Effectiveness of Clinical Diagnosis in Lesions by HPV in Mouth. Useful Clinical Criteria

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Eficacia del diagnóstico clínico en lesiones por VPH en boca. Criterios clínicos útiles

Resumen. La infección por virus del papiloma humano (VPH) en boca se asocia con lesiones como hiperplasia epitelial multifocal (HEM), verruga vulgar (VV) y condiloma acuminado (CA). En este sentido, se evaluó la eficacia del diagnóstico clínico en lesiones por VPH en boca y se propusieron criterios útiles para su diagnóstico. Los resultados sugieren que la evaluación clínica no permite diferenciar las lesiones. Las variables clínicas encontradas para el diagnóstico de HEM fueron edad y presencia de lesiones múltiples. En el caso de la VV, género femenino y lesiones únicas pediculadas ≤ 7.65 mm. Para el CA, edad y género masculino. Aunque estas variables podrían resultar insuficientes en algunos casos, contribuyen al desarrollo de una odontología basada en evidencia.

Palabras clave: VPH, HEM, VV y CA.

Abstract. Infection by human papilloma virus (HPV) in the mouth is associated with lesions as multifocal epithelial hyperplasia (MEH), verruca vulgaris (VV) and condyloma acuminatum (CA). In this study, the effectiveness of clinical diagnosis in lesions caused by HPV in mouth was evaluated, and useful clinical criteria for its diagnosis were proposed. The results suggest, that the clinical evaluation does not allow to differentiate the lesions. Age and the presence of multiple lesions were the clinical variables found for the diagnosis of MEH. In the case of VV, female gender and unique pediculate lesions ≤ 7.65mm, and for the CA, age and male gender. Although, these variables might be insufficient in some cases, they contribute to the development of a dentistry based on evidence.

Key words: HPV, MEH, VV, and CA.

Introduction

The prevalence of oral cancer (OC) has increased in the last decade (Gaitán-Cepeda *et al.,* 2011: e4). Although a number of factors are involved in its etiology, it has been proposed that the human papillomavirus (HPV) is the most important biological factor (Ram *et al.,* 2011: 133). Nevertheless, the behavior of HPV in the mouth is controversial, and independently of its type, its genome can be seen as episomal, integrated, or both (Wilson *et al.,* 2013: 953-4).

Infection by low-risk HPV in the mouth is common and associated with three lesions: multifocal epithelial hyperplasia (MEH) with HPVs 6, 11, 13, and 32; verruca vulgaris (VV) with HPVs 1, 2, 4, and 7; and condyloma acuminatum (CA) with HPVs 6, 11, as well as high-risk HPVs 16 and 18 (Kumaraswamy and Vidhya, 2011: 120-2).

MEH and VV are common lesions in children (Bascones-Martínez *et al.,* 2012: 257; Bharti *et al.,* 2013: 78-9), which often occur simultaneously. The clinical diagnosis of MEH is usually easy when it is presented in its classic form. However, CA shows similar characteristicns, and their presence in children should be investigated as it suggests sexual abuse (Bharti *et al.,* 2013 78-9). On the other hand, single lesions of MEH in adults can be confused with VV and CA (Bermeo *et al.,* 2012: 1028).

Although these lesions most commonly affect the labial mucosa, tongue, soft palate, uvula, and gums, they can appear in any site of the oral mucosa (Kumaraswamy and Vidhya, 2011: 121). The lesions usually correspond to papules and less frequently to nodules, which according to the literature present similar clinical and histopathologic features (Kapoor *et al.,* 2013: 541). The general practitioner and the dentist are the first contacts of patients with lesions by HPV in the mouth; they generally establish the diagnosis based on clinical criteria, which usually do not reveal the nature of the pathology or the type of virus that cause them. Often the lesions removed are not sent for histopathologic study because they are considered as benign tumors without importance (Gallegos-Hernández, 2012: 66).

The purpose of this study was to evaluate the effectiveness of clinical diagnosis in lesions by HPV in mouth: MEH, VV, and CA and propose useful clinical criteria for their diagnosis using logistic regression models.

1. Materials and methods

A retrospective and descriptive study of 355 cases diagnosed histologically as lesions by HPV in the mouth (MEH, VV, and CA) over a period of 20 years was conducted. The cases were obtained from the archive of the Laboratory of Oral Pathology of the Centro de Investigación y Estudios Avanzados en Odontología (CIEAO) of the Universidad Autónoma del Estado de México (UAEMEX).

The characteristics and clinical diagnoses were obtained from requests for histopathologic study of the patients. Fifteen clinical variables were evaluated: Age and gender of the patient, location, number, contour, base, surface, consistency, color, type, border, mobility, pain, diameter, and evolution of lesions.

