HPV implications in oral cancer carcinogenesis: a systematic review

Rusu L.C.[1], Leretter M.T.[2], Roi A.[1], Roi C.I.[3], Mihai L.L.[4], Vardakis M.[1], Rivis M.[3]

[1]*Department of Oral Pathology, Multidisciplinary Center for Research, Evaluation, Diagnosis and Therapies in Oral Medicine, "Victor Babes" University of Medicine and Pharmacy Timisoara*
[2]*Department of Prosthodontics, Multidisciplinary Center for Research, Evaluation, Diagnosis and Therapies in Oral Medicine "Victor Babeş" University of Medicine and Pharmacy Timisoara, 2 Eftimie Murgu Sq., 300041 Timisoara, Romania*
[3]*Department of Anesthesiology and Oral Surgery, Multidisciplinary Center for Research, Evaluation, Diagnosis and Therapies in Oral Medicine, "Victor Babes" University of Medicine and Pharmacy Timisoara*
[4]*Department of Oral Pathology, "Titu Maiorescu" University, Bucharest*

Correspondence to:
Name: Leretter Marius Traian
Address: Eftimie Murgu Sq, no.2, 300041, Timisoara, Romania
Phone: +40 744549345
E-mail address: mariusleretter@yahoo.com

Name: Laurenta Lelia Mihai
Address: Dambovicului Str, no.22, Sector 4 Bucuresti
Phone: +40 72326999
E-mail address: lelia.mohai2000@yahoo.com

Abstract

The topic itself is unknown to the majority of the population and HPV is not yet correlated by non medical people as an infection that can lead to oral carcinogenesis. HPV infection has no borders, in every population over the globe people are infected with at least one subtype of the human papilloma virus even unbeknownst to them. There is extensive research of how subtypes of the human papilloma virus infect the genital area, and especially the female genitalia, due to the fact that it is the main cause of cervical carcinogenesis. The following review of research articles related to the effects of HPV infection on carcinogenesis of the oral squamous cell carcinomas. Research articles were obtained from the database of National Library of Medicine of the United States of America, known as PubMed and the online database Google Scholar, relating to the age and sex of the patient, the site on which oral squamous cell carcinoma developed and the detection method. This systematic review aims to provide a comprehensive analysis of the existing literature regarding HPV-related OSCC, synthesising current knowledge and identifying key research gaps. Further research is needed on the field of cellular and molecular mechanism of infection, prevention, other factors that might influence HPV related carcinogenesis, early detection of biomarkers treatment methods and strategies, long term outcomes of patients treated of oral squamous cell carcinoma and patient that are infected of the subtypes sixteen and eighteen and finally its epidemiological character as an independent type of cancer.

Keywords: Oral cancer, Human papillomavirus, Papillomavirus infections, neck cancers

INTRODUCTION

Human papillomavirus (HPV) is a small, non-enveloped deoxyribonucleic acid (DNA) virus that infects skin or mucosal cells. Its size is approximately 5748 to 8607 base pairs. Of the more than 100 known HPV genotypes, at least 13 have been linked to cervix cancer as well as other anogenital and head and neck cancers [1]. About 70% of all cases of cervical cancer are caused by the two most prevalent "high-risk" genotypes (HPV 16 and 18) [2]. It is the most common sexually transmitted infection (STI) in the United States, with an estimated 79 million Americans currently infected [3]. It is estimated that around 80% of people will be infected with at least one type of HPV at some point in their lives, and the majority of these infections will not cause any serious health problems [4]. Most people will be infected due to the various ways of contamination, since HPV fomites or virions may not even be susceptible to medical or dental clinical disinfectants [5]. Depending on the type of HPV there are different diseases that may evolve, if the patient becomes symptomatic [6].

