ANÁLISE DO PADRÃO DE RECORRÊNCIA DO CARCINOMA DE CÉLULAS ESCAMOSAS DE OROFARINGE RELACIONADO AO PAPILOMA VÍRUS HUMANO

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RESUMO

De Cicco R. **Análise do padrão de recorrência do carcinoma de células escamosas de orofaringe relacionado ao Papiloma Vírus Humano**. São Paulo; 2020. [Tese de Doutorado-Fundação Antônio Prudente].

Introdução: O carcinoma de células escamosas (CEC) de orofaringe associado ao Papiloma vírus humano (HPV) está associado a melhores taxas de resposta ao tratamento, além de melhor prognóstico. Entretanto, observamos que em algumas regiões geográficas, onde a prevalência de CEC de orofaringe relacionado ao HPV é menor, o impacto da infecção pelo HPV é pouco estudado pela literatura. **Objetivos** analisar os padrões de recorrência de carcinomas de células escamosas de orofaringe de acordo com variáveis clinico-patológicas, associação ou não com HPV e tratamento inicial instituído. **Método:** Foram avaliados retrospectivamente 215 pacientes com diagnóstico de CEC classificados como estádio I a IV (sem metástases à distância) através da classificação da American Joint Committee on Cancer (AJCC), tratados no AC Camargo Cancer Center com intenção curativa por meio de cirurgia inicial ou radioterapia, com ou sem quimioterapia associada. Os dados coletados incluíram informações demográficas, status do HPV, consumo de tabaco e álcool, além de variáveis anatomopatológicas e de tratamento. Os padrões de recorrência foram analisados conforme o status do HPV. A sobrevida livre de doença e a sobrevida livre de recorrência foram calculadas usando curvas de Kaplan-Meier seguindo-se da análise multivariada de Cox. **Resultados:** Cento e vinte e sete (59,1%) pacientes foram diagnosticados com carcinoma de células escamosas relacionadas ao HPV; a idade média dos pacientes foi de 56 anos. A tonsila foi o sítio mais acometido (n=131) tanto nos pacientes com tumores HPV positivos (n = 78, 59,5%) como HPV negativos (n = 53, 40,5%). De acordo com a oitava edição da AJCC, 34 (15,8%), 71 (33%), 47 (21,9%) e 60 (27,9%) pacientes apresentavam doença em estádio I, II, III e IV, respectivamente. A cirurgia foi inicialmente realizada em 109 (50,7%) casos, e os esquemas de radioterapia concomitante a quimioterapia foram oferecidos como as opções de tratamento inicial para 104 pacientes (48,4%, p = 0,686). No geral, a sobrevida livre de doença em cinco anos foi de 73,5% para pacientes com tumores

HPV-positivos e 68,1% para pacientes com tumores HPV-negativos; essa diferença não foi estatisticamente significativa (p = 0,227). O status do tabaco foi considerado o único fator prognóstico independente para a sobrevivência. Além disso, o status do HPV não foi associado a diferenças nas taxas de recorrência (p = 0,680). Observamos também que, enquanto todos os casos de metástases a distância nos pacientes HPV-negativos ocorriam nos pulmões, nos HPV-positivos observamos locais incomuns de doença à distância, como fígado, ossos, pele e sistema nervoso central. **Conclusões:** O status do HPV esteve associado a maiores taxas de sobrevida na população investigada. No entanto, o tabagismo foi considerado o único fator prognóstico independente para sobrevida livre de doença. Além disso, pacientes com carcinoma de células escamosas de orofaringe relacionados ao HPV apresentaram doença a distância em locais não comumente observados nos pacientes com tumores HPV-negativos.

Descritores: Carcinoma de Células Escamosas. Neoplasias de Cabeça e Pescoço. Papillomaviridae. Neoplasias Orofaríngeas/mortalidade. Recidiva. Metástase Neoplásica,

SUMMARY

De Cicco R. **[Papilloma Virus: related Oropharyngeal squamous cell carcinoma patterns of recurrence analysis]**. São Paulo; 2020. [Tese de Doutorado-Fundação Antônio Prudente].

Background*:* Human papilloma virus (HPV)-positive oropharyngeal squamous cell carcinoma (OPSCC) is associated with better tumor-response rates and survival outcomes. However, in some geographic regions with a lower prevalence of HPV-positive OPSCC, the impact of HPV infection on prognosis remains unclear. The aim of this study was to describe the patterns of recurrence and survival among patients treated for OPSCC in a geographic region with a reported low prevalence of HPV-related OPSCC. **Methods*:*** We retrospectively evaluated 215 patients diagnosed with American Joint Committee on Cancer (AJCC) stages I to IV (with no distant metastases) OPSCC who were treated with upfront surgery or radiation therapy with or without chemotherapy at AC Camargo Cancer Center, São Paulo, Brazil. The collected data included demographic information, HPV status, tobacco and alcohol consumption, and pathologic and treatment variables. The patterns of recurrence were recorded according to HPV status. Disease-specific survival and recurrence-free survival were calculated using Kaplan-Meier curves and multivariate Cox regression analysis. **Results*:*** One hundred twenty-seven (59.1%) patients were diagnosed with HPV-positive OPSCC; the median patient age was 56 years. The tonsils were the most frequent site (131 cases) in both the HPV-positive (n=78, 59.5%) and HPV-negative (n=53, 40.5%) groups. According to the AJCC eighth edition, 34 (15.8%), 71 (33%), 47 (21.9%), and 60 (27.9%) patients had stage I, II, III, and IV disease, respectively. Surgery was initially performed in 109 (50.7%) cases, and upfront chemoradiation regimens were provided as the initial treatment options in 104 (48.4%, p=0.686) patients. Overall, the 5-year cancer- specific survival was 73.5% and 68.1% for HPV-positive and HPV-negative patients, respectively; this difference was not significant (p=0.227). Tobacco status was considered the only independent prognostic factor for survival. Furthermore, HPV status was not associated with differences in recurrence

rates (p=0.680). While all distant relapses were found to be lung metastases in the HPV-negative group, we observed unusual sites of distant metastases in the HPV-positive group; five patients presented with liver metastasis, four with bone metastasis, one with skin implants, and one with central nervous system (CNS) disease. **Conclusions*:*** HPV status was associated with higher rates of survival among the investigated population. Moreover, smoking status was considered the only independent prognostic factor for survival. Furthermore, patients with HPV-positive tumors were more likely than patients with HPV-negative OPSCC to have unusual sites of distant metastases.

Key-words: Carcinoma, Squamous Cell. Head and Neck Neoplasms. Papillomaviridae. Oropharyngeal Neoplasms/mortality. Recurrence. Neoplasm Metastasis

ÍNDICE

1 INTRODUÇÃO

1.1 EPIDEMIOLOGIA

O câncer de orofaringe é a 25ª neoplasia mais incidente na população mundial (BRAY et al. 2018). Teve incidência global estimada de 92.887 casos, com mortalidade estimada de 51.005 casos para o ano de 2018 (BRAY et al. 2018). Apesar de discreta redução na estimativa global em relação às estimativas para o ano de 2015 (FERLAY et al. 2014) é estimado um aumento na incidência global desta doença em 53,7%, com aumento na mortalidade de 58,5% para o ano de 2040 (International Agency for Research on Cancer-IARC 2018; FERLAY et al. 2019). Nos Estados Unidos, a incidência das neoplasias de orofaringe para 2018 foi de 13.220 novos casos, com mortalidade estimada para o mesmo período em 3.846 pacientes, colocando o país em segundo lugar no mundo entre os mais prevalentes para o câncer de orofaringe (BRAY et al. 2018; FERLAY et al. 2019). Na população brasileira, a incidência anual para 2018 foi estimada em 4.629 casos, sendo a mortalidade estimada em 2.936 pacientes (BRAY et al. 2018), correspondendo a cerca de 5 a 8% das neoplasias malignas em homens e 2% em mulheres, colocando o Brasil como o 5º país em incidência e mortalidade para neoplasias de orofaringe no mundo (BRAY et al. 2018). A estimativa de crescimento na incidência e mortalidade para o câncer de orofaringe no Brasil para os próximos 20 anos é de 63,9% e 77,7%, respectivamente (IARC 2018; FERLAY et al. 2019).

Apesar de não se tratar de neoplasia classificada entre as mais prevalentes no mundo (BRAY et al. 2018), em certas regiões geográficas como Índia e Taiwan as neoplasias de cabeça e pescoço são as mais frequentes (WYSS et al. 2013), sendo que os tumores de orofaringe são a quinta neoplasia com maior incidência estimada para 2015 nestas regiões (BRAY et al. 2018; TORRE et al. 2018). No continente asiático se concentraram 41,7% de todos os novos casos e 53,5% das mortes relacionadas à neoplasia de orofaringe do mundo em 2018 (FERLAY et al. 2019).

A orofaringe compreende os seguintes sítios: As tonsilas palatinas, os pilares amigdalianos, a base de língua, o palato mole, a parede posterior da faringe e a úvula. O sítio mais acometido por neoplasias é a tonsila, sendo que mais de 90% dos casos são do tipo carcinoma de células escamosas (CEC) (CHATURVEDI et al. 2008). O prognóstico dos pacientes portadores desses tumores depende do estádio e sítio acometido. A causa de morte mais frequente pelo CEC de orofaringe é a recidiva locorregional, que comumente ocorre nos dois primeiros anos após o tratamento inicial (MARUR et al. 2010; MEHANNA et al. 2013). No Brasil, cerca de 50% dos casos de pacientes portadores de CEC de orofaringe são diagnosticados nos Estádios III ou IV, sendo considerados localmente avançados (DE MATOS et al. 2015).

Os fatores de risco mais comumente associados para as neoplasias de cabeça e pescoço têm sido o tabagismo e a ingestão de bebidas alcoólicas (CHATURVEDI et al. 2013). Dados epidemiológicos demonstraram um declínio na incidência global destes tumores, principalmente nas décadas de 1990 e 2000 (CHATURVEDI et al. 2011). O consumo de tabaco também sofreu uma redução global neste período (GIOVINO 2007; CHUNG e GILLISON 2009; CHUNG et al. 2014).

Enquanto a incidência nos canceres de laringe, hipofaringe, cavidade oral e nasofaringe sofreu queda (GILLISON et al. 2015; CHATURVEDI et al. 2008), os tumores de orofaringe, principalmente aqueles da região tonsilar e da base da língua, sofreram um importante aumento na sua incidência, especialmente nos Estados Unidos, Reino Unido, Austrália, Japão e Suécia (SHIBOSKI et al. 2005; NÄSMAN et al. 2009; MIRGHANI et al. 2015; O'SULLIVAN et al. 2016; VOKES et al. 2015; YOUNG et al. 2015). Esse importante aumento na incidência destas neoplasias se deve à sua relação com a infecção pelo Papiloma vírus Humano (HPV) (SHIBOSKI et al. 2005; CHATURVEDI et al. 2011, 2013).

1.2 PAPILOMA VÍRUS HUMANO

Descoberto inicialmente em células da pele, na década de 1950, o papillomavírus humano (HPV) é um vírus classificado pela família dos *Papillomaviridiae* (BOYLE et al. 1973). O papel do HPV na carcinogênese do colo do útero foi esclarecido por Harald zur Hausen, pelo qual recebeu o Prêmio Nobel de Medicina de 2008 (ZUR HAUSEN 1976). Mais de 200 tipos de HPV foram reconhecidos com base em dados da sequência de DNA mostrando diferenças genômicas. Oitenta e cinco genótipos de HPV são bem caracterizados. Outros 120 isolados são novos genótipos potenciais parcialmente caracterizados (BURD 2003). Em seu genoma de dupla fita, contém cerca de oito mil pares de bases, que codificam oito proteínas precoces (E1 a E8) e duas tardias (L1 e L2). As proteínas tardias formam o capsídeo viral, enquanto que as precoces estão associadas à patogênese, replicação e regulação (VASSALO et al. 1999). São vírus epiteliotrópicos, ou seja, seu ciclo de

vida está relacionado à diferenciação da célula escamosa. Havendo infecção, a mesma pode permanecer em latência, ou mesmo sofrer replicação ativa, com síntese de partículas infectantes (VASSALO et al. 1999; BURD 2003).