Statistical Analysis: The data were tabulated and analyzed in SPSS v16 (SPSS Inc., Chicago, USA). To evaluate the effectiveness of the clinical criteria diagnosis of each of the lesions, it was obtained the frequency of HPV lesions in the mouth and percentages of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) using Bayes' theorem (diagnostic tests). The concordance between the clinical diagnosis and the definitive histopathologic diagnoses was evaluated by kappa coefficients considering the valuation of Landis and Koch (Cerda and Villarroel, 2008: 57).

Logistic regression models were used to establish the clinical criteria that predict the diagnosis of each lesion. The dependent variables MEH, VV, and CA were converted into three dichotomous variables considering, in each case, one of the lesions as case and the remaining two as control. Each dependent variable was analyzed separately.

Of the fifteen independent variables, 12 were included in the final analysis: age and gender of the patient, location, number, contour, base, surface, consistency, color, pain, diameter, and evolution of the lesions. The type of lesions, border characteristics, and mobility were discarded during the first stage of the analysis, as they were constant variables.

The "Enter" method was chosen for the development of the models; their adjustment was assessed using the Hosmer and Lemeshow test, and the Nagelkerke coefficient of determination was used. We obtained statistically significant coefficients ($p \leq 0.05$), odds ratios (OR), 95% confidence intervals and constructed receiver operating characteristic curves (ROC).

2. Results

The distribution of the lesions was: VV, 58.3% (207); MEH, 22.5% (80); and CA, 19.2% (68). The clinical diagnoses reported for the clinicians in the biopsy request forms included 21 different diagnoses, including reactive lesions, verrucous carcinomas, and melanoma, as shown in table 1.

The prevalence of lesions by HPV in the mouth in the laboratory over the period of 20 years was 7.4%. Of the 355 lesions, 37 were diagnosed clinically as MEH, 14 as VV, and 1 as CA. Only 35 of the 37 MEH, 12 of the 14 VV, and

Table 1. Clinical diagnoses and their frequencies.

Clinical diagnosis	Frequency
Reactive lesion	150
Papilloma	49
Fibrous hyperplasia	45
No diagnosis	37
MEH	**37**
VV	**14**
Mucocele	5
Pyogenic granuloma	3
Verrucous carcinoma	2
Gingival fibrosis	1
CA	**1**
Lymphangioma	1
Ulcerative lesion	1
Papillary hypertrophy	1
Papillary hemangioma	1
Melanoma	1
Pigmented nevus	1
Leukoplakia	1
Geographic tongue	1
Uvula hypertrophy	1
Epulis	1
Hyperkeratosis	1
Total	355

Source: own elaboration.

the CA lesion coincided with the histologic diagnosis (data no shown). The clinical diagnosis of MEH had 43.7% sensitivity, 99.2% specificity, 83.3% PPV, and 95.6% NPV. The diagnosis of VV had 5.8% sensitivity, 98.6% specificity, 24.8% PPV, and 92.9% NPV. The clinical diagnosis of CA had a sensitivity of 1.4%, specificity of 100%, PPV of 100%, and NPV of 92.7%.

The concordance between the clinical diagnosis and the definitive histopathologic diagnosis for MEH was moderate, with kappa = 0.532 at $p < 0.001$. For VV and CA, the concordance was slight, with kappa = 0.037 at $p = 0.03$ and kappa = 0.024 at $p = 0.04$, respectively.

Logistic Regression Analysis: The models built correctly classified 86.2% of the MEH, 72.1% of the VV, and 81.7% of the CA lesions. The model developed for MEH showed an adjustment by Hosmer and Lemeshow of $p = 0.123$ and a Nagelkerke coefficient of determination of 41.1%. The explanatory variables statistically significant for MEH were age and number of lesions. The probability of have MEH, decreased in patients older than 34.74 ± 19.19 years, whereas the presence of multiple lesions increase the possibility of suffer MEH. The sessile base remained in the model; although it was not statistically significant, improved the adjustment of the model (see table 2). The ROC curve for the MEH model showed an area under the curve (AUC) of 0.804 at $p < 0.001$; thus, the clinical variables thrown into the model to differentiate MEH from VV and CA have good diagnosis value. However, the cutoff for a sensitivity of 80% showed 55.3% specificity and 44.7% probability of false positives using the criteria (see figure 1).

The model developed for VV had an adjustment by Hosmer and Lemeshow at $p = 0.562$ and a Nagelkerke coefficient of determination of 25.7%. The statistically significant explanatory variables that increased the probability of have VV, were female gender and unique lesions of pediculated base; whereas in lesions with a diameter greater than 7.65 ± 4.20mm, the probability decreased (see table 3). The ROC curve showed that the clinical variables established to distinguish VV from MEH and CA have a regular diagnostic value (AUC = 0.746, $p < 0.001$). The cutoff for a sensitivity of 81.2% showed 59.5% specificity and 40.5% probability of false positives using the criteria (see figure 2).

