smokers: First steps towards precision medicine. Oral Oncol 2018;78:119-25.
85. Yang Z, Du W, Zhang X, et al. Non-smoking and non-drinking oral squamous cell carcinoma patients: A different entity. Front Oncol 2021;11:558320.
86. Adeoye J, Hui L, Tan JY, et al. Prognostic value of non-smoking, non-alcohol drinking status in oral cavity cancer. Clin Oral Investig 2021.
87. Koo K. Genomic Signature of Oral Squamous Cell Carcinomas from Non-Smoking Non-Drinking Patients. 2021;25:6909-18.
88. Brennan K, Koenig JL, Gentles AJ, et al. Identification of an atypical etiological head and neck squamous carcinoma subtype featuring the CpG island methylator phenotype. EBioMedicine 2017;17:223-36.
89. Foy JP, Bertolus C, Michallet MC, et al. The immune microenvironment of HPV-negative oral squamous cell carcinoma from never-smokers and never-drinkers patients suggests higher clinical benefit of IDO1 and PD1/PD-L1 blockade. Ann Oncol 2017;28:1934-41.
90. Albuquerque R, Lopez-Lopez J, Mari-Roig A, et al. Oral tongue squamous cell carcinoma (OTSCC): alcohol and tobacco consumption versus non-consumption. A study in a Portuguese population. Braz Dent J 2011;22:517-21.