Nas neoplasias malignas relacionadas ao HPV, há evidência de integração entre o DNA viral e do hospedeiro (BURD 2003; VOKES et al. 2015). Para promover a integração do DNA, ocorre a ruptura do genoma no interior das proteínas E1 ou E2. Estes genes ficam inativados, porém ficam mantidas as expressões de genes E6 e E7. As proteínas E6 e E7 se ligam e inativam as proteínas p53 e pRb, respectivamente. Estas proteínas são supressoras de multiplicação celular, sendo que uma vez inativadas, desregulam as ações antiapoptóticas e de proliferação celular, promovendo o aumento de expressão da p16, que é utilizada na clínica para diagnóstico do carcinoma de células escamosas de orofaringe relacionado ao HPV (PANNONE et al. 2011). A exposição ao HPV é tão frequente, que a presença de DNA do HPV em tecidos malignos indica infecção ativa, mas uma célula que se tornou maligna devido à oncogênese do HPV geralmente superexpressa p16. O gene supressor TP53 é comumente mutado nas neoplasias relacionadas ao tabaco e álcool, mas essas mutações não são uma ocorrência comum no carcinoma de células escamosas relacionado ao HPV.

O HPV 16 é responsável por cerca de 90% dos casos de CEC de orofaringe relacionados ao vírus, seguido pelo HPV 18 (NDIAYE et al. 2014). Apesar da vacina contra o HPV de alto risco potencialmente prevenir eventuais CECs de orofaringe, mudanças na incidência desta doença somente devem ser notadas a partir de 2060 (GILLISON et al. 2015).

Os homens tendem a apresentar maior chance de infecção oral pelo HPV (GILLISON et al. 2008). Isso pode ser devido à mucosa genital feminina ter uma carga viral de HPV mais alta do que a mucosa ou pele genital masculina e, portanto, homens que fazem sexo oral com mulheres têm maior exposição a doses do vírus HPV do que o contrário (GIULIANO et al. 2012). Nas últimas décadas observou-se um aumento no número de parceiros sexuais, além da redução da idade de início de vida sexual ativa, e esses fatores podem ter contribuído para o aumento da exposição oral ao HPV (GILLISON et al. 2008). O risco de infecção oral pelo HPV aumenta com o número de parceiros que praticam sexo oral. Devido a alta prevalência de infecção oral pelo HPV (em torno de 7% da população mundial), acredita-se que a maioria das infecções orais são eliminadas pelo sistema imunológico. A depuração tardia da infecção por HPV oral pode ser um fator de risco para o desenvolvimento do CEC de orofaringe relacionado ao HPV.

1.3 CÂNCER DE OROFARINGE E O HPV

O envolvimento do Papiloma Vírus Humano (HPV) na carcinogênese da mucosa da orofaringe foi inicialmente proposto por Syrjanen em 1983, baseado em características morfológicas e propriedades imunohistoquímicas observadas em biópsias de carcinoma de células escamosas de boca e orofaringe (SYRJÄNEN 1983). A associação entre a infecção pelo Papiloma Vírus Humano e o câncer de orofaringe foi confirmada em 2007 (D'SOUZA et al. 2007).

É observado que a prevalência do CEC de orofaringe relacionado ao HPV é diametricamente proporcional à prevalência do CEC de colo uterino relacionado ao

HPV em países desenvolvidos, comparando com países em desenvolvimento (DE MARTEL et al. 2017; PAN et al. 2018). Alguns estudos apontam que a infecção por HPV ultrapassou o tabaco e álcool como fator de risco mais prevalentes sobre o CEC de orofaringe nos Estados Unidos (MARUR et al. 2010; GILLISON et al. 2015; VOKES et al. 2015). Entretanto, ainda é observada menor prevalência de CEC de orofaringe associado ao HPV no Brasil em comparação a outros centros mundiais como a Europa e os Estados Unidos (LOPEZ et al. 2014; MATOS et al. 2015; BETIOL et al. 2016; DE PETITO et al. 2017).

Foi observado que os pacientes com carcinoma de células escamosas de orofaringe associados ao HPV apresentam padrão epidemiológico, histológico, apresentação clínica e resultados terapêuticos distintos ao CEC de orofaringe HPV negativos (CHUNG e GILLISON 2009; GILLISON et al. 2008, 2015). Os tumores HPV positivos se apresentam em pacientes mais jovens, entre 4a e 5a décadas de vida (GILLISON et al. 2008; YOUNG et al. 2015). Eles apresentam comportamento sexual de risco, melhores condições sócio-ecônomicas, geralmente não são tabagistas ou etilistas (GILLISON et al. 2008; YOUNG et al. 2015; CHATURVE et al. 2016) e ocasionalmente observa-se associação com o uso de maconha (DAHLSTROM et al. 2013; GILLISON et al. 2015). Sua histologia apresentar padrão basalóide, diferindo do padrão queratinizado encontrado nos CEC de orofaringe não associados com o HPV (MARUR e FORASTIERE 2008). Observa-se também maior incidência de metástases linfonodais ao diagnóstico nos pacientes com tumores HPV positivos (CHATURVEDI et al. 2013). Apesar da maior incidência de metástases regionais, os pacientes com tumores HPV positivos têm apresentado melhores resultados oncológicos, com taxas de sobrevida global e sobrevida livre de doença superiores aos pacientes com tumores

HPV negativos (ANG et al. 2010). Esta diferença de prognóstico dos pacientes com CEC de orofaringe HPV-positivos em relação aos HPV-negativos foi determinante para recentes mudanças ocorridas na classificação da *American Joint Comission on Cancer* (AJCC), em relação ao estadiamento dos tumores de orofaringe, pela primeira vez dividindo o estadiamento conforme status do HPV (O'SULLIVAN et al. 2016; AMIN et al. 2017; LYDIATT et al. 2017; MIZUMACHI et al. 2017).

1.4 DIAGNÓSTICO DO CÂNCER DE OROFARINGE RELACIONADO AO HPV

Atualmente não existe nenhum teste único que seja padrão para o diagnóstico do HPV nas neoplasias de cabeça e pescoço. Alguns reproduzem satisfatoriamente a atividade viral no hospedeiro e/ou têm maior custo-efetividade, enquanto existem testes que apesar de alta sensibilidade e especificidade, atualmente são tecnicamente difíceis de reprodução, portanto não aplicáveis à prática clínica. De acordo com o estadiamento da AJCC, em sua oitava edição, a imunohistoquímica com avaliação da expressão maior que 70% do p16, é suficiente para classificar o tumor como HPV-positivo.

1.4.1 Detecção do DNA do HPV por reação em cadeia de Polimerase (PCR)

A amplificação por PCR do DNA do HPV é uma tecnologia de amplificação alvo capaz de amplificar seqüências de DNA vestigiais em uma amostra biológica. Trata-se de método com alta sensibilidade, que pode ser obtido através de amostra fresca, parafinada, congelada ou até mesmo de fluidos corporais, como saliva e plasma.

Pode detectar o subtipo do HPV envolvido na tumorigênese, e, além da alta sensibilidade, tem custo-efetividade favorável e pode ser aplicado facilmente na prática clínica. Como desvantagens, apresenta baixa sensibilidade, além do fato de que os métodos baseados em PCR não permitem distinguir se o HPV atuou como fator de transformação maligna, ou foi somente um vírus transcricionalmente silencioso, sem nenhum papel no processo de tumorigênese. Além disso, as amostras clínicas são muito sujeitas a contaminação cruzada durante sua análise, necessitando de cuidado especial para evitar falsos positivos nas amostras (WESTRA et al. 2014).

1.4.2 Detecção do RNA do HPV por PCR

Este método, que avalia a detecção do RNA mensageiro (mRNA) de E6 e E7, é considerado o padrão ouro para caracterização do HPV clinicamente relevante, possuindo alta sensibilidade e especificidade. Entretanto, sua análise através de amostras frescas ou congeladas é um desafio do ponto de vista técnico para aplicação na prática clínica diária, estando restrito a laboratórios de pesquisa atualmente (VENUTI et al. 2012).

1.4.3 Hibridização *in situ* do HPV

A Hibridização *in situ* do DNA do HPV é uma tecnologia de amplificação de sinal que utiliza sondas de DNA marcadas complementares às sondas de DNA viral direcionadas. Tais sondas podem hibridizar com sequências de DNA específicas por tipo de HPV ou por conjunto de subtipos de vírus a serem pesquisados. A partir de tecidos frescos ou parafinados, é método que possui alta especificidade. Entretanto o fato de não se analisar o tecido em si, pode ocasionar leituras de HPV em tecidos não

tumorais vizinhos ou mesmo em falso positivos, não permitindo diferenciação entre ambos (WESTRA et al. 2014).

A Hibridização *in situ* do RNA do HPV tem se mostrado tão sensível quanto a expressão imunohistoquímica do p16 e da análise do DNA do HPV. A sua reprodutibilidade na prática clínica é possível, além de confirmar a presença do vírus transcricionalmente ativo. É considerado método promissor nos diagnósticos de carcinoma HPV-relacionado (WESTRA et al. 2014).

1.4.4 Expressão da proteína p16 através de imunohistoquímica

A análise da expressão da proteína p16 através de imunohistoquímica é considerada ao mesmo tempo exame prático e com alta correlação entre sua alta expressão com a presença de atividade do HPV nos carcinomas de orofaringe. A sua avaliação pode ser realizada através de amostras frescas, congeladas, em parafina, ou mesmo através de análise imunocitoquímica em análises de punções aspirativas, o que facilita sua aplicabilidade clínica. Entretanto para ser verdadeiramente útil como marcador de infecção pelo HPV, sua interpretação deverá ser informada quanto a presença de forte coloração (maior a 70%) e de padrão nuclear. Sua aplicabilidade fica restrita a sítios que não fazem parte da orofaringe(WESTRA et al. 2014; VENUTI et al. 2012).

A AJCC em sua oitava edição, dividiu o estadiamento dos carcinomas de células escamosas de orofaringe em HPV-positivo e HPV-negativo, baseados na análise da expressão da proteína p16.

1.5 TRATAMENTO DO CARCINOMA DE CÉLULAS ESCAMOSAS DE OROFARINGE

Nas últimas décadas, houveram mudanças importantes no paradigma do tratamento do carcinoma de células escamosas de orofaringe (O'SULLIVAN et al. 2013; LING et al. 2016). Em tumores diagnosticados em estádios precoces, a monoterapia, englobando tanto a ressecção cirúrgica quanto a radioterapia, oferecem resultados oncológicos comparáveis (SHIN et al. 2009; DE ALMEIDA et al. 2014). Nos casos diagnosticados com tumores em estádios III e IV as cirurgias radicais, englobando mandibulotomias, mandibulectomias ou acessos por faringotomia seguidas de radioterapia (DENITTIS et al. 2001; WEINSTEIN et al. 2010; BASTOS DE SOUZA et al. 2014; deram espaço ao tratamento associando radioterapia concomitante a quimioterapia baseada em platina, que demonstram resultados oncológicos semelhantes, associados a menor morbidade relacionada ao tratamento (ALLAL et al. 2003; MEHTA e HARRISON 2007; BERMAN e SCHILLER 2017). Os pacientes com tumores HPV-positivos apresentam taxas de sobrevida global superiores a 80% em algumas séries (ANG et al. 2010). Novos desafios surgiram após a instituição desta modalidade terapêutica, como a toxicidade relacionada ao tratamento, e distúrbios tardios de deglutição com necessidade de uso de dieta enteral e gastrostomias permanentes, o que afeta significativamente a qualidade de vida destes pacientes (LANGENDIJK et al. 2008; RUSTHOVEN et al. 2008; VOLKENSTEIN et al. 2015).

Nos últimos anos entretanto, as cirurgias com acesso via oral, como o *Transoral Robotic Surgery* (TORS) (MOORE et al. 2012; WEINSTEIN et al. 2010,

2012; FORD et al. 2014) e o *Transoral Laser Surgery* (TOLS) (MOORE et al. 2009; RICH et al. 2009) têm ganhado espaço, principalmente para tratamento de tumores iniciais (LEE et al. 2014), e posteriormente para alguns casos selecionados com lesões localmente avançadas (MOORE et al. 2009; WEINSTEIN et al. 2010, 2012; FORD et al. 2014), com menor índice de complicações tardias de deglutição e resultados oncológicos semelhantes. Modalidades de radioterapia mais recentes, como a Radioterapia de Intensidade Modulada (DE ARRUDA et al. 2006) (IMRT), terapia de Prótons, também tem contribuído para a diminuição destas complicações (DE ARRUDA et al. 2006; RUSTHOVEN et al. 2008; DALY et al. 2010; O'SULLIVAN et al. 2012).

Devido ao melhor prognóstico encontrado nos pacientes com tumores HPV-positivos, foram propostas estratificações de risco, baseados no status do HPV, tabagismo (ANG et al. 2010; GILLISON et al. 2012) e estadiamento TNM, com o objetivo de identificar grupos que poderiam se beneficiar da desintensificação do tratamento. Essas informações foram a base para o início de estudos com pacientes classificados como portadores de tumores de baixo risco para recorrência utilizando-se menores doses de radioterapia, diminuição de campo irradiado e diminuição, ou até suspensão do uso da quimioterapia nestes grupos (O'SULLIVAN et al. 2013).

