ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 10, Issue, 03, pp. 34272-34276, March, 2020

RESEARCH ARTICLE OPEN ACCESS

HUMAN PAPILLOMAVIRUS (HPV) IN LEUKOPLASIA AND EPIDERMOID CARCINOMA OF THE ORAL CAVITY: A SYSTEMATIC REVIEW

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ARTICLE INFO

Article History:
Received 19th December, 2019
Received in revised form
20th January, 2020
Accepted 02nd February, 2020
Published online 30th March, 2020

Key Words:

Human Papillomavirus, Carcinoma, Squamous Cell Carcinoma of Head and Neck, Oral Leukoplakia.

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ABSTRACT

The main objective of this research was to analyze the frequency of detection of human papillomavirus (HPV) of high oncogenic risk in oral leukoplakias and oral squamous cell carcinoma. This is a systematic review of the literature developed by collecting data from the National Library of Medicine (Medline via PubMed); Elsevier's Scopus (SCOPUS); Web of Science and Cochrane Library with descriptors indexed in the Medical Subject Headings (MeSH). The studies were selected by the relevance test and analyzed according to the GRADE system. Four articles were part of this review, three of which from the Web of Science database and one from Elsevier's Scopus (SCOPUS); all of them with frequency of detection of high oncogenic risk HPV in leukoplakia and squamous cell carcinomas of the oral cavity with analysis regarding the expression of the intracellular protein p-16. The studies have shown the presence of high-risk oncogenic HPV in leukoplakia and oral squamous cell carcinoma with a highly variable frequency of viral detection. The highest frequency was found in carcinomas and leukoplakias with a higher degree of dysplasia, however, more studies are needed of the oncogenic potential of HPV and its synergistic performance with other carcinogens in the development of oral lesions.

Citation: ***Simone Bertozi de Souza Vasconcelos, Inês Aparecida Tozetti, Daniella Moraes Antunes et al.*** **2020.** "Human papillomavirus (hpv) in leukoplasia and epidermoid carcinoma of the oral cavity: a systematic review*", International Journal of Development Research*, 10, (03), 34272-34276.

INTRODUCTION

In the last decades, human papillomavirus (HPV) infection has emerged as a new epidemic and a public health issue because of its involvement in several types of cancer that affect the cervix, the anogenital region and the oropharynx (Boguñá *et al*., 2019). The implantation of HPV in the oral cavity can occur through autoinoculation or oro-sexual contact. Among the associated manifestations are: squamous papilloma, condyloma acuminatum, common wart and focal epithelial hyperplasia. The relationship between the etiology of leukoplakia and oral squamous cell carcinoma remains under investigation (Castro *et al*., 2004). The diagnosis of HPV in oral lesions occurs by viral identification with the techniques of *in situ* Hybridization (ISH) and Polymerase Chain Reaction (PCR) (Castro *et al*., 2004). The development of squamous cell carcinoma involves multiple cumulative genetic changes over a long period, the effect of which exceeds the capacity for cell repair (Farah *et al*., 2014). The etiology is considered multifactorial where intrinsic and extrinsic factors add up to generate a malignant phenotype (Ramos *et al*., 2017). In the oral cavity, leukoplakia is considered the most common lesion and with the highest risk for the development of oral cancer. Oral leukoplakia is characterized as a white plaque adherent to the mucosa that cannot be removed by scraping, not linked to a specific anatomopathological diagnosis (Lee *et al*., 2000). The relation between the clinical aspect and the histopathological characteristics of oral leukoplakias was evaluated and classified according to histopathological aspect in: hyperkeratosis with absence of epithelial dysplasia; mild epithelial dysplasia; moderate epithelial dysplasia; severe epithelial dysplasia, carcinoma in situ and invasive carcinoma (Rodrigues *et al*., 2000). There are more than 200 known HPV subtypes, which are classified according to their potential for