The warts that affect the skin of the body are not the same that affect the mucosal epithelial tissues or even the skin around the anogenital region [7]. Even though there are numerous types of human papilloma virus that manifest as anogenital warts, the main causative types are type 6 and type 11 [8]. When types 6 and 11 infect the larynx and the lining of the respiratory tract they can form warts / papillae on the epithelial lining causing laryngeal papillomatosis [9]. Penile cancers -50% of those caused by HPV- caused from HPV are caused, again, from HPV type 16 [10]. Either cervix or penis, the probability of cancerous lesion multiplies significantly in patients who are also affected by human immunodeficiency virus (HIV) [11]. The virus spreads by desquamation, -also a reason why it is such a common sexually transmitted infection [12]. Detection through biomarkers, such as ctDNA (circulating tumourDNA) or cHPV (circulating HPV DNA), in blood is promising but further advancements on the molecular tools is needed [13]. Diagnosis for men is not commercially ready, but researchers are trying to identify which regions are more prone to be infected by human papilloma viruses [14]. A complete oral oncological screening takes 2’ to complete and can be done also without any additional equipment, although devices like Velscope and OralID are proven that can detect abnormalities of the mucosa on an earlier stage [15]. Condoms may reduce just slightly the probability of transmission of the infection [16]. In total there are three injection vaccines, with the second injection performed at least after 4 weeks from the first dose and the third injection performed at least 5 months after the first dose [17].

As can be quite logical, lack of vitamins A, C, D, E affect directly the oral mucosa and it is believed that deficiencies can induce the pathogenesis of oral lesions, but further investigations are needed for the metabolic pathways to be understood [18]. A cluster of differentiated 8 lymphocytes -T suppressor cells- predominates in the infiltrate, suggesting that immunosuppression is associated with disease progression [19]. The lower lip, the anterior floor of the mouth, and the lateral margins of the tongue are all high-risk areas for oral cancer [20]. It is of importance to mention also verrucous carcinoma -a subtype of OSCC- that can be identified from its distinct clinical features [21]. Fixation of nodes to neighbouring tissue as a result of cell invasion is a late event that indicates aggressive disease [22]. The diagnosis of oral squamous cell carcinoma is based only after histological examination in combination with a proper anamnesis of the patient and the clinical evaluation of the lesion [23]. Staging helps determine the extent of oral cancer, guide treatment decisions, and assess the prognosis of the patient based on the size of the primary tumour, lymph node involvement, and the presence of distant metastasis [24]. Radiation therapy can be used pre- and post- operatively in cases where dimensions of the tumour are wide [25]. Research

reveals that the earlier the stage on which the patient initiates the treatment the less risk for a recurrence [26].

Aim and objectives

This study is intended to identify how the infection with the human papilloma virus affects locations where oral squamous cell carcinoma is more prone to develop. It is intended to investigate the differences between the two sexes and the different age groups of the patients that were positively diagnosed with HPV related OSCC. It is important to be able to differentiate lesions that can have a specific area of infection in order to be able to continue the research on the topic regarding the action and the mechanisms of both the infectious cause-HPV- and the host-humans- of how they interact. With a better understanding and more exposure of the subject it is more likely for techniques to be developed that can improve all the aspects of treatment planning. From an earlier detection, to better understanding the risk factors, to refine treatment approaches, to evaluate prognostic factors for recurrence, to more thorough evaluation of HPV vaccines and how those affect the oral and oropharyngeal expression of the Human papilloma virus.

MATERIAL AND METHODS

Search strategy

The databases of PubMed and Google Scholar were searched to extract the needed research articles. The search for the articles for PubMed, on the search bar was the following: (((HPV [Title]) OR (Human papillomavirus [Title]) OR (Human papillomavirus [Title])) AND ((OSCC [Title]) OR (oral squamous cell carcinoma[Title]))) NOT ((systematic review [Title]) OR (meta-analysis [Title])). Additional parameters were added, the article must be published in the year 2013 or later. The database gave a hundred and nine different articles. While on the Google Scholar database the search was: allintitle: ((HPV) OR (human papillomavirus)) AND ((OSCC) OR (oral squamous cell carcinoma)). Again with the additional parameter to have been published in the year 2013 or later. The database gave us 94 results of articles and reports.