The model of CA presented an adjustment by Hosmer and Lemeshow of $p = 0.458$ and a Nagelkerke coefficient of determination of 12.1%. The statistically significant explanatory variables that increase the probability of presenting CA lesions were the age in patients older than 34.74 years and the male gender (see table 4). The location of the CA presented, in general, a significant statistical association at $p = 0.038$. This variable allowed a better adjustment of the model why was retained; although no significant associations were noted when its different categories were evaluated. According to the ROC curve, the evaluated clinical variables for differentiating CA from MEH and VV have a regular diagnostic value (AUC = 0.690, $p < 0.001$). The cutoff for a sensitivity of 80.9% showed 42.2% specificity and 57.8% probability of false positives using the criteria (see figure 3).

Table 2. Useful variables in the clinical differential diagnosis of MEH.

Variables	Categories	B	OR	95% C. I. for OR	p
Age	Numeric variable	–0.032	0.968	0.952 - 0.985	< 0.001
Number of lesions	Multiple	2.594	13.377	6.975 - 25.656	< 0.001
	Unique	Category of reference			
Base	Sessil	0.661	1.937	0.973 - 3.854	0.060
	Pediculated	Category of reference			

Source: own elaboration.

Table 3. Useful variables in the clinical differential diagnosis of VV.

Variables	Categories	B	OR	95% C. I. for OR	p
Gender	Female	0.647	1.910	1.125 - 3.242	0.017
	Male	Category of reference			
Number of lesions	Unique	2.098	8.152	4.292 - 15.484	< 0.001
	Multiple	Category of reference			
Base	Pediculated	0.813	2.255	0.264 - 0.745	0.002
	Sessile	Category of reference			
Diameter of lesions	Numeric variable	–0.715	0.489	0.275 - 0.871	0.015

Source: own elaboration.

Table 4. Useful variables in the clinical differential diagnosis of CA.

Variables	Categories	B	OR	95% C. I. for OR	p
Age	Numeric variable	0.018	1.018	1.002-1.033	0.024
Gender	Male	0.929	2.532	1.389-4.616	0.002
	Female	Category of reference			

Source: own elaboration.

3. Discussion

The increase in the prevalence of OC in the last decade (Gaitán-Cepeda *et al.*, 2011: e4) requires us to identify the factors involved in its etiology, including infection by HPV. Episodes of immunodeficiency due to malnutrition are related with the etiology of HPV lesions (Feller *et al.*, 2011: 82). The immunodeficiency due to malnutrition is common in patients in the State of Mexico, which

could contribute to the establishment of infectious lesions by HPV, such as those analyzed in this study. The participation of HPVs 6, 11, 16, and 18 has been identified in oral squamous cell carcinoma (Kumaraswamy and Vidhya, 2011: 121), in MEH (Prabhat *et al.,* 2013: 2-3; Bascones-Martínez *et al.,* 2012: 256) and in CA (Cardoso and Calonje, 2011: 149-150; Kapoor *et al.,* 2013: 542). In these cases, the HPV genome can be found as episomal, integrated, or both forms (Wilson *et al.,* 2013: 953-4). This means that the low-risk HPVs that participate in the etiology of MEH, VV, and CA do not exclude the risk of carcinogenesis.

The histopathologic study of lesions caused by HPV is important because the time between the identification of a superficial lesion and its becoming invasive is short. The lesions with dysplastic changes do not show clinical evidence of their nature in the early stages and the role that each type of HPV will play in the lesion is unclear. Therefore, differentiating between MEH, VV, and CA through a clinical-histopathologic study is essential for the proper treatment of patients (Gallegos-Hernández, 2012: 66).

The results of this study suggest that the prevalence of HPV lesions in the mouth is low in our state. Nevertheless, patients do not usually pay attention to oral lesions, although the presence of such lesions sometimes leads them to consult a general practitioner or dentist. Many of these lesions are removed with the use of lasers, cryosurgery, and even surgery, but they are frequently not sent for histopathologic study, which prevents revealing their true prevalence or importance according to the type of HPV (Bermeo *et al.,* 2012: 1028).

In addition, the clinical evaluation does not always allow differentiating between single lesions by HPV and others of a diverse nature (Kallarakkal, 2013: 1), which explains the disparity in clinical diagnoses in this study, their low percentages of sensitivity and the poor concordance between them and the definitive histopathologic diagnosis, especially in the case of VV and CA.

The percentages of specificity, PPV, and NPV obtained to evaluate the efficacy of the clinical criteria in the diagnosis of the three lesions appear to be consistent in the case of MEH and VV. However, the percentages of sensitivity and PPV for CA are high because only one dentist made a clinical diagnosis of CA of the total of the evaluated lesions; this diagnosis was confirmed by the histologic study.