A ocorrência de metástases a distância é algo a ser melhor entendido em pacientes portadores de carcinoma de orofaringe HPV-positivos. Apesar do conhecimento sobre o melhor prognóstico deste grupo, as taxas de metástases a distância são as mesmas tanto para os casos HPV-positivos, como para os HPV-negativos (GOODWIN 2001; HUANG et al. 2012, 2013; O'SULLIVAN et al. 2012). Há relato de ocorrência de metástases a distância em locais não habituais no grupo de

pacientes com tumores HPV-positivos, e depois de longos intervalos de tempo (HUANG et al. 2012). Apesar da taxa de recorrência locorregional encontrada nestes pacientes ser baixa, as metástases a distância são a causa mais frequente de morte nos pacientes portadores de carcinoma de células escamosas de orofaringe relacionados ao HPV (QUON e FORASTIERE 2013).

Este conhecimento acerca do padrão de recorrência destes tumores, com possíveis implicações terapêuticas e no planejamento do seguimento pós tratamento, tem sido ainda pouco explorado na literatura. Além disso, devido à baixa prevalência deste tipo de neoplasia na população brasileira, pouco foi e acerca do real impacto que a infecção do HPV tem no prognóstico do CEC de orofaringe nesta região geográfica.

2 OBJETIVOS

2.1 OBJETIVO PRINCIPAL

Analisar os padrões de recorrência local, regional e à distância dos carcinomas de células escamosas de orofaringe relacionados ao HPV e comparar com os padrões observados em pacientes com tumores HPV negativos.

2.2 OBJETIVO SECUNDÁRIO

Analisar o impacto das variáveis epidemiológicas, clínicas, anatomopatológicas e terapêuticas estudadas na sobrevida livre de doença e sobrevida livre de recorrência dos casos analisados.

3 ARTIGO

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Impact of human papillomavirus (HPV) status on survival and recurrence in a geographic region with a low prevalence of HPV-related cancer: A retrospective cohort study

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Key words: oropharynx, HPV, prognosis, tobacco, survival

Abstract

Background: Human papilloma virus (HPV)-positive oropharyngeal squamous cell carcinoma (OPSCC) is associated with better tumor-response rates and survival outcomes. However, in some geographic regions with a lower prevalence of HPV-positive OPSCC, the impact of HPV infection on prognosis remains unclear. The aim of this study was to describe the patterns of recurrence and survival among patients treated for OPSCC in a geographic region with a reported low prevalence of HPV-related OPSCC.

Methods: We retrospectively evaluated 215 patients diagnosed with American Joint Committee on Cancer (AJCC) stages I to IV (with no distant metastases) OPSCC who were treated with upfront surgery or radiation therapy with or without chemotherapy in a single tertiary Cancer Center in Brazil. The collected data included demographic information, HPV status, tobacco and alcohol consumption, and pathologic and treatment variables. The patterns of recurrence were recorded according to HPV status. Disease- specific survival and recurrence-free survival were calculated using Kaplan-Meier curves and multivariate Cox regression analysis.

Results: One hundred twenty-seven (59.1%) patients were diagnosed with HPV-positive OPSCC; the median patient age was 56 years. The tonsils were the most frequent site (131 cases) in both the HPV-positive (n=78, 59.5%) and HPV-negative (n=53, 40.5%) groups. According to the AJCC eighth edition, 34 (15.8%), 71 (33%), 47 (21.9%), and 60 (27.9%) patients had stage I, II, III, and IV disease, respectively. Surgery was initially performed in 109 (50.7%) cases, and upfront chemoradiation regimens were provided as the initial treatment options in 104 (48.4%, p=0.686) patients. Overall, the 5-year cancer- specific survival was 73.5% and 68.1% for HPV-positive and HPV-negative patients, respectively; this difference was not significant (p=0.227). Tobacco status was considered the only independent prognostic factor for survival. Furthermore, HPV status was not associated with differences in recurrence rates (p=0.680). While all distant relapses were found to be lung metastases in the HPV-negative group, we observed unusual sites of distant metastases in the HPV-positive group; five patients presented with liver metastasis, four with bone metastasis, one with skin implants, and one with central nervous system (CNS) disease.

Conclusions: HPV status was not associated with higher rates of survival among the investigated population. Moreover, smoking status was considered the only independent prognostic factor for survival. Furthermore, patients with HPV-positive tumors were more likely than patients with HPV-negative OPSCC to have unusual distant metastases.

Introduction

Globally, the incidence of oropharyngeal squamous cell carcinoma (OPSCC) is 115,131 cases per year, and an estimated 77,598 deaths occurred in 2015 as a result of OPSCC.[1] Although tobacco and alcohol consumption are still known as the most frequent causes of most head and neck squamous cell carcinoma (HNSCC) cases,[2] infection with human papillomavirus (HPV) plays an important role in OPSCC carcinogenesis.[3] Despite the decreasing prevalence of smoking and alcohol consumption from the 1990s onwards,[4] which has led to a decrease in the incidence of most HNSCC, evidence shows an increase in the incidence of HPV-related OPSCC during the same period.[5] Additionally, epidemiologic evidence demonstrates the role of HPV infection in the carcinogenesis of OPSCC, and currently, HPV is the most common risk factor for OPSCC in the United States.[6-9]

Moreover, the increasing incidence of HPV-related OPSCC over the last few decades has led to the discovery of a "new" disease with a different epidemiology, which has distinct clinical and pathological features.[10,11] HPV-related OPSCC commonly presents in younger[12] patients who are non-smokers; the typical clinicopathological presentation is that of a basaloid pattern (differing from the keratinized SCC presentation in HPV-negative patients)[9,13] accompanied by a higher incidence of regional lymph node metastases. However, HPV-related OPSCC has also been associated with a better tumor response to systemic and local therapies as well as improved disease-free and overall survival compared with patients harboring HPV-unrelated tumors. Such clinical evidence was the basis for the significant modifications that appeared in the most recent tumor, node, metastasis (TNM) classification and for the clinical trials investigating the de-intensification protocols for HPV-positive OPSCC.[14-19]

In some geographic regions, the relationship between HPV and OPSCC has not been clearly established. Recent data from Brazil shows that the prevalence of HPV associated with OPSCC ranges from 5.6-25.6%,[20-22] which is in contrast to the 85% observed in Sweden.[23] Moreover, the role of HPV infection on clinical outcomes in these low HPV-OPSCC-prevalent regions remains unclear. The objective of this study was to describe the clinical patterns of recurrence and survival among patients treated

for HPV-positive OPSCC in Brazil, a geographic region with a reported low prevalence of HPV- related OPSCC.

Materials and methods

Study design and patients

We retrospectively collected data from 215 patients treated for OPSCC at the AC Camargo Cancer Center in São Paulo, Brazil from 1984 to 2014. Patients were considered eligible for inclusion in the study if: they were treated for OPSCC (tonsil, base of tongue, soft palate, uvula, and posterior wall) with a curative intention, if their tumor tissues were tested for HPV at the time of treatment, or if they were able to provide tumor tissues for HPV investigation if they had not been previously tested. Patients were excluded from our study if they received palliative treatment, had distant metastases at admission, or if they did not have tissues available for HPV investigation.

Study procedures

HPV DNA detection and/or p16 immunochemistry was used for HPV diagnosis. DNAs extracted from formalin-fixed paraffin-embedded tissues were subjected to semi-automated HPV genotyping using the INNO-LiPA HPV Genotyping Extra Amp kit (Fujirebio, USA) and AutoBlot-3000H equipment (MedTec Biolab Equipment, USA). A positive hybridization to HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, or 82 defined cases with high-risk HPV DNA. p16 was considered positive when high expression (more than 75% cells stained) of $p16^{INK4a}$ in immuno-stained sections was observed.

Outcome measures

Follow-up time was defined as the time from treatment to the first relapse date for recurrence-free survival, and as the time from treatment to the date of cancer related-death for cancer-specific survival. Patients who were followed-up for at least 18 months were eligible for this study.

Data collection

Collected data included demographics (gender, age), clinical factors (performance status, comorbidities, tobacco, alcohol [more than 10 g daily], hemoglobin levels, body mass index), HPV status, stage according to the seventh and eighth editions of the American Joint Committee on Cancer (AJCC) TNM staging system, pathologic factors (number of metastatic lymph nodes, surgical margins, tumor grade, perineural invasion, vascular invasion, and extracapsular spread) and treatment characteristics (surgery, neck dissection, or chemoradiation regimen). The patterns of local, regional, and distant recurrence were recorded and correlated to HPV status and clinic and epidemiological characteristics to analyze differences between the groups.

Data analysis

SPSS version 23 (SPSS®, Inc.; Illinois, USA) was used for the statistical analysis. HPV prevalence and its coinciding 95% confidence intervals (CI) were calculated; patients were categorized as being HPV-positive or HPV-negative. Differences in epidemiological, clinical, pathological, and treatment variables based on HPV status were calculated using chi-squared tests for categorical variables and Student's t-test for continuous variables. Disease-specific survival and recurrence-free survival were calculated using Kaplan-Meier curves, and log-rank tests were used for comparisons between variables. Finally, a multivariate survival analysis was performed using a Cox proportional hazard regression model. We divided our cohort into three groups (all cases, cases managed with upfront surgical resection, and cases managed with upfront non- surgical resection) before performing the regression models. We then determined the variables that were only present in patients treated with upfront surgical resection. Factors that were statistically significant in the univariate analysis were included in the multivariate analysis. P-values <0.05 were considered statistically significant.

Ethics

The study procedures were approved by the AC Camargo Cancer Center Ethics Committee (#2202/16). Informed consent was waived due to the retrospective nature of the study.

Results

The study population consisted of 215 patients, the majority of whom were males (n=190, 88.4%), and the median age was 56 years. In all, 127 (59.1%) patients were diagnosed with HPV-positive OPSCC. The majority of the patients included did not report any associated comorbidities (n=169, 78.6%); however, cardiovascular disease was the most frequent comorbidity among those who reported their comorbidities (n=24, 11.0%). Most patients reported tobacco consumption (n=130, 60.5%), while the use of alcoholic beverages was reported by 103 (40.9%) patients. The tonsils were the most frequent site (131 cases) for both HPV-positive (n=78 59.5%) and HPV-negative (n=53, 40.5%) patients, followed by the base of the tongue (n=64, 29.8%), the soft palate (n=14, 6.5%), the uvula (n=3, 1.4%), and the posterior wall (n=2, 0.9%). Moreover, the majority of patients in both the HPV-negative and HPV-positive groups presented with locally advanced stage III to IVa/b disease, classified as cT3 (n=31, 35.2% in the HPV-negative and n=50, 40.0% in the HPV-positive group, p=0.580) and cT4 (n=27, 30.7% in the HPV-negative and n=28, 22.4% in the HPV-positive group, p=0.580) (Table 1). When we compared epidemiological and clinical variables based on HPV status (Table 1), a significant difference was observed between the groups based on tobacco ($p<0.01$) and alcohol consumption (p=0.023). Patients who were HPV-negative consumed more tobacco (n=65, 80.2%) and alcohol (n=50, 61.7%) than HPV-positive patients.

According to the eighth edition of the AJCC staging system, 34 (15.8%), 71 (33%), 47 (21.9%), and 60 (27.9%) patients had stage I, II, III, and IV disease, respectively (Table 1).

Surgery was initially performed in 109 (50.7%) patients, while chemoradiation regimens were the initial treatment option in 104 (48.4%) patients. Between 1984 and 2000, all cases in this study underwent surgical resection. Moreover, between 2001 and 2014, 66.2% of the patients received chemoradiation and 32.5% received surgery as the primary treatment.

No significant difference was observed in the treatment option based on HPV status (p=0.686). Despite that surgical treatment was the primary option for initial local disease (n=42, 54.5% for T1-T2) and that non-surgical therapies were preferred for locally advanced tumors (n=71, 52.2% for T3-T4), this difference was not significant

(p=0.393). However, we observed differences in treatment according to N classification (p<0.01), presence of comorbidities (p<0.01), and tobacco and alcohol consumption (p<0.01) (Table 5). Primary neck dissection was performed in 106 cases, while salvage neck dissection was performed in five cases. Nearly half of the pathology reports described a moderate differentiation grade (n=107, 49.8%). In patients who underwent surgical treatment, free margins, close margins, and positive margins were obtained in 85 (76.6%), 11 (9.9%), and 15 (13.5%) specimens, respectively. Vascular invasion, perineural invasion, and extracapsular spread were identified in 14 (13.6%), 33 (31.1%), and 36 (48.6%) patients, respectively, who underwent neck dissection. No difference was observed between the distributions of pathological variables based on HPV status.

