LOW PREVALENCE OF HIGH RISK HUMAN PAPILLOMA VIRUS IN NORMAL ORAL MUCOSA BY HYBRID CAPTURE 2

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ABSTRACT
High risk human papillomavirus (HR-HPV) are recognized as a necessary factor to development cervical cancer. During the last decade many studies have found HR-HPV in oral squamous cell carcinoma (OSCC) and normal oral mucosa, however the association between HR-HPV and OSCC is still uncertain. The aim of the study was to determine DNA HR-HPV in normal oral cavity of healthy adults. A cross-sectional study was performed; samples from 77 patients with normal oral cavity were collected at the Dentistry school, Autonomous University of Yucatan, Merida, Yucatan, México. HR-HPV was detected by hybrid capture 2. One sample out of 77 (1.2%) was positive for HR-PVH. It was from a man of 50 years old. HR-HPV is present in low rate among healthy oral mucosa. Hybrid capture 2 could be a good methodology for large epidemiology studies.

Key words: Papillomavirus, hybrid capture 2, oral mucosa, squamous cell carcinoma, high risk

INTRODUCTION
Human Papillomavirus (HPV) are a large family of small, non enveloped, double-strands DNA virus with 8Kb. They infect the squamous epithelium of the skin or mucosa (3).

Nearly 100 HPV have been described, 35 of them are general found in genital tract. Depending of their association with neoplasias, genital HPV have been classified into high risk or oncogenic, low risk or no oncogenic and probable high risk (3). In 1995 the International Agency for Research on Cancer (IARC) classified HPV types 16 and 18 as human carcinogen (9). Today the presence of oncogenic HPV is a necessary factor to development cervical cancer (12).

Intra-oral squamous cell carcinoma (OSCC) is the eight most common malignancy worldwide (11). DNA HPV has been detected with increased frequency in oral dysplastic and carcinomatous epithelium in comparison with normal oral mucosa (8).

HPV prevalence in oral cavity varies; depending of the methodology used, populations studied and sampling methods. However many studies suggest a relationship between HPV and OSCC (11).

HR-HPV 16 and 18 are the most found into OSCC like in cervical cancer (4). Today the association between HR-HPV and OSCC is still uncertain, knowledge of the prevalence of HPV in normal oral mucosa is an important step among all the epidemiological evidence necessary to establish the association between HPV and OSCC.

The aim of the study was to determine the presence of DNA HR-HPV in normal oral mucosa cells from subjects without oral lesions using hybrid capture 2 (HC2).

MATERIALS AND METHODS
Oral brushings were obtained of 77 consecutive subjects with normal oral cavity. All the subjects were referred to the
Dentistry school, of the Autonomous University of Yucatan, located in Merida, Yucatan, México. A written consent was obtained from all participants before the sampling.

DNA oncogenic papillomavirus detection was done by Hibrid Capture 2 (HC2) (Digene Corporation, Gaithersburg, Md). Briefly, specimens were denatured at 65°C for 45 min and hybridized under high-stringency condition with a mixture of RNA probes that detect 13 oncogenic HPV types. The resultant DNA: RNA hybrids were capture on the superface of the microtiter plate wells coated with anti-DNA-RNA hybrid antibody. The immobilized hybrids reacted with alkaline phosphatase-conjugated antihybrid monoclonal antibody. Light intensity was measured with a luminometer. Three positive and three negative controls were included.

RESULTS AND DISCUSSION

A total of 77 subjects were studied 38 (49.4%) were men and 39 (50.6%) were female. The mean of age was 34.0 (range 19-64 years: SD 10.04); 18/77 (23.4%) were smokers at the moment of the study, 55/77 (71.4%) referred oral sex and 35/77 (45.5%) had knowledge about oral cancer.

The prevalence of high risk HPV was 1/77 (5.71%). The sample was from a 55 years old man, smoker, whom practice oral sex and have knowledge about the presence of oral cancer. The test was successful and validated.

Squamous cell carcinoma of head and neck (HNSCC) is important group of malignant diseases development from the mucosa of the upper aero digestive tract, including oral cavity. Tobacco smoking and alcohol consumption are well know risks factors to development HNSCC. However not all the patients have tobacco or alcohol exposure (2).

In order of the similitude between oral and anogenital epithelium and the well documented knowledge about the oncogenic potential of HR-HPV, many studies to detect HPV DNA in OSCC and premalignat lesions have emerge. However studies from subjects without oral lesions are fewer.

In our study we detected the presence of HR-HPV among subjects with normal oral mucosa. Studies done in different countries have reported low frequency of DNA HPV among healthy subjects. Some studies have reported 5.5% in Italy, 1% in Germany, 0% in Greek, 0.6% in Japan (1,5,7,10). The frequent found in our study (5.71%) is similar to Italian study.

It is important to make know that our sample is small; however the results are important because in Latin-Americans populations there are not enough studies of HR-HPV among subjects without oral lesions.

The role of HPV infection in oral squamous cell carcinoma (OSCC) is not completed defined. Today there are many doubts related to HPV and OSCC but the varying sensitivity of the applied methodologies and the sampling are very important problems to compare results.

HC 2 is a commercially available assay approved by the Food and Drug Administration for the DNA detection of HPV. The HC 2 system is a commercial liquid hybridization kit using RNA probes against HPV DNA genomic targets followed by signal amplification, it detects thirteen carcinogenesis types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) implicated in the pathogenesis of invasive cervical cancer and its precursor lesions (6).

Many studies have demonstrated that HC 2 is a sensitive and specific assay for detecting HPV DNA from cervical specimens. It detects 1.0 pg of HPV DNA target per 1 ml of specimen. One of the most important characteristic of HC 2 is its reproducibility, and there is not possibility of mistake during the testing, because the software invalidity the running when the positives and negatives controls report unexpected readings. One of the limitations of HC2 does not let to know specifics HPV types.

Worldwide HC2 is use for patients with HPV infections or intraepithelial lesions. There are many studies reporting the benefits of used HC2 for cervical cancer screening.

To the best of our knowledge, this is the first study using HC2 to detect DNA PVH in oral mucosa. We consider HC2 as an adequate test for large epidemiology studies because it has reproducibility, good sensitivity and easy sampling, and the results of the different studies will be comparables and the advance of the knowledge about the role of HPV in OSCC will faster.

RESUMO

Baixa prevalência de papilomavírus humano de alto risco na mucosa oral normal através de Captura Híbrida 2

Papilomavírus humano de alto risco (HR-HPV) é um fator reconhecido como necessário para o desenvolvimento de câncer cervical. Na última década vários estudos encontraram HR-HPV em OSCC (oral squamous cell carcinoma) e em mucosa oral normal, mas a associação entre HR-HPV e OSCC não é bem conhecida. O objetivo desse estudo foi determinar DNA de HR-HPV na cavidade oral normal de adultos saudáveis. Realizou-se um estudo cross-sectional com amostras da cavidade oral normal de 77 pacientes da Escola de Odontologia da Autonomus University of Yucatan, Merida, Yucatan, México. HR-HPV foi detectado através de Captura Híbrida 2. Uma amostra em 77 (1,2%) foi positiva para HPV e era proveniente de um homem de 50 anos de idade. Concluiu-se que HR-HPV tem baixa prevalência na mucosa oral normal e a Captura Híbrida 2 pode ser um método adequado para estudos epidemiológicos.

Palavras-chave: Papilomavírus, Captura Híbrida 2, mucosa oral, OSCC, alto risco
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