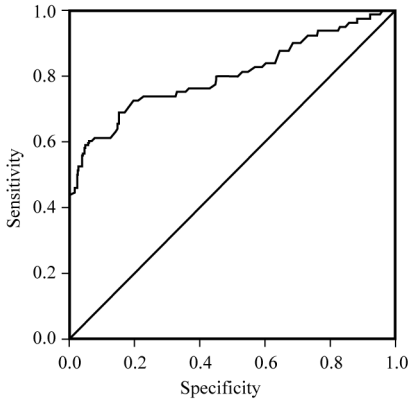
According to the logistic regression, the clinical variables that contribute to the differential clinical diagnosis of MEH with VV and CA are age in patients younger than 34.74 years and the presence of multiple lesions. However, MEH traditionally, is described as multiple lesions having smooth superficies, an oval or round shape, a tendency to coalesce, a sessile base, and a diameter smaller than 0.5cm, and as usually located in labial and vestibular mucosa and tongue, mainly in children and adolescents (Tenore *et al.,* 2013: 43).

The variables that describe VV and show differences with MEH and CA are female gender and unique pediculate lesion with a diameter equal to or smaller than 7.65mm. In contrast, the literature includes in its description a unique or multiple, sessile or pediculate lesion located on the hard palate or gums, with a regular contour, a warty surface, and white color (Kumaraswamy and Vidhya, 2011: 121).

The logistic regression model for CA suggests that it can only be differentiated from MEH and VV taking in account the age of the patients and the gender; the rest of the clinical variables showed no significant differences. Whereas the literature described the CA as a small sessile or pediculate lesion with a warty surface of white color and an oval, round, or even irregular contour, which may appear on the labial mucosa, tongue, or floor of the mouth as asymptomatic pathology (Bharti *et al.,* 2013: 78-9).

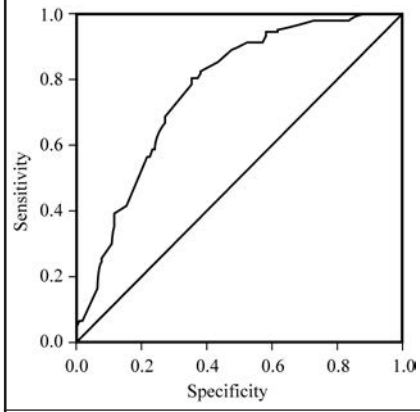
The cutoffs in the ROC curves for a sensitivity of 80% to 81% show low percentages of specificity and high probabilities of

Figure 1. ROC Curve of the MEH.



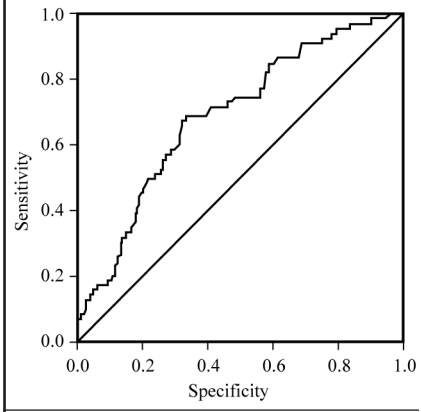
Note: diagonal segments are produced by ties.

Figure 2. ROC Curve of the VV.



Note: diagonal segments are produced by ties.

Figure 3. ROC Curve of the CA.



Note: diagonal segments are produced by ties.

false positives when the criteria described for each one of the lesions are used. In contrast, the clinical diagnoses showed low percentages of sensitivity and high percentages of specificity for the three lesions.

It is advisable to surgically remove lesions which do not heal over a prudent time period and to send them for histopathologic study. Molecular biology tests must be used when there is suspicion of sexual abuse or of lesions with dysplastic changes developed by HPV independently of their classification as a virus of low or high risk (Rautava *et al.,* 2012: e42171).

Conclusions

The HPV behavior in mouth and the increased prevalence of OC in the last decade require a change of attitude on the part of dentists and general practitioners of primary care when they evaluate lesions considered of low risk. Thereby, the identification and clinic-histopathologic study are mandatory in any oral lesion.

The results of this study will contribute to the development of a dentistry based in evidence and provide a better care to patients, through the demonstration of two points. First, the lack of efficacy of the clinical diagnosis to differentiate not only the oral HPV lesions as MEH, VV and CA but also to differentiate those of reactive lesions and even verrucous carcinoma and melanoma; and the second one is proposing objective variables that can be used in the differential diagnosis of the HPV lesions. However, according to the models developed, a low percentage of clinical variables are useful for establishing the diagnosis of oral lesions by HPV. Age and the presence of multiple lesions in the case of MEH and unique lesions in the case of VV were observed to be highly significant ($p \leq 0.001$), which can lead to type II errors, especially using these three variables. The ROC curves also showed low percentages of specificity and high percentages of false positives using the clinical criteria in each case; for this reason, we recommend using them with caution.

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