The median follow-up time was 47 months (interquartile range [IQR] 43-53). Among the HPV-negative patients, 8 (9.3%), 12 (14%), and 12 (14.0%) had local recurrences, regional relapses and distant metastasis, respectively. Among HPV-positive patients, 8 (6.5%), 13 (10.5%), and 16 (12.9%) had local recurrences, regional relapses and distant metastasis, respectively. The patterns of recurrence did not significantly differ between the groups (p=0.680). The mean time until recurrence was 45.1 months (95% CI 35.6-54.5) and 50.6 months (95% CI 42.1-60.1) in the HPV-negative and HPV-positive groups, respectively; this time was not significantly different (p=0.42). When we compared the sites of distant metastasis, all 12 HPV-negative patients had pulmonary distant disease, while in HPV-positive patients, five had liver metastasis, four had bone metastasis, one had skin implants, and one had central nervous system disease (Table 2).

The overall median disease-specific survival was 113.8 months (95% CI 75.9 - 151.7) and the 5-year disease-specific and recurrence-free survival was 71.5% and 64.0%, respectively. The 5-year disease-free survival of patients with HPV-negative versus HPV-positive OPSCC was 68.5% and 73.1%, respectively (p=0.227). When we used the stratification proposed by Ang et al.[24] we observed a significant difference in the survival rates between groups, particularly in those who smoked (p=0.023)(Table 3). After the whole cohort was analyzed by univariate analysis using a Cox regression model (Table 4), we found that tobacco use (hazard ratio [HR] 5.37, 95% CI 2.14-13.42), alcohol consumption (HR 3.02, 95% CI 1.66-5.84), T stage (HR 1.35, 95% CI

1.02-1.78), and Karnofsky's performance status (KPS) (HR 2.56, 95% CI 1.14-5.88) were significantly associated with higher death risk. However, only tobacco use (HR 5.14, 95% CI 1.05-25.00, p=0.04) was considered an independent prognostic factor for disease-specific survival in the multivariate analysis. Moreover, after an analysis of the surgical cases by univariate analysis (Table 4), we found that smoking tobacco (HR 3.68, 95% CI 1.13-12.00), alcohol intake (HR 2.39, 95% CI 1.11-5.16), N stage (HR 1.22, 95% CI 1.04-1.43) and number of positive lymph nodes (HR 1.26, 95% CI 1.12-1.46) were statistically associated with higher death risk, while the number of metastatic nodes (HR 1.27, 95% CI 1.07-1.50) was considered the only independent prognostic factor for disease-specific survival according to a multivariate analysis for this group. Furthermore, as for the whole cohort, in the cases treated without surgery (Table 4), only tobacco use (HR 5.29, 95% CI 1.04-28.83 p=0.04) was considered as independent prognostic factor for disease-specific survival in the multivariate analysis.

Discussion

Previous studies suggest that HPV-related OPSCC is associated with improved oncologic outcomes compared with HPV-negative tumors. Epidemiologic features such as age, smoking status, and alcohol consumption differ for HPV-positive and HPV-negative patients. Furthermore, the high prevalence of HPV-related OPSCC has been widely described during the last decade in Europe and the United States. However, in some geographic regions such as Brazil, the prevalence and epidemiological and clinical characteristics of HPV-related OPSCC seem to differ from those reported during the last several decades in Europe and the United States.[22] In 2014, Lopez at al.[21] described the prevalence of HPV-positive OPSCC as 10.5% in a Brazilian population. Moreover, in 2016, Betiol et al.[20] found that the prevalence of HPV-related OPSCC was 17.7% in a tertiary Cancer Center in São Paulo, while Petito et al.[25] found that 25.6% of OPSCC patients tested positive for HPV. Furthermore, a systematic review of HPV infection in the Brazilian population found a 27.4% HPV positivity rate amongst patients with oral and OPSCC.[22] In the current study, an HPV positivity rate of 59.1% was found, which was much higher than the prevalence rates reported in previous regional studies. This result might be explained in that previous studies were conducted at public institutions, which treat patients with a lower

socioeconomic status, while our hospital mainly provides private care. Despite the lower prevalence of HPV positivity in Brazil compared with American and European studies, we observed a slow growth in the incidence of HPV-related OPSCC over the last decade.[20-22,25]

We also found a higher prevalence of tobacco (55.1%) and alcohol (40.9%) consumption among patients with HPV-related OPSCC compared with findings in recent American studies.[4,11] A significant difference was observed in the distribution of smokers, according to HPV status; 55.1% and 80.2% were HPV-positive and HPV-negative, respectively. However, the prevalence of smoking status even in our HPV-positive OPSCC group was much higher than what is usually observed in America.[11] Additionally, 45.7% and 61.7% of HPV-positive and HPV-negative patients, respectively, reported alcohol consumption. Although we observed an overall decreasing prevalence in alcohol and tobacco intake, the prevalence remains very high amongst patients with OPSCC in the Brazilian population; according to a study conducted by de Matos et al.[22] the prevalence of smoking and alcohol consumption was 87.8% and 75.2%, respectively.

In our cohort, we observed no difference in the age and sex distribution based on HPV status. Furthermore, the mean age of patients in the HPV-positive group was slightly higher than that of patients in the HPV-negative group. These findings differed from the studies conducted by Marur et al.[6] and Gillison et al.[11,26] which found that HPV was related to OPSCC in younger patients. Based on our findings, we believe that in some geographic regions such as Brazil, epidemiologic features of HPV-related OPSCC may not differ from those of HPV-negative OPSCC, as they have been reported to be widely related in North America and Europe over the last several decades.[6,11,23]

Importantly, the definition of HPV status differs among studies; in the current study, we used p16 and/or HPV DNA positivity. The available evidence suggests that 70% staining by p16 immunohistochemistry followed by high-risk HPV DNA positivity is the gold standard for a diagnosis of HPV-associated OPSCC.[27-29] However, for HPV analysis, only p16 immunochemistry is recommended in the eighth edition of the AJCC TNM classification. Furthermore, strong p16 staining is considered a surrogate marker for HPV-related OPSCC.[24,30] Since we conducted a

retrospective study that used data from previous studies, we did not use one single method; this may be a limitation when considering the HPV DNA positivity of HPV-related OPSCC. However, when we stratified the cohort based on the HPV detection methods, we did not observe differences in survival, regardless of the method used for detection.

Although epidemiological data support that there is a higher risk of regional metastasis in patients with HPV-positive OPSCC,[9-11] no evidence in the literature has indicated that extracapsular spread has a significant impact on survival among patients with HPV-positive tumors,[16] which was also supported by our findings. Although we found that N stage was not related to survival according to the multivariate analysis, the number of positive lymph nodes was considered an independent prognostic factor for survival (Table 4).

When we compared staging according to the seventh and eighth editions of the AJCC TNM classification, 86.5% of patients were determined to have stage III and IV disease according to the seventh edition, while 49.8% had stage III and IV disease according to the eighth edition. A study conducted by Zhan et al.[31] suggested that HPV positive patients are “down staged” in the new edition compared with the AJCC seventh edition. We did not see a significant difference in the 5-year disease-specific survival between the two staging groups. However, we observed a difference in cancer-specific survival and recurrence-free survival when we compared tobacco status (Table 3).

Between 1984 and 2000, all included cases underwent surgical resection, which can be explained by the fact that surgery was considered the primary treatment for oropharyngeal cancer at our institution. Moreover, HPV testing was not performed routinely at that time. Despite the lack of a difference in HPV status between the 1980-1990s and 2000-2010s (p=0.194), we observed significant differences in the 5-year disease-specific survival (from 1984-2000 was 61.4% and from 2001-2014 was 76.6%, p=0.01).

The patterns of recurrence did not differ based on the type of recurrence when HPV status was analyzed. However, our findings were similar to those of Huang et al.[32] who found unusual sites of distant metastasis (i.e., skin, liver, and central nervous system disease) in HPV-positive patients.

Disease and recurrence-free survival were not affected by HPV status (Figure 1), as mentioned in Table 4. However, both disease- and recurrence-free survival were significantly affected by tobacco intake when we used the stratification methods proposed by Ang et al.[24] and the Kaplan-Meier methods (Figure 2). Moreover, we observed that patients who smoked had lower disease- and recurrence-free survival rates (Figure 2). According to the Cox regression models we performed, HPV status was not significantly associated with higher death risk in either the univariate or multivariate analysis. However, tobacco intake was considered a significant independent prognostic factor in the multivariate analysis for disease-specific survival; thus, tobacco use was related to a higher risk of cancer-related death than HPV status itself.

This study has several limitations. First, this was a retrospective study conducted at a single institution, thus limiting the validity and generalizability of our findings. The heterogeneity found in our study population was obvious treatment selection bias over time. That is, because data were collected from 1984 to 2014, our study includes patients from the surgical era (1980s and 1990s) in which all patients were treated surgically; the chemoradiation era (2000s); and the TORS era (2010s), each with significant differences in survival. Hence, we need different approaches to the same disease since each approach may lead to different overall results.

Conclusions

Our study suggests that the burden of HPV-related OPSCC may be increasing in the Brazilian population according to recent data[20-22,25] and the HPV-positive OPSCC prevalence. However, HPV status in OPSCC patients was not associated with distinct epidemiological features, with the exception of tobacco and alcohol consumption, in the studied population. Furthermore, HPV status was not associated with higher survival rates. Moreover, tobacco use was considered an independent prognostic factor for disease-specific survival, regardless of HPV status. Finally, patients with HPV-positive OPSCC were more likely to present with unusual distant metastasis.

Disclosure statement

The authors have no conflicts of interest to declare

Abbreviations

AJCC: American Joint Committee on Cancer

CI: confidence interval

HPV: human papilloma virus

OPSCC: oropharyngeal squamous cell carcinoma

TNM: tumor-node-metastasis classification

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Table 1. Epidemiological, clinical, and pathological distributions stratified by human papilloma virus status

Variable	HPV - (%)	HPV + (%)	p-value
Male	79 (89.8%)	111 (87.4%)	0.379
Female	9 (10.2%)	16 (12.6%)	
Mean age	54.6	56.2	0.500
Tobacco	65 (80.2%)	65 (55.1%)	**<0.01**
Alcohol	50 (61.7%)	53 (45.7%)	**0.019**
KPS - mean	88	90	0.233
Hemoglobin	14.0	14.3	0.257
CV disease	7 (8.0%)	17 (13.4%)	0.153
Diabetes	5 (5.7%)	8 (6.3%)	0.548
COPD	5 (5.7%)	6 (4.7%)	0.493
Hepatopathy	2 (2.3%)	2 (1.6%)	0.542
Nephropathy	2 (2.3%)	0 (0.0%)	0.166
Site			
Tonsil	53 (60.2%)	78 (61.9%)	
Base of tongue	25 (28.4%)	39 (31.0%)	
Palate	7 (8.0%)	7 (5.6%)	0.833
Posterior Wall	2 (2.3%)	1 (0.8%)	
Uvula	1 (1.1%)	1 (0.8%)	
Pathological Grade			
I	30 (34.9%)	52 (41.9%)	
II	48 (55.8%)	59 (47.6%)	0.568
III	8 (9.3%)	12 (9.7%)	
Margins			
Free	33(73.3%)	52 (78.8%)	0.799
Insufficient	5 (11.1%)	6 (9.1%)	
Positive	7 (15.6%)	8 (12.1%)	
Vascular invasion			
Negative	34 (85.0%)	55 (87.3%)	0.479
Positive	6 (15.0%)	8 (12.7%)	
Perineural invasion			
Negative	27 (65.9%)	46 (70.8%)	0.374
Positive	14 (34.1%)	19 (29.2%)	
ECS			
Negative	12 (46.2%)	26 (54.2%)	0.339
Positive	14 (53.8%)	22 (45.8%)	
Stage			
cT1	10 (11.4%)	14 (11.2%)	0.580
cT2	20 (22.7%)	33 (26.4%)	
cT3	31 (35.2%)	50 (40.0%)	
cT4	27 (30.7%)	28 (22.4%)	
cN0	24 (27.3%)	32 (25.6%)	**<0.01**
cN1	15 (17.0%)	70 (56.0%)	
cN2	36 (40.9%)	13 (10.4%)	
cN3	13 (14.8%)	10 (8.0%)	
Clinical Stage			
I	6 (6.8%)	28 (22.6%)	
II	8 (9.1%)	63 (50.8%)	**<0.01**
III	14 (15.9%)	33 (26.6%)	
IV	60 (68.2%)	0 (0.0%)	

Abbreviations: HPV, human papilloma virus; CI, confidence interval; CV, Cardiovascular; COPD, chronic obstructive pulmonary disease; KPS, Karnofsky performance scale; BOT, base of tongue; ECS, extracapsular spread.