Inclusion and exclusion criteria

In order for the research articles to be included in the review they needed to have the following criteria. First and foremost, the articles must be published in the year two thousand thirteen and later. Inside their content, the researchers must specify the age and sex of the patients that the cancerous lesion was documented and its location. It also must contain the detection method of HPV of the tumour and it must contain high risk types of HPV. The tumours included must be primary, non recurrent and not previously treated by chemotherapy or radiation therapy. The studies must be in vivo and contain clear data regarding the age and sex of the patients as well as the location of the OSCC. Studies that are secondary (systematic reviews, literature reviews and meta-analyses) are excluded. Excluded are also letters to editors, case reports and articles that are not written in the English language. In its contents the articles that describe detection through serological analysis are excluded. If any of the criteria are not met, the article is not included in the review.

RESULTS

From the articles that were the outcome of the search from the PubMed database, the selection process began. Each individual's article abstract was read and depending on the content and its relevance to the goal thesis review was determined. Once an article's abstract showed relevance, it was read in its complete form to determine the presence of the inclusion

criteria. If the articles were relative to the subject but did not include all the inclusion criteria they were not added on the review. In total out of the 109 articles that resulted from the search in the PubMed database only 9 had all the inclusion criteria. Then, the selection process began on the Google Scholar database. In a similar manner, articles that were relevant to the subject had their abstract read and if they showed similarities to the goal of the review, they were read in full content to determine their inclusion. From the database of Google Scholar, 94 articles were the result of the search bar. After selection of the articles that align with the inclusion criteria and subtracting the duplicate articles that were found from the PubMed database, the resulting articles were 4. The selection and determining of inclusion criteria was done by the undergraduate student writing this review and their evaluation was done by the coordinating professor. Examples of the articles that were included are Chen et al. Oral human papillomavirus infection, sexual behaviours and risk of oral squamous cell carcinoma in southeast China: A case-control study [40]. Even though it has all but one criteria met - the location of the HPV related oral squamous cell carcinoma- it can not be included with the rest of the articles. Another is Yang et al. [41]. Even though the article contains valuable data, it is not specific to the sites of the OSCC and lacks the detail to collect the independent data of the HPV-related patients.

Starting with the articles that were retrieved from Google scholar there are: Silveira et al. [27], Tsimplaki et al. [28], Tokuzen et al [29] and Phusingha et al. [30]. Silveira et al. [27] research is based on the survivability of previously treated patients with oropharyngeal or oral squamous cell carcinoma. The patients included were HPV tested to divide in subgroups the patients infected or not and if infected with which type of the human papilloma viruses. In its immunohistochemical method there were p16INK4a, cyclin D1, p53 and Ki-67 antibodies used for detection of a tissue microarray. Out of all the data received, in the current review we extracted the patients that were tested positive to the antibodies of the human papillomavirus. That resulted in 12 cases of oral squamous cell carcinoma and in 32 cases of oropharyngeal squamous cell carcinoma. The first group consists of 12 males whose average age is 47 years old. The malignant lesions were previously excised from various locations of the oral cavity. Specifically, out of the 12 OSCC, 5 of those were excised from the tongue, 4 from the floor of the mouth, 2 from the retromolar area and 1 from the gingiva of the patient. The other group, HPV related OPSCC, consists of 25 male and 7 female patients, that average 60 years of age. The malignant lesions were found and excised by the following locations. From the 32 OPSCC, 13 were found on the pharyngeal tonsils, 6 on the base of the tongue, 5 on the soft palate, 1 in uvula and 7 in other non disclosed locations.

Following, Tsimplaki et al. [28], is a study that focuses on the OSCC that occurs in the tongue and how high risk HPV infection and expression of E6/E7 mRNA affects 53 Greek patients. In the data correlating the research includes if the patients were smokers or consumed regular alcohol. The detection was done by, analysis of HPV DNA and E6/E7 mRNA presence, PapilloCheck® for HPV detection and genotyping. Out of the 53 samples just 6 were positive for HPV DNA, 2 of those in male and 4 in female patients. 4 of the patients were younger than 45 years old while 2 were older with an average of 51 years. In the collected data only one patient (M73) from the HPV related was a smoker and consumed alcohol. Thus concluding that 9.4% of the 53 patients with OSCC of the tongue were positive for E6/E7 mRNA expression.

Tokuzen et al. [29], concentrates on the HPV infection and the expression of the enzyme p16. Through immunohistochemical processes, RNA extraction and qualitative reverse transcription-PCR (qRT-PCR) HPV infection by p16 expression was evaluated. 100 cases were reported, 10 of which were positive for p16 expression, 7 cases were from male and 3 cases were from female patients. The median age of the positive patients is 71 years old.

