Presence of HPV 16 sequences in oral lichen planus lesions

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RESUMEN

La lichen plan bucal es una alteración de la mucosa bucal de origen inmunológico. La infección de HPV ha sido sugerida como un posible factor etiológico. Nos hemos estudiado 17 lesiones de lichen plan bucal en utilizando la PCR — una puissante technique pour la détection des séquences d’ADN de différentes lesions — pour detectar HPV–16–ADN séquences. Nous avons trouvé un pourcentage de 11.76% de lésions de lichen plan bucal HPV–16 positives (2 de 17). Dans les études histopathologiques nous avons trouvé aucun effet cytologique d’infection de HPV. Il est possible que l’état intégré de HPV–16–ADN soit responsable de la perte de la capacité de réplication, et conséquemment des effets biologiques de la réplication virale.

INTRODUCTION

Oral lichen planus (OLP) is a chronic inflammatory mucosal disorder of unknown origin (Scully and El-Korm, 1985). Nevertheless, diverse exogenous agents including generalized medical disorders, mainly diabetes, hypertension and liver disorders (Tyldesley, 1974; Lacy et al., 1983); systemic medications (Robertson and Wray, 1992); viral infections (Maitland et al., 1987; Biersterfeld et al., 1991) and a number of immunologic disorders (Walsh et al., 1990) are often associated with the initial onset and subsequent exacerbations of OLP lesions.

The possible viral etiology is also proposed by the recent demonstrations of the presence of various viruses like HSV-1 and HPV in a high percentage of oral lesions including OLP (Tyldesley, 1974; Maitland et al., 1987; Cox et al., 1993). Despite these studies on the presence of HPV in OLP lesions, the real etiological role of this virus remains to be established. HPV infections have been demonstrated in several oral disorders (Löning et al., 1985; Syrjänä et al., 1986; Milde and Löning, 1986; Eversole and Lairops, 1988; Scully et al., 1988; Syrjänä et al., 1988; Greer and Shrayer, 1990; Zeuss et al., 1991; Young and Min, 1991); however, detection rates varied, dependent on the type of study and especially on the method involved.

The present study was undertaken to examine the presence of HPV type 16 in OLP lesions using the polymerase chain reaction (PCR), a more sensitive and specific molecular technique than ISH.

**MATERIAL AND METHODS**

**Patients and protocols**

Formalin-fixed paraffin-embedded oral biopsies from 17 patients with OLP lesions attended in the Oral Medicine Department of School of Dentistry of Granada were included in this study. 11 patients were women (mean age = 45.36 yr, range = 20-72 yr) and 6 were men (mean age = 45.16 yr, range = 21-72 yr).

Clinical parameters collected include lesion type, symptoms, lesion location and other related factors. The histopathological examination included observation of epithelial alterations, basal layer vacuolization, intensity of the inflammatory infiltrate, number of Civatte’s bodies and intraepithelial lymphocytes, and the presence de Max-Joseph’s spaces and keratinocytes cells. Histopathological study was performed by the same oral pathologist and quantifying of basal layer vacuolization and intensity of inflammatory infiltrate was based on his personal experience.

**Cell culture**

The CaSkii cell line was obtained from the American-type culture collection (Rockville, MD) and was cultured in an RPMI 1640 medium supplemented with 10% fetal calf serum. CaSkii contains HPV 16 sequences (Howley, P.M., 1987).

**Amplification of HPV sequences**

Multiple (3 to 5) 10 µm sections of the paraffin-embedded tissues obtained from each patient were minced into 0.5 ml. Eppendorf microcentrifuge tubes, dehydrated with xylene, washed with ethanol and digested for 24 h at 37°C in an extraction buffer containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM KCl, 0.1 mg/ml gelatin, 0.45% Nonidet-P40, 0.45% Tween 20 and 60 µg/ml proteinase K. The samples were heated to 95°C for 8 min to inactivate the proteinase K. centrifuged (10,000 g x 10 min) to pellet the debris, and 10 µl of the supernatants were analyzed by PCR. PCR amplifications were performed using a DNA thermal cycler (Perkin-Elmer-Cetus, Norwalk, CT). The total reaction volume was 100 µl, containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 µM each of dATP, dCTP, dGTP, and dTTP, together with 1 µM of each primer. Primers specific for a segment of the E6 gene (HPV 16) were synthesized by Eurogentec (Liège, Belgium) (see Tab. 1).

Finally, 2.5 units of thermostable Taq polymerase (Amplitaq, Perkin-Elmer-Cetus) were added and the whole mixture was submitted to 32 cycles of amplification with the following profile; denaturation for 2 min at 94°C; primer annealing for 2 min at 55°C; and primer extension for 1 min at 72°C.

