European Journal of Obstetrics & Gynecology and Reproductive Biology 170 (2013) 468–473

Contents lists available at SciVerse ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Human papillomavirus infection and spontaneous abortion: a case–control study performed in Mexico

Laura Conde-Ferráez [a,*], Alberto de A. Chan May [b], Jorge R. Carrillo-Martínez [b], Guadalupe Ayora-Talavera [a], María del Refugio González-Losa [a]

[a] Centro de Investigaciones Regionales, Universidad Autónoma de Yucatán, Mérida, Yucatán, Mexico
[b] Centro Médico Nacional "Lic. Ignacio García Téllez", Instituto Mexicano del Seguro Social, Mérida, Yucatán, Mexico

ARTICLE INFO

Article history:
Received 28 November 2012
Received in revised form 12 April 2013
Accepted 2 July 2013

Keywords:
HPV
Spontaneous abortion
Pregnancy
Young women

ABSTRACT

Objective: To investigate if HPV cervical infection is associated with spontaneous abortion in a Mexican population.
Study design: Case control study including 281 women from two Social Security Hospitals in Merida, Mexico. Cases were women with spontaneous abortion attending for curettage, and controls were pregnant women at term who attended for delivery. HPV molecular detection and typing of HPV 16, 18, 58 and 6/11 was performed on cervical samples, and TORCH serology IgM tests (against *T. gondii*, CMV, HSV) were performed on cases. Data were analyzed using Chi square, odds ratio and linear regression tests.
Results: HPV global prevalence was 19.8% (24.4% in cases and 15.2% in controls). HPV types 16 and 58 were the most frequently detected in both groups. Multiple HPV types concurrent infection were found in 31.4% of typified samples. Amongst cases 27.3% of HPV positive women reported at least one previous pregnancy loss; compared to 17.43% amongst HPV negative women. Nevertheless, HPV was not significantly associated with spontaneous or to repetitive abortion. Cases were 60.2% positive to any TORCH agent, although it was not significantly associated to referred miscarriage history. Spontaneous abortion was associated to a previous pregnancy loss and to women's age older than 35 years old. HPV infection was significantly associated to alcohol intake before pregnancy and to multiple sexual partners.
Conclusion: HPV cervical infection was not associated with spontaneous abortion. HPV in spontaneous abortion and other adverse pregnancy outcomes merits further study.

1. Introduction

Spontaneous abortion is one of the most frequent adverse pregnancy outcomes, estimated to occur from 12% up to 76% of all pregnancies [1–3]. The most common causes of pregnancy loss in the first trimester are of genetic origin [4]. Amongst other important risk factors are maternal age younger than 20 or older than 35 years old, placental inflammation and infection [5,6], but the etiology is often uncertain.

Some of the infectious agents believed to be involved in pregnancy loss and congenital malformations are included in TORCH screening: *Toxoplasma gondii*, cytomegalovirus (CMV), rubella, and herpes simplex virus (HSV), amongst others. Other infectious agents that are not routinely screened for in infertile couples could be associated with spontaneous abortion. This is the case with human papillomavirus (HPV), which is the most frequent sexually transmitted virus, and considered the necessary cause of cervical cancer [7]. HPVs have been reported to infect trophoblasts and to be involved in abnormal placentation [6]. Some studies have described HPV infection in placenta and curettage material with high frequencies from 60% up to more than 80%, whilst HPV DNA has been found in 70% of spontaneous abortion products in contrast to 20% of elective abortion specimens [6,8–10].

In vitro experiments have achieved productive infection of trophoblast cell lines with different HPV types and suggested that HPV could be related to placental dysfunction and to altered endometrial adherence [11–13]. Early experimental evidence with murine models suggested that HPV could affect the survival or apoptosis of embryos, depending on the developmental stage involved [14,15]. Moreover, clinical studies have found HPV DNA in the amniotic fluid and peripheral blood, supporting the ascending infection and possibly the transplacental transmission hypotheses [16,17].

* Corresponding author at: Centro de Investigaciones Regionales, Universidad Autónoma de Yucatán, Edificio Inalámbrica Calle 96 s/n x Ave Jacinto Canek & calle 47, Paseo de Las Fuentes, Merida, Yucatan, CP 97225, Mexico.
Tel.: +52 99 924 68 09x1213; fax: +52 99 9236120.
E-mail addresses: laura.conde@uady.mx, lauracf@gmail.com (L. Conde-Ferráez).

0301-2115/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ejogrb.2013.07.002

Epidemiological evidence on the role of HPV in spontaneous pregnancy loss, however, is still scarce. We therefore performed a case–control study to investigate the possible association of HPV cervical infection and spontaneous abortion.

2. Materials and methods

2.1. Population

A case–control study was performed including patients from the obstetric care services of two social security hospitals belonging to Instituto Mexicano del Seguro Social (IMSS): Hospital General Regional # 1 Licenciado Ignacio García Téllez and Hospital General Regional #12 Benito Juárez García. Both are located in Merida, Yucatan (Southeast Mexico). Sample size was calculated using EpiInfo, with a 1:1 cases-controls ratio (95% confidence, 90% power), giving a total of 127 cases and 127 controls. Cases were defined as women experiencing spontaneous abortion up to 20 weeks of gestation, who attended for medical attention and/or a curettage procedure. Controls were defined as women attending for delivery at term, with viable products.