The primary sites of the OSCC are 3 in mandibular gingiva, 2 in maxillary gingiva, 2 in the floor of the mouth, 1 in the buccal mucosa, 1 at the tongue and 1 on the lip.

Phusingha et al. [30], is a case control study in which its main focus is the increasing trend of HPV-related OSCC in Northeast Thailand. Through buccal mucosa cells, DNA was extracted and with PCR was assessed for HPV DNA detection. Samples that were positive, through reverse line blot hybridisation (RLBH) were genotyped. All samples were sectioned as tissue microarray (TMA), stained, a tumour biopsy punched in each. block to perform in situ hybridisation (ISH). Out of the case control patients the only results taken are from the formalin-fixed paraffin-embedded lesions that were HPV positive (82). Out of the 82 cases, 29 were male and 53 were female patients, with a mean age of 62, that form the following age groups. Below and equal to 50 years old there were 14 patients, between 51 and 64 there were 16 patients and equal and above 65 years old there were 52 patients. The sites of the OSCC that were identified are 39 at the buccal mucosa, 17 at the tongue, 13 on the lip, 7 on the palate, 5 on the floor of the mouth and 1 on a non-specified location. With the different ways of examining the HPV DNA and mRNA on exfoliated cells and on lesion cells from FFPE, they create a reproducible study that can be repeated in later years in cohort studies.

Kouketsou et al. [31], is a cohort study that fixates on the detection of the HPV in OSCC on Japanese patients. There were 174 OSCC cases that were examined immune-histochemically for p16 expression levels. The positive p16 OSCCs were by in situ hybridisation (ISH) for HPV DNA and their genotypes by PCR. The results were associated with the clinical-pathological characteristics. From the samples from 174 cases were only 24 that tested positive on p16 suppressor enzyme expression. The age groups were sectioned by decades. Between the ages 30 to 39 there were 2 patients, ages 40 to 49 only 1 patient, ages 50 to 59 there were 2 patients. Starting from 60 to 69 years there were 7 patients that tested positive to expression of the p16 enzyme. Between the ages 70 to 79 there were 4 positive patients, from age 80 to 89 were 7 patients and finally from age 90 to 99 just 1 patient. The split between the two sexes was 8 male and 16 female patients. The site of the OSCC that was HPV related was on the following sites and amount. On the tongue there are 8 cases, 7 cases are on the lower gingiva, 6 on the buccal mucosa, 2 on the lower lip and 1 on the floor of the mouth. The study concludes that p16 immunohistochemistry and HPV genotyping by PCR may be beneficial for assessing HPV infection in OSCC.

Kane et al [32], focuses on the predictability of the positivity of HPV infection on advanced OSCC. Out of 124 patients 16 patients were tested HPV-positive. Another factor that was tested was tobacco use that was statistically correlated with HPV positivity. The HPV detection was done by immunohistochemical analysis of p16 enzyme expression in the tumour cell nucleus. From the 16 HPV positive samples, 14 corresponded to male patients and 2 to female patients, while all of those patients were above the age of 30. The locations of the sites were determined to the tongue, with 3 samples, and other sites with 13 sites. The study concludes on the result that, a 12.9% of the samples of T4 OSCC is associated with p16 enzyme expression, oral tobacco consumers are the one third of HPV-related OSCC and clinical-pathological variables can not on their own be used as predictors for HPV related OSCC.

Chuerduangphuiet al. [33], targets on the relationships between HPV-related OSCC and the amplification of genes in the epidermal growth factor receptor signalling cascade (EGFR). From 142 FFPE tissues with OSCC, DNA was extracted to investigate the number of EGFR, KRAS, c-myc and cyclin-D1 genes by real time-polymerase chain reaction (RT PCR). Immunohistochemical examination of TMA OSCC samples was performed to detect c-myc expression and HPV infection. HPV E6/E7 RNA detection was done by in situ hybridization. HPV infection was investigated also by PCR and RT-PCR. Of 142 samples, 81 were HPV positive. 24 of those were from male patients and 57 from female patients. The majority of the

patients were women (57) and men numbered 24. The age groups were divided to those below 60 years old (22) and those above 60 years old (59). The main site that was tested HPV-positive was the buccal mucosa with 38 samples. Following were the tongue and lip with 16 each. The gums numbered 2 samples while the floor of the Mouth and the hard palate numbered 5 and 4 respectively. The study concludes that in the EGFR signalling cascade, amplified genes - c-myc, cyclin D1, EGFR- are partially caused by HPV infection.

