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Absence of DNA Human Papillomavirus Type 16 in Mexican Women with Normal Pap Smear

¹Maria del Refugio González-Losa, ¹Ana C. Hidalgo-Martínez, ¹Luis Manzano-Cabrera,
²Aremi Estrada-Saldivar and ¹Marylin Puerto-Solis

¹Laboratorio de Virología, Centro de Investigaciones Regionales Dr. Hideyo Noguchi,
Universidad Autónoma de Yucatán. Mérida, Yucatán, México

²Hospital General del Instituto de Seguridad al Servicio de los Trabajadores
del Estado (ISSSTE) Mérida, Yucatán, México

Abstract: To determine the prevalence of human papillomavirus type 16 in women with normal pap-smear. All women self-referring for pap smear were enrolled sequentially during 6 months. During the pap smear, an extra sample of cervical cells were taken and suspended in cold PBS. HPV diagnosis was done by polymerase chain reaction using MY09/MY11 primers. Genotyping was done by specific nested PCR. Four hundred ninety five women were included in the study, 80 (16.16%) were negative to β -globin. The prevalence of HPV was 6.5%. Any sample amplified with specific PCR. The imminent use of the vaccine against HPV 16 makes indispensable epidemiological studies in focus to have data of HPV 16 prevalence and distribution in representative samples with the same methodology in order to have a solid evidence to evaluate the results of HPV vaccination.

Key words: Human papillomavirus, pap smear, Mexican women

Introduction

Cervical Cancer (CC) is the second most common cancer in women worldwide. It is the leading cause in cancer deaths among Mexican women; more than 4000 women died every year (Flores *et al.*, 2003).

Actually human papillomavirus (HPV) infection is recognized as a necessary event to develop cervical cancer. The prevalence of HPV DNA in invasive cervical cancer is 99.7% with the best methodology (Walboomers *et al.*, 1999).

Today, the existence of approximately 200 different types of HPV have been documented; 30-40 have been found in the female genital tract and 18 are referred to as high-risk HPV types due to their association with anogenital cancer (García-Carranca *et al.*, 2003; Calleja-Macias *et al.*, 2005).

HPV prevalence and types distribution varies among diverse world populations; however HPV 16 is the predominant type found in more than 50% of women with a diagnosis of cervical neoplasia (Walboomers *et al.*, 1999).

Although the infectious nature of cervical cancer and the identification of specific viral types associated to development of the neoplasia, it is therefore reasonable to assume that a vaccine that prevents infection will reduce the incidence of cervical cancer (Mandic *et al.*, 2004).

The current study was conducted to gain the prevalence of HPV 16 in women with normal pap smear.

Corresponding Author: María del Refugio González-Losa, Centro de Investigaciones Regionales Dr. Hideyo Noguchi, Avenida Itzáez con calle 59 No. 490, Centro, C.P. 97000 Mérida, Yucatán, México Tel: (99) 24 64 12/137 Fax: (99) 23 61 20

Materials and Methods

All women self-referring for pap smear were enrolled sequentially from the department of medicine preventive at Hospital Regional ISSSTE, Mérida, Yucatán, México, from September 2002 to February 2003. Hospital Regional ISSSTE is a referral center for state workers. After a written consent was obtained from all participants a questionnaire including socio-demographic variables, sexual, reproductive and contraceptive history was answered for each woman. During the pap smear an extra sample of cervical cells were taken. Those cells were suspended in cold PBS. The quality of DNA sample was assessed by PCR using β -globin primers GH20 and PCO4. The presence of HPV DNA was determined by L1 consensus primers polymerase chain reaction (PCR) assay using MY09/MY11 primers. The HPV positive samples were typed by specific nested PCR as describe Wheeler CM (1997). One-tube nested PCR were performed to amplify a 610 bp region of the E6 gene from HPV 16. A mixture of 100 μ L containing 10 mM Tris, pH 8.5, 50 mM KCL, 200 mM of each oligonucleotide (dATP, dCTP, dGTP, dTTP), 2.5 mM MgCl₂, 2.5 U TaqDNA polymerase, Two primer pairs were added to the initial amplification reactive mixture, outer primer pair CEG 70/71 (31-662 pb) were added at 0.1 μ M. concentration. The inner primer pair CEG112/113 (56-640 pb) were added at 1 μ M concentration. The cycles were as follows: one denaturalization cycle at 95°C for 9 min, 35 cycles of 94°C (30 sec), 57°C (30 sec) and 72°C (60 sec). Subsequently, we used 40 cycles of 94°C (30 sec), 47°C (30 sec), 72°C (60 sec) and 5 min at 72°C for final elongation (Wheeler *et al.*, 1997). Two cervical samples previously typed by reverse blot strip assay were used as positives control (González-Losa *et al.*, 2004).