carcinogenesis as high or low oncogenic risk (Gheit, 2019). HPV acts with its oncogenic potential through the expression of oncogenes E6 and E7 since the integration of HPV in the cell genome causes an abnormal epithelial proliferation (Ferraz *et al*., 2012). The P16INK4a gene encodes protein 16 (p16) which negatively regulates the progression of the cell cycle, blocking the phosphorylation of the Retinoblastoma protein (pRB). The pRB controls the transition between the G1 and S phases of the cell cycle. In its active, hypophosphorylated form, the pRB keeps the cell in the G1 phase, whereas, when phosphorylated, it releases the E2F factor that induces the progression of the cell cycle to the S phase (Ferraz *et al*., 2012; Oguejiofor *et al*., 2013; Pannone *et al*., 2012). The relation between HPV and benign and malignant proliferative epithelial lesions is well known in genital lesions (Castro *et al*., 2004). The association of HPV with oropharyngeal cancer is well described in the literature, but its relation with the development of lesions of the oral cavity is still controversial. Thus, this review aims to relate the presence of types of HPV of high oncogenic risk with leukoplakia and squamous cell carcinoma in the oral cavity by discussing its oncogenic potential.

MATERIALS AND METHODS

This is a study of systematic literature review, carried out according to the instructions of the Preferred Reporting Items for Systematic Reviews and Meta-Analyzes - PRISMA (Moher, 2009). To elaborate the leading question of the study, the PICO strategy was adopted: P - Patients with leukoplakia and oral carcinoma; I - HPV detection; C - HPV not detected; O - Positive results for HPV of high oncogenic risk. Thus, the leading question was defined: "Which studies have shown results of detection of HPV of high oncogenic risk in patients with leukoplakia and squamous cell carcinoma of the oral cavity?"

The search took place from October to December 2019 by two reviewers independently. The electronic databases used were Medline via PubMed; Elsevier's Scopus (SCOPUS); Web of Science and Cochrane Library. Descriptors indexed in the Medical Subject Headings (MeSH) were used: "Mouth cancer"; "Mouth Neoplasms"; "Papillomavirus Infections"; "Carcinoma, Squamous Cell", "Leukoplakia, Oral". Observational studies were included in this research without restriction of period and language, in which there was the identification of high-risk oncogenic HPV in leukoplakia and carcinoma of the oral cavity by using the p-16 immunostaining technique. Some studies were excluded: those that did not present the objective of the present review and those that carried out research in vitro, in animal models or in form of case reports, letter to the editor and/or editorials, books, book chapters, indexes and abstracts. The data collection was carried out by two researchers in two distinct phases: in the first, the titles and abstracts were systematically examined to check the relevance test using the Rayyan QCRI application. In the second phase, the eligible studies had their full texts evaluated in order to double-check the relevance test criteria. The data were extracted in a standardized way, in a Microsoft Excel spreadsheet containing: authorship, year of publication, type and frequency of HPV of high oncogenic risk detected, sample characteristics and immunostaining by p-16. The quality of evidence was performed using the Evidence Quality Rating System and the strength of the recommendations - GRADE (Balshem, 2011).

RESULTS

In the databases analyzed, 112 articles were initially selected, all of which were screened by the reading of their titles and abstracts. The selection was based on the relevance test and careful analysis that resulted in four articles for the final sample, three from the Web of Science and one from Scopus (Figure 1).

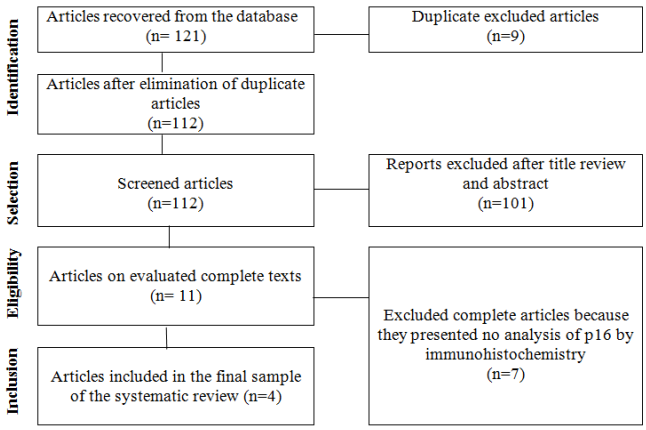

Figure 1. Flow diagram of the search and composition process of the final sample of studies of high-risk oncogenic HPV detection, with analysis of p-16 expression in the oral cavity, 2019