Kim et al. [34], investigates the HPV subtypes in OSCC via microarray technology (TMA). With a DNA chip kit 187 samples were tested for high and low risk HPV after histopathological analysis of the main mass of the tumour. Of those, 13 were tested positive at HPV DNA presence. The high risk HPV infections were 8 out of those 13 samples. Patients' age was mostly below 65 years old (7 patients) while above 65 years just 1 split in two locations. Four of the OSCC were on the tongue and additional four were on the gums of the patients. It finalises the study by noting that TMA can accurately detect simultaneously known subtypes of HPV but it is limited to detect new subtypes.

Purwanto et al. [35], focuses on the prevalence of HPV infection in Indonesian patients with OSCC. 78 OSCC FFPE samples were tested for HPV DNA plus 79 samples from normal healthy individuals. PCR was performed to identify HPV gene regions of L1 HPV16 and HPV16/18. From the 78 samples 14 were tested positive for HPV DNA. 9 samples were obtained from male and 5 samples from female patients. The age groups were about even with 8 patients below the age of 47 and 6 patients above the age of 47. The site of the OSCCs was mainly the tongue with 11 samples, then the lips with 2 samples and finally from the maxilla mucosa just 1 sample. The study ends with the verdicts that the prevalence of HPV16/18 n OSCC cases is significantly high (17.9%). HPV18 occurred more often, in HPV-related OSCC patients, than HPV16 (86%). This is supported by the high HPV18 prevalence among Indonesian cervical cancer patients studied in 1995 and 2006. Contrary, the prevalence of HR-HPV remains low in the normal Indonesian population (3.8%), but is more frequently detected in non cancer patients. De Lima et al. [36], is a study that centres on the expression of p16, MLH1 and MYC, in HPV-related OSCC. One hundred OSCC samples were sent for in situ hybridisation and later immunohistochemical detection of the HPV proteins. Thirty one samples were detected with HPV proteins. Twenty of those came from male patients and 11 from female patients. The age groups show that 8 patients were below 54 years old and 23 above 54 years old. The location of OSCC of the patients is mainly on the tongue with 8 samples, the floor of the mouth with 8 more samples and also their combination is represented by another 7 samples. There are 4 samples removed from the lips, two samples from alveolar ridge and further 2 from the hard plate. The study reinforces the hypothesis of HPV-related OSCC carcinogenesis from the expression of p16 and MYC and suppression of MLH1.

Saleh et al. [37], fixates on the localisation of the HPV16 on Oral squamous cell carcinoma. From archives, 114 OSCC specimens were obtained and tested for HPV16 by p16INK4A immunohistochemistry (IHC) and HPV16 E6/E7 mRNA by in situ hybridisation (ISH). A comparison of the two techniques was done and revealed that 16 samples were positive by p16INK4A while 14 were positive by HPV16 mRNA ISH. It was taken in consideration of the 14 samples by ISH. Four of those were in male patients while 10 in female patients. The main site was the hard palate with 5 samples. Then, the tongue with 4 samples, the gingiva with 3, followed by the floor of the mouth and. the buccal mucosa with 1 sample each. The study recommends the use of p16INK4A as a substitute marker for HPV detection in OSCC, complemented by RNA ISH to identify the HPV subtype if needed.

Pérez-Islas et al. [38], is a cohort study that puts on the spotlight the DNA of the Human papilloma virus in patients with OSCC. Formalin fixed paraffin embedded tissues of OSCC were used to extract DNA and evaluate the presence of HPV DNA. Of the total 119

sample tissues 23 were tested positive on HPV DNA, 13 of which came from male patients and the rest of 10 from female patients. The mean age of all patients is 61 years old while on the HPV positive patients, 55 years old. The main sites are the tongue and the hard palate that number 10 and 4 tissue samples respectively. Then, tissues obtained from the gums and the lips are 3 samples each and finally in the retromolar area there are 2 samples and 1 from the floor of the mouth. The study concludes that HPV positivity is strongly associated with better prognosis and recurrence of the OSCC.