Results

Four hundred ninety five women were included in the study, 80 (16.16%) were negative to β -globin, so the results are of 415 patients. The overall prevalence of HPV was 27/415 (6.5%). The highest frequency was found in 36-45 years old women.

The epidemiological characteristics of HPV positive women were: 74% were between 26 and 55 years old, 74% were married, 52% live in urban areas, 52% have at least high school education. Sixty six percent had been pregnant at least one time, 26% reported 4 or more live births and 92.6% had their first pregnancy after 18 years. Eighty five percent has there first intercourse older than 18 years, 77% reported only one lifetime sexual partner. No body uses contraceptive method, only 7.5% were current smokers. When specific nested PCR for HPV 16 were done for the 27 HPV positive samples, none were positive.

Discussion

CC is a major worldwide health problem; 500,000 new cases are reported every year, 50% of these women die.

The Mexican National Cervical Cancer Screening Program (NCCSP) was implemented in 1974; however the mortality rates have remained stable. More than 4,000 women die every year (Bosch, 2003; Flores *et al.*, 2003). This makes prevention, control and treatment a national priority.

After decades of studies, it has been established that CC is a sexually transmitted disease, HPV infection is a necessary event for it develops. Actually, more than 35 strains of HPV have been identified in the genital tract and classified into low and high-risk viruses according to their capacity to induce malignant transformations in the epithelial cells of the cervix. The distribution and prevalence of the different HPV types vary from one geographical region to another, however type 16 is considered to be responsible for more than 50% of the CC throughout the world (Clifford *et al.*, 2003).

The knowledge of the infectious nature of CC has altered scientist's view in respect to the prevention of this pathology, proposing vaccinating women against HPV 16 before the onset of their sexual life as an important alternative to reducing the incidence of CC (García-Carranca, 2003).

The majority of HPV studies carried out in Mexico have focused on women with CC and/or precursory lesions; very few have included women without any cervical pathology. Likewise, in the last few years, studies have focused on determining high risk HPV using the capture of hybrids with probes for high-risk viruses and the determination of specific types has passed to a second level. This has resulted in the specific information on HPV 16 being limited.

The prevalence of HPV 16 reported in Mexican women without cervical pathology fluctuates between 0 and 13.7% (Hernández-Avila *et al.*, 1997; Torroela-Kouri *et al.*, 1998; Lazcano-Ponce *et al.*, 2001; Rodríguez-Reyes *et al.*, 2003) a difference, which could be explained by the different techniques used, or the individual characteristics of each population.

Giuliano *et al.* (2002) have demonstrated that risk factors for high and low risk HPV are different. Most of the women HPV positive in our study does not present the epidemiological profile associated with high risk HPV, which could explain the absence of HPV 16.

Mexico is a large country composed of 32 states with a population consisting of different cultural groups. Data on HPV prevalence in general and type 16 in particular is very limited. The imminent use of the vaccine against HPV 16 makes awareness of this fact indispensable.

In this context we consider our study to be important, since a better understanding of the epidemiology of HPV 16 infection, constitutes a relevant point for planning and reinforces preventative strategies.

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