Table 2. Patterns of recurrence stratified by human papilloma virus status

	HPV-negative N (%)	HPV-positive N (%)	p-value
Recurrences			
Local	8 (9.3%)	8 (6.5%)	
Regional	12 (14%)	13 (10.5%)	0.680
Distant	12 (14%)	16 (12.9%)	
Distant Metastasis			
Lung	12 (100%)	9 (45.0%)	
Liver	0 (0.0%)	5 (25.0%)	
Bone	0 (0.0%)	4 (20.0%)	**0.018**
Skin	0 (0.0%)	1 (5.0%)	
CNS	0 (0.0%)	1 (5.0%)	

Abbreviations: HPV, human papilloma virus; CNS, central nervous system

Table 3. Cox's univariate and multivariate analyses - cancer-specific survival

Univariate	HR	95% CI		p-value	Multivariate	HR	95% CI		p-value
All cases									
Age	1.016	0.99	1.04	0.20					
Sex	0.88	0.40	1.94	0.76					
Comorbidities	0.98	0.48	2.01	0.97					
Tobacco	5.37	2.14	13.42	**0.01**		5.14	1.05	25.00	**0.04**
Alcohol	3.021	1.66	5.84	**0.01**		1.67	0.58	4.82	0.33
KPS	0.93	0.89	0.97	**0.02**		0.97	0.93	1.02	0.34
Hemoglobin	0.737	0.74	1.52	0.73					
T stage	1.35	1.02	1.78	**0.03**		1.55	0.92	2.60	0.09
N stage	1.11	0.97	1.28	0.13					
HPV	0.74	0.46	1.20	0.22					
Surgical cases									
Age	0.99	0.97	1.03	0.88					
Sex	0.90	0.38	2.12	0.80					
Comorbidities	0.72	0.09	5.34	0.75					
Tobacco	3.68	1.13	12.0	**0.03**		2.70	0.69	10.60	0.15
Alcohol	2.39	1.11	5.16	**0.02**		1.42	0.58	3.49	0.43
KPS	0.72	0.24	2.06	0.52					
Hemoglobin	1.19	0.26	5.32	0.81					
T stage	1.26	0.92	1.73	0.14					
N stage	1.22	1.04	1.43	**0.01**		1.16	0.97	1.38	0.10
HPV	0.65	0.37	1.15	0.14					
Number LN	1.28	1.12	1.46	**0.01**		1.27	1.07	1.50	**0.01**
Grade	1.25	0.87	1.81	0.22					
Margins	1.28	0.92	1.78	0.14					
Vascular inv	0.51	0.24	1.08	0.08					
Perineural inv	1.58	0.88	2.83	0.12					
ECS	0.03	0.01	18.97	0.29					
Non-Surgical cases									
Age	1.06	1.01	1.10	**0.01**	1.04	0.99	1.09	0.08	
Sex	0.51	0.07	3.90	0.52					
Comorbidities	1.56	0.61	3.97	0.34					
Tobacco	8.77	2.01	38.18	**0.01**	5.29	1.04	28.83	**0.04**	
Alcohol	3.57	1.33	9.58	**0.01**	1.40	0.48	4.10	0.53	
KPS	0.93	0.90	0.98	**0.01**	0.97	0.93	1.02	0.37	
Hemoglobin	1.08	0.75	1.56	0.67					
T stage	1.68	0.96	2.93	0.06					
N stage	0.89	0.65	1.22	0.47					
HPV	1.07	0.41	2.79	0.87					

Abbreviations: HR, hazard ratio; CI, confidence interval; HPV, human papilloma virus; Inv, invasion; ECS, extracapsular spread

Table 4. Five-year survival stratified by human papilloma virus and tobacco status

	5-year cancer specific survival %	p-value	5-year recurrence-free survival %	p-value
HPV -	68.5	0.22	58.9	0.24
HPV+	73.1		66.7	
Tobacco - / HPV -	100	0.01	100	0.01
Tobacco - / HPV +	93.2		82.9	
Tobacco + / HPV -	65.3		55.6	
Tobacco + / HPV +	58.2		53	

Table 5. Differences in the characteristics of patients treated surgically vs nonsurgically

Variable	Non-surgical treatment (%)	Surgical treatment (%)	p-value
Male	93 (87.7)	97 (89.0)	0.47
Female	13 (12.3)	12 (11.0)	
Comorbidities	38 (35.8)	8 (7.3)	**0.01**
Tobacco	55 (52.4)	75 (79.8)	**0.01**
Alcohol	42 (40.4)	61 (65.6)	**0.01**
Site			
Tonsil	60 (56.6)	71 (65.7)	0.11
Base of tongue	39 (36.8)	25 (23.1)	
Palate	5 (4.7)	9 (8.3)	
Posterior Wall	2 (1.9)	1 (0.9)	
Uvula	0 (0.0)	2 (1.9)	
T Classification			
cT1	12 (11.3)	12 (11.2)	0.30
cT2	23 (21.7)	30 (28.0)	
cT3	43 (40.6)	38 (35.5)	
cT4	28 (26.4)	27 (25.2)	
N Classification			
cN0	13 (12.3)	44 (40.4)	**0.01**
cN1	48 (45.3)	38 (34.9)	
cN2	34 (32.1)	15 (13.8)	
cN3	11 (10.4)	12 (11.0)	
Clinical Stage			
I	18 (17.1)	16 (15.0)	0.16
II	29 (27.6)	42 (39.3)	
III	22 (21.0)	25 (23.4)	
IV	36 (34.3)	24 (22.4)	
HPV-Positive	62 (58.5)	65 (59.6)	0.865
Recurrence	30 (28.3)	38 (34.9)	0.39
Date of treatment			
1984 -2000	0 (0.0)	58 (53.2)	**0.01**
2001-2014	106 (100)	51 (46.8)	

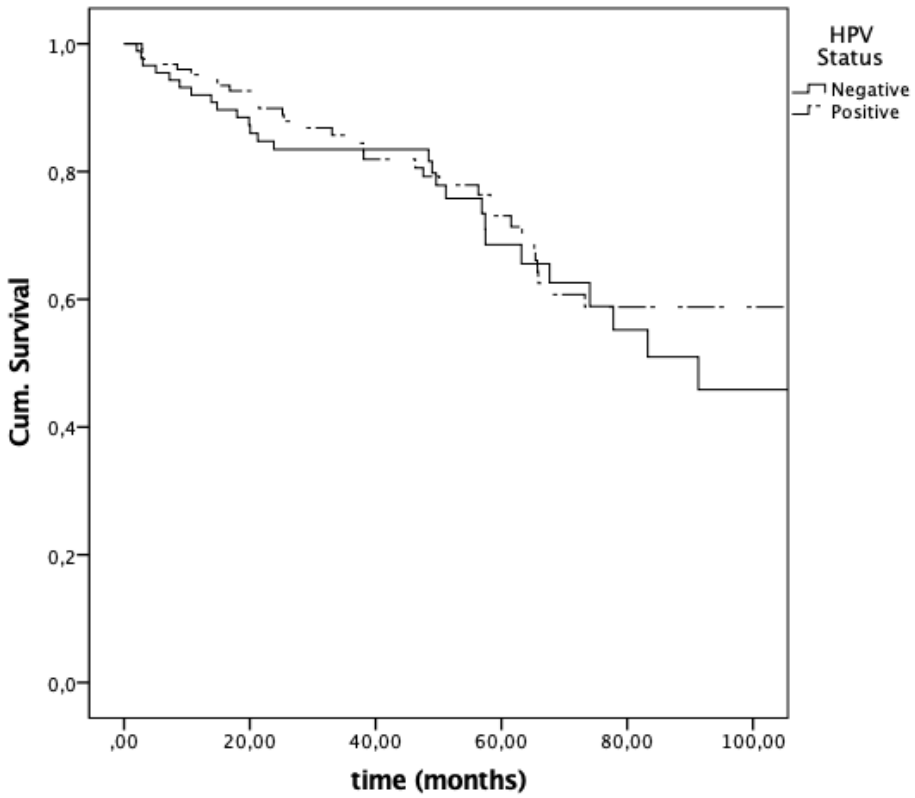

Figure 1. Cancer-specific survival stratified by Human papilloma virus (HPV) status

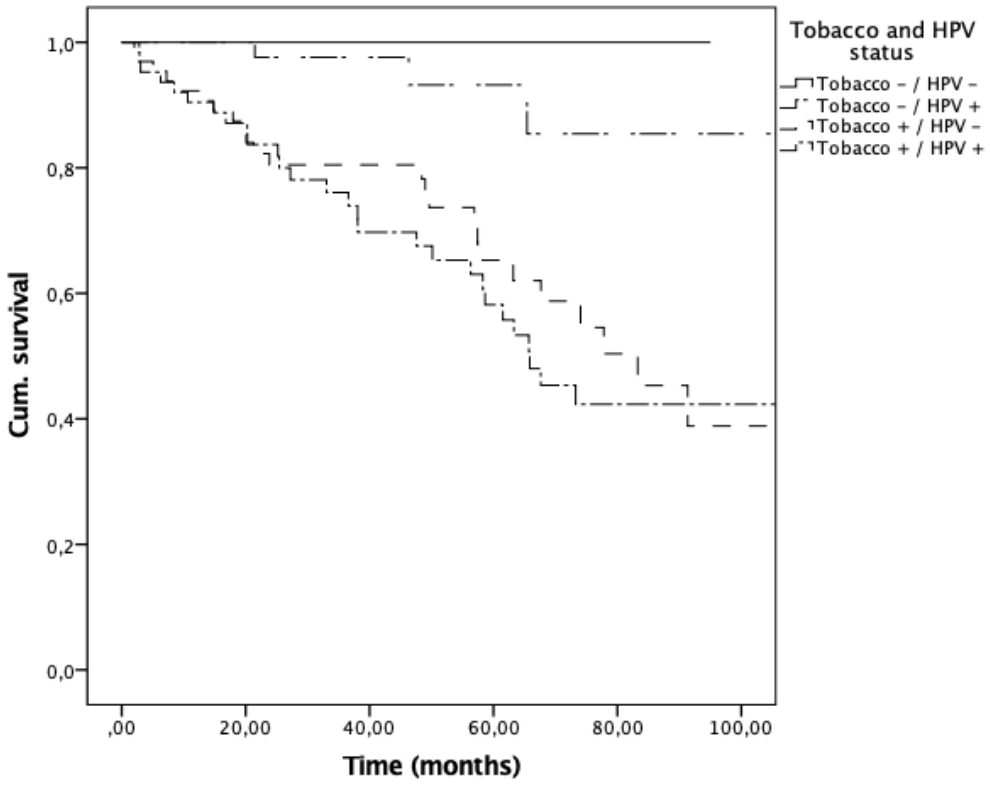

Figure 2. Cancer-specific survical stratified by Human papilloma virus (HPV) and tobacco status

4 COMENTÁRIOS GERAIS

O carcinoma de células escamosas de orofaringe associado ao Papiloma vírus humano têm sido alvo de diversos estudos em todo o mundo nas últimas décadas. Observamos que estes tumores apresentam um perfil epidemiológico diferente (GILLISON et al. 2015) e melhor prognóstico, se comparados aos casos HPV-negativos (ANG et al. 2010). Apesar de o status do HPV se mostrar um fator prognóstico independente para mortalidade, a associação do tabagismo é também associada a piores taxas de sobrevivência, mesmo em pacientes HPV-positivos (GILLISON et al. 2012), porém com o tabaco não sendo considerado, como em nosso estudo, um fator prognóstico independente para mortalidade ou recorrência. No Brasil observamos uma prevalência menor de pacientes portadores de tumores de orofaringe HPV-positivos e uma alta prevalência de tabagistas nesta população (DE MATOS et al. 2015). Além disso, pouco foi estudado a respeito dos padrões de recorrência dos pacientes com carcinoma de células escamosas de orofaringe relacionados ao HPV, comparado aos tumores HPV-negativos e do impacto que o status do HPV tem na mortalidade destes pacientes.