Khalesi et al. [39], is a cross-sectional study of 40 samples that emphasises on the cervical metastasis of the HPV-related OSCC. The samples were paraffin embedded and Immunohistochemical processed for the analysis of the p16 enzyme in the sample cells. In those 40 samples 19 were collected from males and 21 from females. The mean age of the patients is 59 years old. The OSCC site of the samples is mainly the tongue with 27 samples, followed by the alveolar mucosa with 10 samples. On a smaller amount are sites of buccal mucosa with 2 cases and the floor of the mouth with 1 sample. The study results in the statement that p16 expression is significantly higher in non-lymph node metastasis in comparison with lymph node metastasis, which can ultimately affect the prognosis of the patient.

Combining the articles, the sum of them is thirteen with a total of 361 patients of both male and female sexes, in a ratio 167/194, that HPV DNA or proteins was found from the affected cells of the tumour.

The sites of the oral cavity that are documented are the tongue, the buccal mucosa, the alveolar mucosa/gums, the lips, the floor of the mouth, the hard palate and added the option of others for further inclusion. Once added from all the above articles there are 120 samples that were collected from the tongue (there are 7 additional that are described as tongue and floor of the mouth but are not included in this category), 87 samples from the buccal mucosa, 38 samples of alveolar mucosa or gingiva or gums or alveolar ridge, 40 samples from the lip, 28 samples from the floor of the mouth (the 7 additional that are described as tongue and floor of the mouth are not included in this category), 22 samples from the hard palate and 25 on other. On the other are the retromolar areas that amount to 4, the described tongue and floor of the mouth samples that amount to 7 and 14 more samples that were not described in their reviews making a true ratio of non described to known 14: 347.

Table 1. Total acquired data of the included articles

Study	Malignancy	No, Sex(M/F)	Age Mean/ Groups	Location
[27]	OSCC	12, 12/0	x=47	Tongue: 5,FoM: 4, Retromolar: 2, Gingiva: 1
[28]	OSCC	6, 2/4	x=51/4<45, 4>45	Tongue: 6
[29]	OSCC	10, 7/3	x=71/ 14<50, 16 51-64, 52>65	Tongue: 1, Buc. Mucosa: 1, Gingiva: 5, Lip: 1, FoM: 2
[30]	OSCC	82, 29/53	x=62	Tongue: 17, Buc. Mucosa: 39, Lip: 13, FoM: 5, Hard Palate: 7, Other: 1
[31]	OSCC	24, 8/16	x=68/30-39: 2 40-49: 1 50-59: 2 60-69: 7 70-79:4 80-89: 7 90-99:1	Tongue: 8, Buc. Mucosa: 6, Gingiva: 7 Lip: 2, FoM: 1
[32]	OSCC	16, 14/2	16 >30	Tongue: 3, Other: 13
[33]	OSCC	81, 24/57	22<60, 59>60	Tongue: 16,, Buc. Mucosa: 38, Gingiva:2 Lip: 16, FoM: 5, Hard Palate:4
[34]	OSCC	8, 6/2	7< 65, 1>65	Tongue: 4, Gums: 4

[35]	OSCC	14, 4/10	8<47, 6>47	Tongue: 11, Buc. Mucosa: 1, Gingiva:2 Lip: 2
[36]	OSCC	31, 20/11	1 15-44, 7 45-54, 9 55-64, 14 >64	Tongue: 8, Gingiva:1 Lip: 4, FoM: 8, Hard Palate: 2, Tongue+FoM: 7
[37]	OSCC	14, 4/10	x=65 / 1<50, 2 51-50, 5 61-70. 4 71-80, 1 81-90, 1 91-99	Tongue: 4, Buc. Mucosa: 1, Gingiva:3,FoM: 1, Hard Palate:5
[38]	OSCC	23, 23/10	x=63	Tongue: 10, Gingiva:3 FoM: 1, Hard Palate:5
[39]	OSCC	40, 19/21	x=59	Tongue: 27, Buc. Mucosa: 2, Gingiva:10 FoM: 1, Hard Palate:4, Retromolar:2