2.2. Ethical considerations

All participants signed an informed consent form. The project was approved by the corresponding scientific and bioethical committee.

2.3. Sampling procedure for HPV detection

After informed consent from the woman, cervical cell samples were collected with a cytobrush and deposited in 5 ml of PBS with penicillin 500 U/ml, streptomycin 500 μg/ml, and gentamicin 4 mg/ml. Samples were kept at 4 °C during transportation to the Centro de Investigaciones Regionales for further analysis.

2.4. Molecular methods

Cervical samples were washed twice with PBS, the cellular pellet was resuspended in 200 μl of PBS, and total DNA was extracted using DNAeasy Blood and Tissue Kit (Qiagen).

PCR detection of HPV DNA was performed using My09-My11 universal primers which amplify a 450 bp region from the L1 gene [18]. As internal control, a 268 bp region from the human β-globin gene was amplified using GH20 and PCO4 primers [19]. PCRs were performed in a volume of 50 μl, with 200 μM dNTP's, 3 mM $MgCl_2$, 50 pmol each oligonucleotide, 1 U GoTaq polymerase (Promega). Thermal cycler conditions as follows: 95 °C, 9 min; 38 cycles of 95 °C 1 min, 55 °C 1 min, 72 °C 1 min; final elongation 72 °C, 5 min.

HPV positive samples were typified according to conditions reported by Sotlar et al. [20], but using only primers specifically to detect high risk HPV types 16, 18 and 58, and low risk types 6/11. Other genotypes were not tested for.

2.5. Serology

To detect the possibility of TORCH infectious agents that could be involved in spontaneous abortion, blood-serum samples were obtained by venepuncture, to perform IgM detection against *T. gondii*, CMV and HSV active infection using commercial kits (HERPES 1 & 2 ELISA IgM Kit, MP Biomedicals; CMV and *T. gondii* EIA IgM kit, Calbiotech). IgM against rubella was not screened, as the incidence has been significantly reduced since a universal vaccination program started in Mexico in 1998 [21]. Only cases were analyzed because blood samples were not available for the control group.

2.6. Database analysis

An instrument was applied to collect data concerning socio-demographic data, clinical, sexual and reproductive histories, and tobacco, alcohol and contraceptive use. All information and laboratory results were included in a database using SPSS software. Frequency of variables, chi square and crude odds ratios were calculated using EpiInfo2000. Linear regression analyses were performed to all variables that resulted significant in the bivariate tests.

3. Results

3.1. Characteristics of the studied population

In total, 281 women were included in this study; corresponding to 143 cases and 138 controls. From these, 87% were of urban origin, and the rest were from suburban and rural communities. The age range was 14–47 years (mean 27 years).

Regarding the cases, the age mean was 28.6 years, ranging from 14 to 47 years: 79.7% (114/143) were younger than 35 years old, and only 7.7% (11/143) were younger than 20 years old. Eighty-six per cent (123/143) were in the first trimester of gestation and 14% (20/143) from 13 to 20 weeks of gestation. From the 143 cases, 20.56% (29/141, 2 missing data) had a previous pregnancy loss (from 2 to 3).

Regarding controls, the age average was 25.3 years, ranging from 16 to 38 years, and the average gestation was 39 weeks (range 36–42). Most of the women delivered vaginally (83.3%), with only 16.7% delivering by cesarean section.

3.2. HPV infection

Three samples were excluded because they did not amplify the internal control β-globin, and the cervical sample from one patient was not available. Therefore 277 were included in HPV testing (139 cases and 138 controls). From these, 55 were HPV positive (34 cases and 21 controls) indicating a prevalence of 19.8% in the studied population.

HPV prevalence was 24.4% in cases and 15.2% in controls. The analysis of risk factors for HPV infection included: number of sexual partners, age at first sexual intercourse; age at first pregnancy; use of hormonal contraceptives in the last 2 years; history of sexually transmitted diseases (STDs); number of gestations; alcohol consumption and tobacco exposure. Results are presented in Table 1: as shown, on bivariate analysis HPV infection is significantly associated with more than two sexual partners, alcohol consumption and passive tobacco exposure. Linear regression analyses showed significance for the following independent variables: number of sexual partners in the last six months ($p = 0.045$) and alcohol consumption before pregnancy ($p = 0.003$). Total number of sexual partners and tobacco exposure were not significant ($p = 0.101$ and $p = 0.198$ respectively).

From the 55 HPV positive samples, 34 tested positive with the specific primers used (21 cases and 13 controls): the distribution of the genotypes is shown in Table 2. Overall, 23 samples had single infections detected and 11 had multiple infections; 21 were negative with these primers.

3.3. Serology against TORCH agents

Blood-serum samples were obtained from 143 cases. Two of them were excluded for being insufficient or inadequate (i.e. haemolysis) and therefore 141 serum samples were finally processed in EIA IgM assays against TORCH agents. The results