Como limitações do presente estudo, trata-se de uma série de casos coletados retrospectivamente, onde foram utilizados dados de outros estudos prévios realizados pelos pesquisadores do A.C.Camargo Cancer Center e da Faculdade de Medicina da USP, além de análise de prontuários de pacientes já tratados nesta mesma Instituição, os quais apresentavam algum tipo de pesquisa para HPV. Alguns estudos pesquisavam somente o DNA do HPV, enquanto que em outros havia somente a análise do p16. Com isso, foram considerados HPV-positivos, pacientes que apresentavam algum tipo de teste positivo (HPV-DNA, hibridização *in* situ ou p16). Apesar de o p16 ser

considerado como marcador de infecção pelo vírus HPV (ANG et al. 2010), é recomendado que, após identificação de casos p16 positivos por Imunohistoquímica, seja confirmada a presença do DNA do HPV no tumor (CHUNG e GILLISON 2009) para considerar positividade para o HPV. No entanto, na mais recente classificação da AJCC, o p16 é considerado suficiente para classificar o paciente quanto ao status do HPV (AMIN et al. 2017). Com o objetivo de diminuir o viés causado pela metodologia empregada em nosso estudo, dividimos a coorte de acordo com o teste empregado (p16, DNA HPV e testes negativos), e aplicando as curvas de Kaplan Mayer e teste de Log-rank para comparar os resultados, não observamos diferença em relação à sobrevida livre de doença ou sobrevida livre de recorrência, de acordo com o método empregado.

Outra limitação observada em nosso estudo é a de uma coorte historicamente longa, com estudo dos pacientes tratados desde 1984, onde eram sistematicamente submetidos a tratamento cirúrgico, até 2014, onde os pacientes têm à sua disposição maior opção de tratamentos com resultados oncológicos semelhantes, seja por meio de combinação de radioterapia com quimioterapia baseada em platina, seja por meio de acessos cirúrgicos transorais com auxílio da cirurgia robótica. Diante disso, dividimos nossa amostra em dois grupos de pacientes tratados entre 1984 a 2000 e 2001 a 2014. Observamos nos pacientes tratados entre as últimas duas décadas uma maior prevalência de casos HPV-positivos (61,1%) e menor quantidade de tabagistas (65,4%) e etilistas (43,5%), em comparação aos pacientes tratados nas décadas de 1980 e 1990 (53,4%, 88,2% e 78%, respectivamente). Pudemos observar também melhores taxas de sobrevida livre de doença em cinco anos nos pacientes submetidos a tratamento entre 2001 e 2014 (83,4%), em relação aos pacientes tratados entre 1984 e 2001 (61,4%).

Observamos que a prevalência de pacientes HPV-positivos em nosso estudo é maior do que previamente reportado em outros estudos brasileiros (DE MATOS et al. 2015; LOPEZ et al. 2014; BETIOL et al. 2016; PETITO et al. 2017). Acreditamos que isso se deva ao fato que o AC Camargo Cancer Center, por ser uma instituição que atenda tanto pacientes do Sistema Único de Saúde como pacientes provenientes da saúde suplementar, apresente perfil epidemiológico e de condições socioeconômicas distintos das instituições onde foram realizados os outros estudos, estes com pacientes

exclusivamente provenientes do Sistema Único de Saúde. Ainda sim, observamos em nossa coorte uma expressiva prevalência de tabagistas e etilistas, mesmo nos casos considerados HPV-positivos, diferente do perfil epidemiológico apontado nos estudos americanos (GILLISON et al. 2008, 2015). Não somente observamos diferença na prevalência de tabagistas comparando nossa amostra com os dados encontrados na literatura, como também observamos que a quantidade de ano/maço nos nossos pacientes é maior (média de 51,69 anos/maço). Além disso, a presença do tabagismo teve impacto significante na sobrevida livre de doença, sendo identificado como o único fator prognóstico independente, ao contrário de outros relatos de instituições norteamericanas e européias que enfatizam o status do HPV (ANG et al. 2010).

Considerando a aplicabilidade clínica destes resultados, estando inserido na realidade do sistema de saúde brasileiro, onde somente a minoria dos centros terciários consegue realizar rotineiramente a pesquisa do HPV, podemos considerar mais importante a informação sobre o histórico de tabagismo do paciente, do que o status do HPV propriamente dito, para avaliação de prognóstico e possivelmente no futuro também para planejamento terapêutico.

Avaliando o padrão de recorrência dos pacientes com tumores HPV-positivos, não observamos diferença em relação à taxa de recorrência locorregional, comparado aos pacientes com tumores HPV-negativos. Não foram observadas alterações em relação a proporção de casos com metástases linfonodais, ou maior prevalência de metástases cervicais contralaterais, considerando o status do HPV. A presença de extravasamento capsular linfonodal também não teve impacto em mortalidade ou recorrência na coorte estudada.

Nos pacientes com tumores HPV-negativos, o pulmão foi o sítio acometido por metástases à distância em todos os casos avaliados, enquanto que nos pacientes com tumores HPV-positivos, mais da metade dos casos (55%) de falha à distância ocorreu em sítios extra-pulmonares, como fígado (25%) e ossos (20%). Devido a alta incidência de metástases à distância observada nestes sítios, nosso estudo sugere que, para os pacientes HPV-positivos, sejam utilizados exames específicos de seguimento oncológico, como PET-CT ou alternativamente ultrassonografia ou tomografia computadorizada abdominal e cintilografia óssea.

A partir dos resultados obtidos, fica clara a necessidade de realização futura de estudos colaborativos incorporando um maior número de amostras para análises genômicas, metabolômicas e imunológicas tanto dos tumores primários como das metástases à distância de casos tumores HPV-positivos e HPV-negativos para entendimento dos diferentes mecanismos associados aos desfechos oncológicos e contribuir para aprimoramento de métodos diagnósticos (incluindo biopsias liquidas) e possivelmente terapêuticos.

4 CONCLUSÕES

Com base nos resultados alcançados, pode-se afirmar que:

- O risco e o padrão de recorrências locorregionais pós tratamento de carcinomas de células escamosas de orofaringe não se alteram baseado no status do HPV;
- Observamos diferença no padrão de recorrência a distância, com presença de metástase à distância em pulmão em pacientes com tumores HPV-negativos e em pulmão e outros sítios não habituais nos pacientes com tumores HPV-positivos;
- O status do HPV nos pacientes portadores de carcinoma de células escamosas não teve impacto na sobrevida livre de doença e sobrevida livre de recorrência em 5 anos, na amostra analisada. A presença de histórico positivo para tabagismo foi o único fator prognóstico independente para sobrevivência.

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Anexo 1 - Carta de aprovação do Comitê de Ética em Pesquisa-CEP

A.C.Camargo Cancer Center

Comitê de Ética em Pesquisa - CEP

APROVAÇÃO

Os membros do Comitê de Ética em Pesquisa em Seres Humanos da Fundação Antonio Prudente – A.C. Camargo Cancer Center, em sua última reunião de **12/07/2016**, após analisarem as respostas aos questionamentos realizados em reunião de **10/05/2016** **aprovaram** a realização do projeto nº **2202/16** intitulado: "Análise do padrão de recorrência do carcinoma de células escamosas de orofaringe relacionado ao Papiloma Virus Humano".

Pesquisador responsável: Dr. Luiz Paulo Kowalski
Aluno: Rafael de Cicco (Doutorado)

Informações a respeito do andamento do referido projeto deverão ser encaminhadas ao CEP dentro de 06 meses em relatório (modelo CEP).

São Paulo, 15 de julho de 2016.

Atenciosamente,

Prof. Dr. Jefferson Luiz Gross
Vice-Coordenador do Comitê de Ética em Pesquisa

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Anexo 2 - Artigo publicado

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ORIGINAL ARTICLE

WILEY

Impact of human papillomavirus status on survival and recurrence in a geographic region with a low prevalence of HPV-related cancer: A retrospective cohort study

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Abstract

Background: Human papillomavirus (HPV)-positive oropharyngeal squamous cell carcinoma (OPSCC) is associated with better tumor-response rates and survival outcomes. However, in some geographic regions, the impact of HPV infection on prognosis remains unclear. The aim of this study was to describe the patterns of recurrence and survival among patients treated for OPSCC in a geographic region with a reported low prevalence of HPV-related OPSCC.

Methods: We retrospectively evaluated 215 patients diagnosed with American Joint Committee on Cancer (AJCC) stages I to IV OPSCC who were treated with upfront surgery or radiation therapy with or without chemotherapy in a tertiary Cancer Center in Brazil. The collected data included demographic information, HPV status, tobacco and alcohol consumption, and pathologic and treatment variables. The patterns of recurrence were recorded according to HPV status. Disease-specific survival and recurrence-free survival were calculated.

Results: One hundred twenty-seven (59.1%) patients were diagnosed with HPV-positive OPSCC. According to the AJCC eighth edition, 34 (15.8%), 71 (33%), 47 (21.9%), and 60 (27.9%) patients had stage I, II, III, and IV disease, respectively. Surgery was performed in 109 (50.7%) cases, and upfront chemoradiation regimens were provided in 104 (48.4%, $P = .69$) patients. Overall, the 5-year cancer-specific survival was 73.5% and 68.1% for patients positive and negative to HPV, respectively. Tobacco status was considered the only independent prognostic factor for survival. Furthermore, HPV status was not associated with differences in recurrence rates ($P = .68$). While all distant relapses were found to be lung metastases in the HPV-negative group, we observed unusual sites of distant metastases in the HPV-positive group.

Conclusions: HPV status was not associated with higher rates of survival among the investigated population. Moreover, smoking status was considered the only independent prognostic factor for survival. Furthermore, patients with HPV-positive tumors were more likely than patients with HPV-negative OPSCC to have unusual distant metastases.

Paper presented at the sixth World Congress of the International Federation of Head and Neck Oncologic Societies, 2018.

KEYWORDS
HPV, oropharynx, prognosis, survival, tobacco

1 | INTRODUCTION

Globally, the incidence of oropharyngeal squamous cell carcinoma (OPSCC) is 115 131 cases per year, and an estimated 77 598 deaths occurred in 2015 as a result of OPSCC.[1] Although tobacco and alcohol consumption are still known as the most frequent causes of most head and neck squamous cell carcinoma (HNSCC) cases,[2] infection with human papillomavirus (HPV) plays an important role in OPSCC carcinogenesis.[3] Despite the decreasing prevalence of smoking and alcohol consumption from the 1990s onwards,[4] which has led to a decrease in the incidence of most HNSCC, evidence shows an increase in the incidence of HPV-related OPSCC during the same period.[5] Additionally, epidemiologic evidence demonstrates the role of HPV infection in the carcinogenesis of OPSCC, and currently, HPV is the most common risk factor for OPSCC in the United States.[6-9]

Moreover, the increasing incidence of HPV-related OPSCC over the last few decades has led to the discovery of a "new" disease with a different epidemiology, which has distinct clinical and pathological features.[10,11] HPV-related OPSCC commonly presents in younger[12] patients who are nonsmokers; the typical clinicopathological presentation is that of a basaloid pattern (differing from the keratinized SCC presentation in patients with HPV-negative OPSCC)[9,13] accompanied by a higher incidence of regional lymph node metastases. However, HPV-related OPSCC has also been associated with a better tumor response to systemic and local therapies as well as improved disease-free and overall survival compared with patients harboring HPV-unrelated tumors. Such clinical evidence was the basis for the significant modifications that appeared in the most recent TNM classification and for the clinical trials investigating the de-intensification protocols for HPV-positive OPSCC.[14-19]

In some geographic regions, the relationship between HPV and OPSCC has not been clearly established. Recent data from Brazil shows that the prevalence of HPV associated with OPSCC ranges from 5.6% to 25.6%,[20-22] which is in contrast to the 85% observed in Sweden.[23] Moreover, the role of HPV infection on clinical outcomes in these low HPV-OPSCC-prevalent regions remains unclear. The objective of this study was to describe the clinical patterns of recurrence and survival among patients treated for HPV-positive OPSCC in Brazil, a geographic region with a reported low prevalence of HPV-related OPSCC.

2 | MATERIALS AND METHODS

2.1 | Study design and patients

We retrospectively collected data from 215 patients treated for OPSCC at the AC Camargo Cancer Center in São Paulo, Brazil, from 1984 to 2014. Patients were considered eligible for inclusion in the study if they were treated for OPSCC (tonsil, base of tongue, soft palate, uvula, and posterior wall) with a curative intention, if their tumor tissues were tested for HPV at the time of treatment, or if they were able to provide tumor tissues for HPV investigation if they had not been previously tested. Patients were excluded from our study if they received palliative treatment, had distant metastases at admission, or if they did not have tissues available for HPV investigation.