Table 2. Representation of the sites of the HPV-related OSCC corresponding to the study from which they were obtained

Study	Tongue	Buccal Mucosa	Alveolar Mucosa/ Gums	Lip	Floor of the Mouth	Hard Palate	Other
[27]	5	-	1	-	4	-	Retromolar:2
[28]	6	-	-	-	-	-	-
[29]	1	1	5	1	2	-	-
[30]	17	39	-	13	5	7	1
[31]	8	6	7	2	1	-	-
[32]	3	-	-	-	-	-	13
[33]	16	38	2	16	5	4	-
[34]	4	-	4	-	-	-	-
[35]	11	-	1	2	-	-	-
[36]	8	-	2	4	8	2	Tongue+FoM: 7
[37]	4	1	3	-	1	5	-
[38]	10	-	3	3	1	4	Retromolar: 2
[39]	27	2	10	-	1	-	-
Total	120	87	38	40	28	22	25

Individually each article came to the following conclusions. Silveira et al. [27], concluded that when assessing for HPV DNA both low risk and high risk can be detected and high risk HPV associated also with low risk HPV- related carcinomas of Oropharynx and oral cavity showed worse survival than just high risk HPV-related OSCC. Kouketsu et al. [31]finalised the study by stating that p16 immunoreactivity and HPV genotyping by RT-PCR, may be used as markers for HPV detection in OSCC.Kane et al. [32] states that standard clinicopathological variables are not able to predict HPV-positivity. Although history of no smoking showed statistical trends towards predicting HPV-positivity in oral cancer patients. Chuerduangphui et al. [33] completes the study with the amplification of genes in the EGFR signalling cascade, cyclin D1 should be noted, is partially induced by a HPV infection. Kim et al. [34] validates that TMA can efficiently detect known subtypes of human papilloma viruses but is limited to detect new ones. Purwanto et al. [35], resolved to identify a higher prevalence of HPV-18 on OSCC - rather than HPV-16 which is detected more frequently in non-cancerous populations- which is supported by data of cervical cancer occurrence resulting from HPV-18 infection. de Lima et al. [36], ends with a conclusion that exclusively cytoplasmic staining for p16, mlh1 and myc were associated with advanced tumours. Saleh et al. [37], concludes that p16 marker can be used for detection of HPV in

OSCC and for identifying the HPV subtype in situ hybridisation. Pérez-Islas et al. [38] states that HPV-positivity is not associated with a longer overall survival of OSCC patients, but a better prognosis was associated and so was recurring or progressing disease. Khalesi et al. [39], the study states that OSCCs without cervical lymph node metastasis had a significant increase in p16 enzyme expression compared to samples with cervical lymph node metastasis. The presence of HPV DNA was higher in samples with less lymph node metastasis and possibly a better prognosis.