All articles were published in English and dated from 2013 to 2019. The countries of origin were the United States of America, the United Kingdom, Canada and Sweden. The journals were: Anticancer Research; Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology; Modern Pathology; and Head and Neck - Journal for the Sciences and Specialties of the Head and Neck. When analyzing the individual level of evidence using the GRADE system (Balshem, 2011), one study was considered high, two moderate and one low (Figure 2). In the studies analyzed, differences were found in the frequency of detection of high-risk oncogenic HPV between leukoplakia and oral carcinoma. As for age, a wide age range was observed (15 - 84 years), with an estimated average between 55 and 58 years among the works (Mc Cord *et al*., 2013; Caley *et al*., 2015; Lerman *et al*., 2017; Sundberg *et al*., 2019). Two studies presented patients over 65 (Mc Cord *et al*., 2013; Lerman *et al*., 2017). The predominant lesions affected males (Mc Cord *et al*., 2013; Caley *et al*., 2015; Lerman *et al*., 2017).

Regarding histopathological aspects, in the four sources, the following cases were found: 53 cases of leukoplakia and/or erythroplasia with dysplasia, 15% of which associated with invasive carcinomas (Lerman *et al*., 2017); 74 cases of leukoplakia ─ 14 with dysplasia and 60 without dysplasia ─ and 13 cases of carcinoma (Sundberg *et al*., 2019); 5 cases of carcinoma (Caley *et al*., 2015); and 77 cases of leukoplakia (40 cases with high-grade dysplasia and 37 with low-grade dysplasia) (Mc Cord *et al*., 2013). Considering the site of most lesions, they occurred on the tongue and on the buccal floor (Mc Cord *et al*., 2013; Caley *et al*., 2015; Lerman *et al*., 2017). In one study, there was a predominance of leukoplakia and carcinomas in the gums, followed by tongue lesions (Sundberg *et al*., 2019). In leukoplakia, the frequency of viral detection varied from 9.1% to 91% when using HIS and, for carcinomas, from 38% to 100% by the PCR method (Mc Cord *et al*., 2013; Caley *et al*., 2015; Lerman *et al*., 2017; Sundberg *et al*., 2019).

Article	Level	Definition	Implications	Information source
Mc Cord *et al.,* 2013	High	Strong confidence that the true effect is near the estimated	It is improbable that further works will modify the confidence in the effect estimate.	Observational study
Caley *et al.,* 2015	Low	Limitations because of the low number of cases of oral cavity lesions and the methodology utilized in molecular biology	Future works will have important impact on the estimate and analysis of risk factors associated with HPV infection.	Observational study
Lerman *et al.,* 2017	Moderate	Limitations in the methods of DNA extraction and statistical analysis.	Future works could modify the confidence referring to dysplasias associated with carcinomas.	Observational study
Sundberg *et al.,* 2019	Moderate	Limitations in the methods of DNA extraction and characteristics of the samples.	Future works could modify the confidence in the effect estimate. There is need for further information of p-16 in the samples.	Observational study

Figure 2. GRADE classification (Balshem, 2011), for studies of high-risk oncogenic HPV detection, with analysis of p-16 expression in the oral cavity, 2019