2.2 | Study procedures

HPV DNA detection and/or p16 immunochemistry was used for HPV diagnosis. DNAs extracted from formalin-fixed paraffin-embedded tissues were subjected to semi-automated HPV genotyping using the INNO-LiPA HPV Genotyping Extra Amp kit (Fujirebio) and AutoBlot-3000H equipment (MedTec Biolab Equipment). A positive hybridization to HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, or 82 defined cases with high-risk HPV DNA. p16 was considered positive when high expression (more than 75% cells stained) of $p16^{INK4a}$ in immunostained sections was observed.

2.3 | Outcome measures

Follow-up time was defined as the time from treatment to the first relapse date for recurrence-free survival and as the time from treatment to the date of cancer related-death for cancer-specific survival. Patients who were followed-up for at least 18 months were eligible for this study.

2.4 | Data collection

Collected data included demographics (sex, age), clinical factors (performance status, comorbidities, tobacco, alcohol [more than 10 g daily], hemoglobin levels, body mass index), HPV status, stage according to the seventh and eighth editions of the American Joint Committee on Cancer (AJCC) TNM staging system, pathologic factors (number of metastatic lymph nodes, surgical margins, tumor grade, perineural invasion, vascular

invasion, and extracapsular spread), and treatment characteristics (surgery, neck dissection, or chemoradiation regimen). The patterns of local, regional, and distant recurrence were recorded and correlated to HPV status and clinic and epidemiological characteristics to analyze differences between the groups.

2.5 | Data analysis

SPSS version 23 (SPSS, Inc, Chicago, Illinois) was used for the statistical analysis. HPV prevalence and its coinciding 95% confidence intervals (CI) were calculated; patients were categorized as being HPV-positive or HPV-negative. Differences in epidemiological, clinical, pathological, and treatment variables based on HPV status were calculated using chi-squared tests for categorical variables and Student's *t* test for continuous variables. Disease-specific survival and recurrence-free survival were calculated using Kaplan-Meier curves, and log-rank tests were used for comparisons between variables. Finally, a multivariate survival analysis was performed using a Cox proportional hazard regression model. We divided our cohort into three groups (all cases, cases managed with upfront surgical resection, and cases managed with upfront nonsurgical resection) before performing the regression models. We then determined the variables that were only present in patients treated with upfront surgical resection. Factors that were statistically significant in the univariate analysis were included in the multivariate analysis. *P*-values <.05 were considered statistically significant.

2.6 | Ethics

The study procedures were approved by the AC Camargo Cancer Center Ethics Committee (#2202/16). Informed consent was waived due to the retrospective nature of the study.

3 | RESULTS

The study population consisted of 215 patients, the majority of whom were men (n = 190, 88.4%), and the median age was 56 years. In all, 127 (59.1%) patients were diagnosed with HPV-positive OPSCC. The majority of the patients included did not report any associated comorbidities (n = 169, 78.6%); however, cardiovascular disease was the most frequent comorbidity among those who reported their comorbidities (n = 24, 11.0%). Most patients reported tobacco consumption (n = 130, 60.5%), while the use of alcoholic beverages was reported by 103 (40.9%) patients. The tonsils were the most frequent site (131 cases) for patients in both HPV-positive (n = 78 59.5%) and HPV-negative (n = 53, 40.5%) groups, followed by the base of the tongue (n = 64, 29.8%), the soft palate (n = 14, 6.5%), the uvula (n = 3, 1.4%), and the posterior wall (n = 2, 0.9%). Moreover, the majority of patients in both the HPV-negative and HPV-positive groups presented with locally advanced stage III to IVa/b disease, classified as cT3 (n = 31, 35.2% in the HPV-negative and n = 50, 40.0% in the HPV-positive group, $P = .58$) and cT4 (n = 27, 30.7% in the HPV-negative and n = 28, 22.4% in the HPV-positive group, $P = .58$) (Table 1). When we compared epidemiological and clinical variables based on HPV status (Table 1), a significant difference was observed between the groups based on tobacco ($P < .01$) and alcohol consumption ($P = .02$). Patients who were HPV-negative consumed more tobacco (n = 65, 80.2%) and alcohol (n = 50, 61.7%) than those who were HPV-positive.

According to the eighth edition of the AJCC staging system, 34 (15.8%), 71 (33%), 47 (21.9%), and 60 (27.9%) patients had stage I, II, III, and IV disease, respectively (Table 1).

Surgery was initially performed in 109 (50.7%) patients, while chemoradiation regimens were the initial treatment option in 104 (48.4%) patients. Between 1984 and 2000, all cases in this study underwent surgical resection. Moreover, between 2001 and 2014, 66.2% of the patients received chemoradiation and 32.5% received surgery as the primary treatment.

No significant difference was observed in the treatment option based on HPV status ($P = .69$). Despite that surgical treatment was the primary option for initial local disease (n = 42, 54.5% for T1-T2) and that nonsurgical therapies were preferred for locally advanced tumors (n = 71, 52.2% for T3-T4), this difference was not significant ($P = .39$). However, we observed differences in treatment according to N classification ($P < .01$), presence of comorbidities ($P < .01$), and tobacco and alcohol consumption ($P < .01$; Table 2). Primary neck dissection was performed in 106 cases, while salvage neck dissection was performed in five cases. Nearly half of the pathology reports described a moderate differentiation grade (n = 107, 49.8%). In patients who underwent surgical treatment, free margins, close margins, and positive margins were obtained in 85 (76.6%), 11 (9.9%), and 15 (13.5%) specimens, respectively. Vascular invasion, perineural invasion, and extracapsular spread were identified in 14 (13.6%), 33 (31.1%), and 36 (48.6%) patients, respectively, who underwent neck dissection. No difference was observed between the distributions of pathological variables based on HPV status.

The median follow-up time was 47 months (interquartile range, 43-53). Among the patients in HPV-negative group, 8 (9.3%), 12 (14%), and 12 (14.0%) had local recurrences, regional relapses, and distant metastasis, respectively. Among the patients in HPV-positive group, 8 (6.5%), 13 (10.5%), and 16 (12.9%) had local recurrences, regional relapses, and distant metastasis, respectively. The patterns of recurrence did not significantly differ between the groups ($P = .68$). The mean time until recurrence was 45.1 months (95% CI 35.6-54.5) and 50.6 months (95% CI, 42.1-60.1) in the HPV-negative and HPV-positive groups, respectively;

TABLE 1 Epidemiological, clinical, and pathological distributions stratified by HPV status

Variable	HPV-negative (%)	HPV-positive (%)	*P*
Male	79 (89.8)	111 (87.4)	.38
Female	9 (10.2)	16 (12.6)	
Mean age	54.6	56.2	.50
Tobacco	65 (80.2)	65 (55.1)	<.01
Alcohol	50 (61.7)	53 (45.7)	.02
KPS—mean	88	90	.23
Hemoglobin	14.0	14.3	.26
CV disease	7 (8.0)	17 (13.4)	.15
Diabetes	5 (5.7)	8 (6.3)	.55
COPD	5 (5.7)	6 (4.7)	.49
Hepatopathy	2 (2.3)	2 (1.6)	.54
Nephropathy	2 (2.3)	0 (0.0)	.17
Site			
Tonsil	53 (60.2)	78 (61.9)	
Base of tongue	25 (28.4)	39 (31.0)	
Palate	7 (8.0)	7 (5.6)	.83
Posterior wall	2 (2.3)	1 (0.8)	
Uvula	1 (1.1)	1 (0.8)	
Pathological grade			
I	30 (34.9)	52 (41.9)	
II	48 (55.8)	59 (47.6)	.57
III	8 (9.3)	12 (9.7)	
Margins			
Free	33(73.3)	52 (78.8)	.80
Insufficient	5 (11.1)	6 (9.1)	
Positive	7 (15.6)	8 (12.1)	
Vascular invasion			
Negative	34 (85.0)	55 (87.3)	.48
Positive	6 (15.0)	8 (12.7)	
Perineural invasion			
Negative	27 (65.9)	46 (70.8)	.37
Positive	14 (34.1)	19 (29.2)	
ECS			
Negative	12 (46.2)	26 (54.2)	.34
Positive	14 (53.8)	22 (45.8)	
Stage			
cT1	10 (11.4)	14 (11.2)	.58
cT2	20 (22.7)	33 (26.4)	
cT3	31 (35.2)	50 (40.0)	
cT4	27 (30.7)	28 (22.4)	
cN0	24 (27.3)	32 (25.6)	<.01
cN1	15 (17.0)	70 (56.0)	
cN2	36 (40.9)	13 (10.4)	
cN3	13 (14.8)	10 (8.0)	

(Continues)

TABLE 1 (Continued)

Variable	HPV-negative (%)	HPV-positive (%)	*P*
Overall stage			
I	6 (6.8)	28 (22.6)	<.01
II	8 (9.1)	63 (50.8)	
III	14 (15.9)	33 (26.6)	
IV	60 (68.2)	0 (0.0)	

Abbreviations: COPD, chronic obstructive pulmonary disease; CV, cardiovascular; ECS, extracapsular spread; HPV, human papillomavirus; KPS, Karnofsky performance scale.

TABLE 2 Differences in the characteristics of patients treated surgically vs. nonsurgically

Variable	Nonsurgical treatment (%)	Surgical treatment (%)	*P*
Male	93 (87.7)	97 (89.0)	.47
Female	13 (12.3)	12 (11.0)	
Comorbidities	38 (35.8)	8 (7.3)	.01
Tobacco	55 (52.4)	75 (79.8)	.01
Alcohol	42 (40.4)	61 (65.6)	.01
Site			
Tonsil	60 (56.6)	71 (65.7)	.11
Base of tongue	39 (36.8)	25 (23.1)	
Palate	5 (4.7)	9 (8.3)	
Posterior Wall	2 (1.9)	1 (0.9)	
Uvula	0 (0.0)	2 (1.9)	
T classification			
cT1	12 (11.3)	12 (11.2)	.30
cT2	23 (21.7)	30 (28.0)	
cT3	43 (40.6)	38 (35.5)	
cT4	28 (26.4)	27 (25.2)	
N classification			
cN0	13 (12.3)	44 (40.4)	.01
cN1	48 (45.3)	38 (34.9)	
cN2	34 (32.1)	15 (13.8)	
cN3	11 (10.4)	12 (11.0)	
Overall stage			
I	18 (17.1)	16 (15.0)	.16
II	29 (27.6)	42 (39.3)	
III	22 (21.0)	25 (23.4)	
IV	36 (34.3)	24 (22.4)	
HPV-positive	62 (58.5)	65 (59.6)	.86
Recurrence	30 (28.3)	38 (34.9)	.39
Date of treatment			
1984–2000	0 (0.0)	58 (53.2)	.01
2001–2014	106 (100)	51 (46.8)	

TABLE 3 Patterns of recurrence stratified by human papillomavirus status

Recurrence	HPV-negative, N (%)	HPV-positive, N (%)	*P*
Local	8 (9.3)	8 (6.5)	.68
Regional	12 (14)	13 (10.5)	
Distant	12 (14)	16 (12.9)	
Distant metastasis			
Lung	12 (100)	9 (45.0)	.02
Liver	0 (0.0)	5 (25.0)	
Bone	0 (0.0)	4 (20.0)	
Skin	0 (0.0)	1 (5.0)	
CNS	0 (0.0)	1 (5.0)	

Abbreviations: CNS, central nervous system; HPV, human papillomavirus.

this time was not significantly different ($P = .42$). When we compared the sites of distant metastasis, all 12 patients with HPV-negative tumors had pulmonary distant disease, whereas in patients with HPV-positive tumors, 5 had liver metastasis, 4 had bone metastasis, 1 had skin implants, and 1 had central nervous system (CNS) disease (Table 3).