DISCUSSIONS

It is apparent that research regarding HPV-related oral squamous cell carcinoma is being conducted all over the globe. In the research out of the thirteen articles there are ten different countries that add on the research work and innovation for a better understanding of the OSCC. The articles originating from Brazil [27], [36] and Japan [29], [31] should be mentioned that are of different university entities. Such an achievement is quite admirable because it shows that there are active biomedical personnel that advance into scientific research. The more research done by professionals the better expertise that can be provided to the future doctors, dentists, biologists for a better understanding of physiology and pathophysiology, hence a constant evolution and improvement in the field of medicine. The latter have been considered as a high risk factor for OSCC because of their addictive nature and harmful to the oral mucosa effects. It is also important to note that the two studies with the most patients [30], [33] were both from Thailand from the same University. Such behaviours can not only create oral tumours but transform oral lesions to malignancies [43]. It is also apparent that the countries involved have the following aspect in common, the majority of the population is placed in the main cities of the country with minimal rural areas that can provide sufficient education and prevention of the HPV infection. The next factor, age can reveal the character of which an infection like HPV acts on the mucosa. On the review the articles were each one with its own subgroups that make the valuation as a whole much more difficult. Studies like [31], [37] chose to create subgroups per decade and it was seen scarcely on the articles that were examined for inclusion. The articles have made their subgroup into older or you than an age around the 45th to the 65th year of age. Their reasoning is that this age is 'hot' for malignancies to form. The immune system is generally weakened, bodies usually fatigued in the modern era of stress and lack of exercise influence the state of the immune system. Hence, it is quite difficult to determine if HPV-related OSCC is the single and independent cause or it is a cause of the cumulative exposure to the HPV DNA in combination with other risk factors or age related changes in the body's defences against carcinogenesis. Age can impact the treatment considerations due to the overall health status, physiological changes associated with ageing that can impact treatment tolerance, response and outcome. The data from the articles regarding which sex is more prone to develop HPV-related OSCC shows less than 10% difference between the two sexes. Specifically, the 167 male samples represent 46.2% of the samples, while the female samples amount to 53.7%. The main focus of the study is portrayed on the location from which the HPV-related OSCC originated (Table 4.17). As was seen out of 361 samples 120, a third, of those were on the tongue. The tongue being a receptor of all the external factors is mainly affected because of its position. Vicious habits such as nail biting, thumb sucking, biting of objects that are exposed to microbiota such as pencil caps or other types of plastic, may be a factor for a microbiological change in the oral flora. The buccal mucosa is the next site with the higher number of HPV related OSCC, which are 87. The anatomy of the buccal mucosa is related to a thin epithelium which is often subjected to trauma from stress biting. Such habits have a direct correlation to the prevalence of ORSCC [42]. The buccal mucosa is in direct

contact and is immediately affected by any substance entering the oral cavity. Smoking and alcohol being the major high risk factor in non HPV OSCC that after chronic cumulative interaction it the mucosa is influenced especially when combined with drinking (common as seen in a social interaction that combines both alcohol consumption and smoking) alcohol due to the synergetic factor that they have. Hence, if the patient is not punctual or he does not visit the dentist or the otorhinolaryngologist such lesions may be not diagnosed until they are apparent to the patient which otherwise could have been avoided. Following, with almost the same amount of samples, is the lip with 40 samples and the alveolar mucosa/gingival with 38. The lip is more exposed to risk factors such as sunlight, which is a high risk factor for OSCC, smoking and trauma from biting or accidents. Most importantly though, the epithelial lining of the lip is much thinner relative to the gingiva, making it much more susceptible to host viruses as a primary infection site. Further, there are 28 samples that were obtained from HPV-positive OSCC of the floor of the mouth and 22 samples from the hard palate. The hard palate due to its wide keratinised layer of the epithelium, is harder for micro wounds that reach the basal layer to form, thus making it harder for HPV to have as a primary site of infection the hard palate. On the other hand, the floor of the mouth is not subjected to many direct risks such as smoking, alcohol or chewing tobacco. Also, the sublingual and submandibular glands excrete saliva onto the floor of the mouth thus creating clearance of the site. The percentages out of 100 the prevalence of HPV-related OSCC on the locations are as follows: tongue 30%, buccal mucosa 24%, lip 11%, alveolar mucosa/ gums 10.5%, floor of the mouth 7.7%, Hard palate 6%, other site 6.9%.

CONCLUSIONS

The review used strict search methods and inclusion criteria on the databases PubMed and Google Scholar in order to collect the 13 articles. The contents of those articles provide us with the following conclusions. HPV-related Oral Squamous Cell Carcinoma can be found in most populations of the earth. The subtypes of HPV that cause most of the HPV-related OSCC are HPV-16 and HPV-18. Such tumours are more often seen on the tongue (30%) and the buccal mucosa (24%) while sites like the lip (11%), the gingival (10,5 %), the hard palate (6%) and the floor of the mouth (7,7%) can also be seen but more rare. An important clinical finding is that the age range that is mostly affected is between the age of 45 to 65 years old but also with occasions occurring in younger and older ages than that. Between the two sexes the review reveals that women are slightly more likely to develop HPV related OSCC with a percentage of 53.7%, making the difference with men (46.2%), less than 10%. By addressing research in the direction of prevention -vaccination and education- and diagnosis -molecular advancement- we can advance our knowledge of HPV-related OSCC and significantly improve the outcome of future patients.

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