Article	Sample characteristics	Lesion sites	Imuno marking p-16 and viral detection
Mc Cord *et al.,* 2013	Cases of leukoplakia = 77 Male = 40 Female = 37 Age range: 15 to 84 years.	32 in tongue, 29 in mouth floor and 16 elsewhere.	- 11 samples were p16 positive (40 cases of high-grade dysplasia) - 1 sample p16 positive (37 cases of low-grade dysplasia) - HIS: 7 of 77 dysplasia cases were positive for high grade HPV.
Caley et al., 2015	Cases of mouth carcinoma = 5 Male = 5 Age range: 43 to 63 years	4 in tongue base and 1 in mouth floor.	- All samples were p16-positive. - HIS: 5 cases were HPV positive for high oncogenic risk. - EIA: HPV-16 in 3 of 4 cases, and HPV-33 in 1 case.
Lerman et al., 2017	Cases of leukoplakia = 53 Male = 47 Female = 06 Age range: 41 to 81 years.	28 in tongue; 9 in mouth floor; 3 in more than two sites; 1 in lingual frenulum; 5 in oral mucosa; 2 in gum; 1 in lip; 2 in soft palate; 1 in retromolar piriform papilla	- All samples were p-16 positive. - HIS: 48 of 53 cases were HPV- positive for high risk oncogenic. - PCR/RFLP: Of 22 cases of dysplasias, 20 were detected HPV-16, 1 HPV-33 and 1 HPV-58. Of 2 invasive carcinomas, 1 was detected HPV-16 and 1 HPV-33.
Sundberg et al., 2019	Cases of leukoplakia = 74 Male = 36 Female = 38 Age range: 50 to 60 years.	Leukoplakias: 21 in tongue; 5 in mouth floor; 27 in gum; 15 in oral mucosa; 3 in lips and 3 in palate. Carcinoma: 2 in tongue; 1 in mouth floor; 8 in gum and 2 in palate.	- 13 cases de leukoplakia were p16 positive. - all carcinomas were p16 positive - PCR/Real Time for HPV: No case of leukoplakia was positive, but 5 cases of carcinoma were positive for HPV-16.

Figure 3. Characterization of studies of high-risk oncogenic HPV detection, with analysis of p-16 expression in the oral cavity, 2019

In the study that used the Immunoenzymatic Assay (EIA), 75% of the carcinomas were positive for high-risk oncogenic HPV (Caley *et al*., 2015). Among the viral types identified, there was a predominance of HPV type 16, of high oncogenic risk; types 33 and 58 were less frequently found. All studies evaluated the expression of the p16 protein by immunohistochemical method and observed the variation in the immunostaining positivity. In two studies, 100% of the cases were p16-positive in leukoplakia and oral carcinomas (Caley *et al*., 2015; Lerman *et al*., 2017) (Figure 3).

DISCUSSION

The studies analyzed in this review demonstrate the need for further research that can suport scientific evidence in the face of the presence of types of high-risk oncogenic HPV with leukoplakia and oral squamous cell carcinoma, with p-16 immunostaining. Only one classification of the GRADE system (Balshem, 2011) was high, and it was observed that the number of articles is still limited. Among the anatomical sites of the lesions in which HPV of high oncogenic risk was detected, both in leukoplakias and carcinomas, it was possible to observe a greater number of cases in the tongue, oral floor and gums (Mc Cord *et al*., 2013; Caley *et al*., 2015; Lerman *et al*., 2017; Sundberg *et al*., 2019).