The overall median disease-specific survival was 113.8 months (95% CI, 75.9-151.7) and the 5-year disease-specific and recurrence-free survival was 71.5% and 64.0%, respectively. The 5-year disease-free survival of patients with HPV-negative vs HPV-positive OPSCC was 68.5% and 73.1%, respectively ($P = .23$). When we used the stratification proposed by Ang et al,[24] we observed a significant difference in the survival rates between groups, particularly in those who smoked ($P = .02$; Table 4). After the whole cohort was analyzed by univariate analysis using a Cox regression model (Table 5), we found that tobacco use (hazard ratio [HR] 5.37; 95% CI, 2.14-13.42), alcohol consumption (HR, 3.02; 95% CI, 1.66-5.84), T stage (HR, 1.35; 95% CI, 1.02-1.78), and Karnofsky's performance status (HR, 2.56; 95% CI, 1.14-5.88) were significantly associated with higher death risk. However, only tobacco use (HR 5.14;

TABLE 4 Five-year survival stratified by human papillomavirus (HPV) and tobacco status

	5-year cancer specific survival, %	*P*	5-year recurrence-free survival %	*P*
HPV-negative	68.5	.2	58.9	.24
HPV-positive	73.1		66.7	
Tobacco-negative/HPV-negative	100	.01	100	.01
Tobacco-negative/HPV-positive	93.2		82.9	
Tobacco-positive/HPV-negative	65.3		55.6	
Tobacco-positive/HPV-positive	58.2		53	

95% CI, 1.05-25.00; $P = .04$) was considered an independent prognostic factor for disease-specific survival in the multivariate analysis. Moreover, after an analysis of the surgical cases by univariate analysis (Table 5), we found that smoking tobacco (HR, 3.68; 95% CI, 1.13-12.00), alcohol intake (HR, 2.39; 95% CI, 1.11-5.16), N stage (HR, 1.22; 95% CI, 1.04-1.43), and number of positive lymph nodes (HR, 1.26; 95% CI, 1.12-1.46) were statistically associated with higher death risk, whereas the number of metastatic nodes (HR, 1.27; 95% CI, 1.07-1.50) was considered the only independent prognostic factor for disease-specific survival according to a multivariate analysis for this group. Furthermore, as for the whole cohort, in the cases treated without surgery (Table 5), only tobacco use (HR, 5.29; 95% CI, 1.04-28.83; $P = .04$) was considered as the independent prognostic factor for disease-specific survival in the multivariate analysis.

4 | DISCUSSION

Previous studies suggest that HPV-related OPSCC is associated with improved oncologic outcomes compared with HPV-negative tumors. Epidemiologic features such as age, smoking status, and alcohol consumption differ for patients with HPV-positive and HPV-negative tumors. Furthermore, the high prevalence of HPV-related OPSCC has been widely described during the last decade in Europe and the United States. However, in some geographic regions such as Brazil, the prevalence and epidemiological and clinical characteristics of HPV-related OPSCC seem to differ from those reported during the last several decades in Europe and the United States.[22]

In 2014, Lopez et al[21] described the prevalence of HPV-positive OPSCC as 10.5% in a Brazilian population. Moreover, in 2016, Betiol et al[20] found that the prevalence of HPV-related OPSCC was 17.7% in a tertiary Cancer Center in São Paulo, whereas Petito et al[25] found that 25.6% of patients with OPSCC tested positive for HPV. Furthermore, a systematic review of HPV infection in the Brazilian population found a 27.4% HPV positivity rate among patients with oral cancer and OPSCC.[22] In the current study, an HPV positivity rate of 59.1% was found, which was much higher than the prevalence rates reported in previous regional studies. This result might be explained in that previous studies were conducted at public institutions, which treat patients with a lower socioeconomic status, while our hospital mainly provides private care. Despite the lower prevalence of HPV positivity in Brazil compared with American and European studies, we observed a slow growth in the incidence of HPV-related OPSCC over the last decade.[20-22,25]

We also found a higher prevalence of tobacco (55.1%) and alcohol (40.9%) consumption among patients with HPV-related OPSCC compared with findings in recent American studies.[4,11] A significant difference was observed in the distribution of smokers, according to HPV status; 55.1% and 80.2% were HPV-positive and HPV-negative, respectively. However, the prevalence of smoking status even in our HPV-positive OPSCC group was much higher than what is usually observed in the United States.[11] Additionally, 45.7% and 61.7% of patients with HPV-positive and HPV-negative tumor, respectively, reported alcohol consumption. Although we observed an overall decreasing prevalence in alcohol and tobacco intake, the prevalence remains very high among patients with OPSCC in the Brazilian population; according to a study conducted by de Matos et al,[22] the prevalence of smoking and alcohol consumption was 87.8% and 75.2%, respectively.

In our cohort, we observed no difference in the age and sex distribution based on HPV status. Furthermore, the mean age of patients in the HPV-positive group was slightly higher than that of patients in the HPV-negative group. These findings differed from the studies conducted by Marur et al[6] and Gillison et al,[11,26] which found that HPV was related to OPSCC in younger patients. Based on our findings, we believe that in some geographic regions such as Brazil, epidemiologic features of HPV-related OPSCC may not differ from those of HPV-negative OPSCC, as they have been reported to be widely related in North America and Europe over the last several decades.[6,11,23]

Importantly, the definition of HPV status differs among studies; in the current study, we used p16 and/or HPV DNA positivity. The available evidence suggests that 70% staining

TABLE 5 Cox's univariate and multivariate analyses—cancer-specific survival

Univariate	HR	95% CI		P	Multivariate	HR	95% CI		P
All cases									
Age	1.016	0.99	1.04	0.20					
Sex	0.88	0.40	1.94	0.76					
Comorbidities	0.98	0.48	2.01	0.97					
Tobacco	5.37	2.14	13.42	0.01		5.14	1.05	25.00	0.04
Alcohol	3.021	1.66	5.84	0.01		1.67	0.58	4.82	0.33
KPS	0.93	0.89	0.97	0.02		0.97	0.93	1.02	0.34
Hemoglobin	0.737	0.74	1.52	0.73					
T stage	1.35	1.02	1.78	0.03		1.55	0.92	2.60	0.09
N stage	1.11	0.97	1.28	0.13					
HPV	0.74	0.46	1.20	0.22					
Surgical cases									
Age	0.99	0.97	1.03	0.88					
Sex	0.90	0.38	2.12	0.80					
Comorbidities	0.72	0.09	5.34	0.75					
Tobacco	3.68	1.13	12.0	0.03		2.70	0.69	10.60	0.15
Alcohol	2.39	1.11	5.16	0.02		1.42	0.58	3.49	0.43
KPS	0.72	0.24	2.06	0.52					
Hemoglobin	1.19	0.26	5.32	0.81					
T stage	1.26	0.92	1.73	0.14					
N stage	1.22	1.04	1.43	0.01		1.16	0.97	1.38	0.10
HPV	0.65	0.37	1.15	0.14					
Number of lymph nodes	1.28	1.12	1.46	0.01		1.27	1.07	1.50	0.01
Grade	1.25	0.87	1.81	0.22					
Margins	1.28	0.92	1.78	0.14					
Vascular inv	0.51	0.24	1.08	0.08					
Perineural inv	1.58	0.88	2.83	0.12					
ECS	0.03	0.01	18.97	0.29					
Nonsurgical cases									
Age	1.06	1.01	1.10	0.01	1.04	0.99	1.09	0.08	
Sex	0.51	0.07	3.90	0.52					
Comorbidities	1.56	0.61	3.97	0.34					
Tobacco	8.77	2.01	38.18	0.01	5.29	1.04	28.83	0.04	
Alcohol	3.57	1.33	9.58	0.01	1.40	0.48	4.10	0.53	
KPS	0.93	0.90	0.98	0.01	0.97	0.93	1.02	0.37	
Hemoglobin	1.08	0.75	1.56	0.67					
T stage	1.68	0.96	2.93	0.06					
N stage	0.89	0.65	1.22	0.47					
HPV	1.07	0.41	2.79	0.87					

Abbreviations: CI, confidence interval; ECS, extracapsular spread; HPV, human papillomavirus; HR, hazard ratio; Inv, invasion.

by p16 immunohistochemistry followed by high-risk HPV DNA positivity is the gold standard for a diagnosis of HPV-associated OPSCC.[27-29] However, for HPV analysis, only p16 immunochemistry is recommended in the eighth edition of the AJCC TNM classification. Furthermore, strong p16 staining is considered a surrogate marker for HPV-related

OPSCC.[21,30] Since we conducted a retrospective study that used data from previous studies, we did not use one single method; this may be a limitation when considering the HPV DNA positivity of HPV-related OPSCC. However, when we stratified the cohort based on the HPV detection methods, we did not observe differences in survival, regardless of the method used for detection.

Although epidemiological data support that there is a higher risk of regional metastasis in patients with HPV-positive OPSCC,[9-11] no evidence in the literature has indicated that extracapsular spread has a significant impact on survival among patients with HPV-positive tumors,[16] which was also supported by our findings. Although we found that N stage was not related to survival according to the multivariate analysis, the number of positive lymph nodes was considered an independent prognostic factor for survival (Table 5).

When we compared staging according to the seventh and eighth editions of the AJCC TNM classification, 86.5% of patients were determined to have stage III and IV disease according to the seventh edition, whereas 49.8% had stage III and IV disease according to the eighth edition. A study conducted by Zhan et al[31] suggested that patients with HPV-positive tumors are "down-staged" in the new edition compared with the AJCC seventh edition. We did not see a significant difference in the 5-year disease-specific survival between the two staging groups. However, we observed a difference in cancer-specific survival and recurrence-free survival when we compared tobacco status (Table 5).

Between 1984 and 2000, all included cases underwent surgical resection, which can be explained by the fact that surgery was considered the primary treatment for oropharyngeal cancer at our institution. Moreover, HPV testing was not performed routinely at that time. Despite the lack of a difference in HPV status between the 1980-1990s and 2000-2010s ($P = .19$), we observed significant differences in the 5-year disease-specific survival (from 1984 to 2000 was 61.4% and from 2001 to 2014 was 76.6%; $P = .01$).

The patterns of recurrence did not differ based on the type of recurrence when HPV status was analyzed. However, our findings were similar to those of Huang et al[32] who found unusual sites of distant metastasis (ie, skin, liver, and CNS disease) in patients with HPV-positive tumors.

Disease- and recurrence-free survival were not affected by HPV status (Figure 1), as mentioned in Table 5. However, both disease- and recurrence-free survival were significantly affected by tobacco intake when we used the stratification methods proposed by Ang et al[24] and the Kaplan-Meier methods (Figure 2). Moreover, we observed that patients who smoked had lower disease- and recurrence-free survival rates (Figure 2). According to the Cox regression models we performed, HPV status was not significantly associated with higher death risk in either the univariate or multivariate analysis. However, tobacco intake was considered a significant independent prognostic factor in the multivariate analysis for disease-specific survival; thus, tobacco use was related to a higher risk of cancer-related death than HPV status itself.

This study has several limitations. First, this was a retrospective study conducted at a single institution, thus limiting

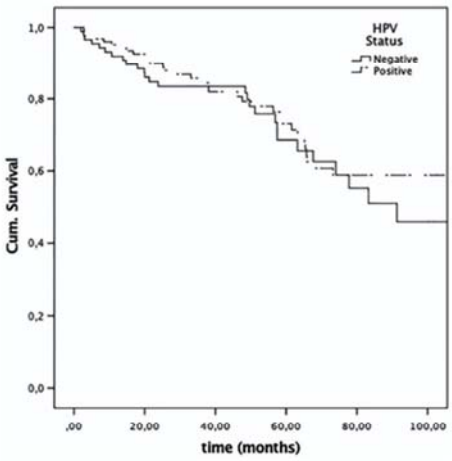

FIGURE 1 Cancer-specific survival stratified by human papillomavirus (HPV) status

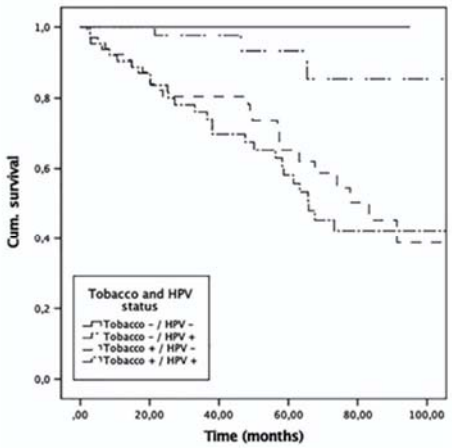

FIGURE 2 Cancer-specific survival stratified by human papillomavirus (HPV) and tobacco status

the validity and generalizability of our findings. The heterogeneity found in our study population was obvious treatment selection bias over time. That is, because data were collected from 1984 to 2014, our study includes patients from the surgical era (1980s and 1990s) in which all patients were treated surgically; the chemoradiation era (2000s); and the TORS era (2010s), each with significant differences in survival. Hence, we need different approaches to the same disease since each approach may lead to different overall results.

5 | CONCLUSIONS

Our study suggests that the burden of HPV-related OPSCC may be increasing in the Brazilian population according to recent data[20-22,25] and the HPV-positive OPSCC prevalence. However, HPV status in patients with OPSCC was not associated with distinct epidemiological features, with the exception of tobacco and alcohol consumption, in the studied population. Furthermore, HPV status was not associated with higher survival rates. Moreover, tobacco use was considered an independent prognostic factor for disease-specific survival, regardless of HPV status. Finally, patients with HPV-positive OPSCC were more likely to present with unusual distant metastasis.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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