PCR assay was performed using both positive and negative controls. Positive controls consisted of DNA extracted from HPV-containing Caski cell line. The reaction products were analysed by a dot-blot hybridization assay using 32P-labelled oligomer probes specific for HPV 16 (Tab. 1) (Seedorf, K. et al., 1985). The filter was pre-hybridized for 1 h at 42°C in 3 x SSPE, 5 x Denhardt’s, 0.5% SDS before adding 32P-end-labelled oligomer (Roychoudhury. R. and Wu, R., 1980) and then hybridized for 1 h at 42°C in the same solution. The filter was washed in 1 x SSPE, 0.1% SDS 3 times for 5 min at room temperature, once at 55°C for 10 min and finally again at room temperature for 5 min. Autoradiography

<table>
<thead>
<tr>
<th>HPV type</th>
<th>Primer location</th>
<th>Sequence</th>
<th>Product length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-upstream</td>
<td>E6 (320-339)</td>
<td>5’-ATTAGT GAG TAT AGA CAT TA-3’</td>
<td>109</td>
</tr>
<tr>
<td>16-downstream</td>
<td>E6 (410-429)</td>
<td>5’-GGCTTT TGA CAG TTA ATA CA-3’</td>
<td></td>
</tr>
<tr>
<td>16-probe</td>
<td>E6 (354-393)</td>
<td>5’-ATGGAA CAA CAT TAG AAC AGC AAT ACA AAC CGT GGT G-3’</td>
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</tr>
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</table>

Tab. 1: Oligonucleotide primer and probe sequences used to detect HPV 16 DNA by PCR
Tab. 2: HPV-16 analysis in OLP lesions.

<table>
<thead>
<tr>
<th>Sample no</th>
<th>Sex</th>
<th>Age</th>
<th>Lesion type</th>
<th>Symptoms</th>
<th>Site</th>
<th>Related factor</th>
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</thead>
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<td>56</td>
<td>atrophic</td>
<td>discomfort</td>
<td>1.3</td>
<td>stress</td>
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<tr>
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<td>23</td>
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<td>none</td>
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<tr>
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<td>M</td>
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<tr>
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</tr>
<tr>
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<tr>
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<tr>
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<td>29</td>
<td>reticular</td>
<td>none</td>
<td>1</td>
<td>stress</td>
</tr>
</tbody>
</table>


was performed at -70°C with Kodak X-AR film, and exposed for 1 h. Samples showing amplification below or equal to the negative controls cells were considered negative.

RESULTS

Tab. 2 summarizes the clinical description and the presence of HPV DNA type 16 in the 17 OLP lesions studied using the PCR technique. Overall, 11.76% of OLP lesions examined (2 out of 17) were found to contain HPV-16-related sequences. The autoradiograph of the PCR amplification product is presented in figure 1. Positive HPV-16 biopsy specimens came from women and were erosive clinical type of OLP.

Tab. 3 shows the histopathological parameters of OLP lesions analyzed. Positive HPV-16 cases showed a maximal vacuolization of the basal layer and an intense inflammatory infiltrate. No morphologic cellular alterations of HPV infection such as koilocytosis, were found in the positive HPV-16 cases.

DISCUSSION

Oral lichen planus (OLP) is a chronic inflammatory mucocutaneous disease of unknown origin (Scully and El-Korm, 1985). Diverse etiological factors have been...
related with this disorder and HPV infection has recently been suggested (Jontell, M. et al., 1990).

The present authors found 11.76% of OLP lesions to contain HPV-16-related sequences. This rate is included in recently published data. Cox et al., 1993 found 75% of cases to be positive for HPV-16 (3 out of 4) using in situ hybridization; Syrjänen et al., 1986 found one positive case with the same technique. Other researchers found no HPV positive OLP cases (Kashima, H.K. et al., 1990).

Jontell et al., 1990 using PCR, found 65% of erosive OLP cases to contain HPV DNA types 6, 11, 16 or 18 and 15% erosive OLP containing HPV-16-related sequences. In the present authors' series, all of the OLP lesions which tested HPV-16 positive were of the erosive type.

It is generally believed that basal cells are the targets for HPV infection. The viruses may get direct access to the basal cells if exposed to a wound or an abrasion (Chang F., et al., 1991). This fact might explain that only erosive forms of OLP lesions were found positive for HPV-16 in the present study. However, erosive OLP is usually resistant to permanent cure and requires regular steroid treatment and examinations since this form is considered to be a premalignant condition (Barnard N.A. et al., 1993).

Steroids are known to decrease the density of Langerhans cells which express class II antigens thereby opening ways to viral antigens (Jontell M. et al., 1988).

In addition, a transcriptional activation element inducible by glucocorticoids has been identified in the regulatory regions of the HPV-16 genome which, in treated patients with virus could lead to over-expression of HPV transforming genes (Pater M.M. et al., 1988).

Moreover, histopathological examination of HPV-16 positive specimens showed no morphological characteristics of HPV infection such as koilocytosis (Greer R. et al., 1987). Koilocytosis is suggested to be a cyopathic effect of HPV and shows productive HPV infection (Koss, L.G. and Durfee, G.R., 1956). HPV-16 DNA is usually integrated into the cellular genome loosing its replicative capability. This might, perhaps be a possible explanation for the absence of koilocytosis in HPV-16 positive OLP lesions (Jontell, M. et al., 1990).

Thus, further evidence is required in order to uncover an etiological role for HPV-16 in the pathogenesis of OLP.

REFERENCES


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