In the literature, a higher rate of detection of the virus is seen in tongue lesions, followed by those of the oral floor (Miller; Johnstone, 1982; Nemes *et al*, 2006; Cruz *et al*., 2006). In leukoplakias, the lesion site tends to vary according to the habits of each individual (Parise *et al*, 2000). There was a wide variation in the age range of the studies analyzed (15 to 84 years), and the average ages found were around 55 to 58 years (Mc Cord *et al.*, 2013; Caley *et al*., 2015; Lerman *et al*., 2017; Sundberg *et al*., 2019). Oral leukoplakias are generally observed to affect individuals over 40 years of age, between the fifth and seventh decades of life (Parlatescu *et al*. 2014; Neville *et al*., 2009; Farenzena *et al*., 2012). In three studies analyzed, the cases of leukoplakia and carcinoma predominantly involved male patients (Mc Cord *et al*., 2013; Caley *et al*., 2015; Lerman *et al*., 2017). The prevalence of leukoplakia increases with age, especially in men (Sobral *et al*., 2014; Parlatescu *et al*. 2014, Neville *et al*., 2009; Farenzena *et al*., 2012). However, in one study, the female gender stood higher in cases of leukoplakia and lower in carcinoma, which may demonstrate the influence of the patients' habits. The articles demonstrated high-risk oncogenic HPV in both leukoplakia and carcinomas, with HPV frequencies being higher in carcinomas, reaching 100% (Caley *et al*., 2015), and ranging from 0% to 9.1% in leukoplakia (Mc Cord *et al*., 2013; Sundberg *et al*., 2019). The frequencies were

lower among the leukoplakias when taking into account especially the minor degree of dysplasia or those that did not present dysplasia. In one article, HPV was not identified in any case of leukoplakia, according to the authors, because of age (Sundberg *et al*., 2019). Another article detected high-risk HPV in 91% of cases of leukoplakia with dysplasia (Lerman *et al*., 2017). The difference in frequencies can be explained by the patients' epidemiological characteristics, anatomical sites and methodologies aplied in viral detection (Simonato, 2006; Sobral *et. al*. 2014). All the studies analyzed demonstrated the relation between the expression of the p-16 protein and the detection of high-risk oncogenic HPV in carcinomas (Mc Cord *et al*., 2013; Caley *et al*., 2015; Lerman *et al*., 2017; Sundberg *et al*., 2019). As for leukoplakias with or without dysplasia, in one study all p16-positive patients were negative for HPV detection, the technique had no predictive value for the diagnosis of HPV infection (Sundberg *et al*., 2019).

One study indicated the highest viral involvement in severe or moderate dysplasia, 27.5% of high-grade dysplasia samples and 2.5% (one case) of low-grade dysplasia were p16-positive (Mc Cord *et al*., 2013). The increase in p16 expression is related to the oncogenic potential of HPV that occurs through oncogenesis E6 and E7 inducing the expression of inhibitory proteins. However, its increase can occur in the presence of dysplasia regardless of viral interference (Angiero *et al*. 2008). The analysis in this study demonstrates that the predominant viral type was HPV-16 of high oncogenic risk; and types 33 and 58 were less frequently cited (Mc Cord *et al*., 2013; Caley *et al*., 2015; Lerman *et al*., 2017; Sundberg *et al*., 2019). HPV-16 and 18 have been identified in some oral leukoplakias (Ramos *et al*., 2017), in oral dysplasia with histopathological characteristics and demographic data similar to HPV-positive oropharyngeal carcinoma (Lee *et al*., 2000), but they can also be found in normal cells of the oral epithelium (Neville *et al*., 2009). Other works report that HPV-16 has been identified in squamous cell carcinomas of the oropharynx (Kreimer *et al*., 2005; Marur *et al*., 2010).

Conclusion

The analyzed studies demonstrated a quite variable frequency of viral detection, which is higher in carcinomas and leukoplakias with a higher degree of dysplasia. This fact suggests that HPV plays an important role in oral carcinogenesis since only studies with high oncogenic risk viruses have been evaluated. Thus, it is possible to associate the oncogenic potential and the synergistic performance with other carcinogens, when the lesions appear.
It is essential to carry out new studies that allow considering the oncogenic potential of HPV in leukoplakias with different degrees of dysplasia and performance in the malignant transformation of these lesions.

Acknowledgements: to the Post-graduate Program of Health and Development of the Center Western Region, School of Medicine, Federal University of Mato Grosso do Sul, Mato Grosso do Sul, Brazil.

Funding: This study was financed in part by the Fundação Universidade Federal de Mato Grosso do Sul – UFMS/MEC – Brazil.

Conflict of interest: none

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