Human papillomavirus (HPV) in the genital area is thought to be transmitted primarily through direct mucosal contact and is causal in the development of cervical cancer (KANODIA et al., 2007), in a larger proportion of other carcinomas in the lower genital tract, and in benign lesions (SINAL; WOODS, 2005). Persistent infection with oncogenic, high-risk (HR) HPV types is particularly associated with development of these preneoplastic and malignant lesions and evidence suggests that progression of cervical lesions is almost always associated with persistence of HPV-HR types (BURD, 2003; HEBNER, LAIMINS, 2006; YU et al., 2005).

Resumo: mais de 130 tipos de HPV foram identificados. Somente um terço destes tende a causar infecções anogenital, oral ou laríngea, predominantemente os tipos 6, 11, e 16. A infecção perinatal pode ocorrer através do líquido amniótico durante a gestação e na exposição direta com as lesões na cérvix uterina durante o nascimento. Este estudo objetivou detectar o genoma do HPV em mulheres grávidas.

Palavras-chave: HPV. Gravidez. PCR.
The majority of papillomatous lesions (warts) regress spontaneously within 2 years when immunocompetent hosts mount an effective cell-mediated immune response. More than 130 types of HPV have been identified to date. Only one third of these tend to cause anogenital, oral, or laryngeal infections, with types 6, 11, and 16 predominating among these (DRAGANOV et al., 2006).

The HPVs stimulate the papillomatosis proliferation in airways, compromising respiratory and larynx regions. A concerning factor is the dissemination through the tracheobronchial tree evolving to pulmonary papillomatosis, resulting in a fatal and uncontrollable infection (MEDEIROS et al., 2005; CONEJO et al., 2001). The illness gains importance as it evolves and attacks children and preadolescents, presenting serious morbidity with complications and reduction of life quality, in result of many surgeries, cirurgical incisions and tracheostomy needed having a bad prognostic, including dead (DRAGANOV et al., 2006; WIATRACK et al., 2004; GOMEZ et al., 1995; KRAMER et al., 1985).

Children may acquire anogenital, laryngeal, or oral HPV infections in a variety of ways (SYRIPINEN & PURONEN, 2000). Perinatal infection may occur transplacentally (hematogenously) via amniotic fluid during gestation and delivery and through direct exposure to cervical and genital lesions during birth (RUFFIN et al., 2006; KUI et al., 2003). The HPV is considered the ethiological agent of the laryngeal papillomatosis (SILVA et al., 2003; AUBORN et al., 1994).

A pilot study was done aiming to detect the HPV prevalence in 45 pregnant women (in the pregnancy 3rd trimester) with no history of sub clinic HPV lesions aiming to evaluate the possibility of transmission to the newborn.

MATERIAL AND METHODS

Sample Group

A random study including 45 women in their third trimester was performed. All the participants were volunteers from the Medical Service of Family Health Program (PSF) in Campinorte - GO. All the patients signed a commitment agree attesting their voluntary participation.
Attainment Of Samples

After signing the commitment agree the individuals answered a questionnaire about their sexual life. Then the samples were harvested with bristles swab, after scrubbing the cervix region, the swab was washed with 3% alcohol - acid solution (methanol - acetic acid) to loosen the cells adhered to the swab bristles and for the harvested cells preservation. The samples were kept between 2-8 ° C until the DNA extraction.

DNA Extraction

For the extraction the samples were submitted to centrifugation at 14 000 rpm for 30 minutes to separate the cells from the alcohol-acid solution. After, the samples were submitted to the DNA extraction with the genomic purification kit Wizard (Promega Corporation, USA).

Polymerase Chain Reaction

The PCR reactions were done accordingly to the protocol considered by Levi et al. (1988), using the final volume 25 μl, contenting proximally 100 g of genomic DNA, 10 mM Tris - HCL, pH 8.3, 50 mM KCL, 2.5 mM MgCl2, 200 uM of with dNTP, 0.5 U of Taq polymerase DNA. For the detection of HPV genome universal consensus primers GP5/6 (F: 5’ - TTT GTT ACT GTG GTA GAT ACT AC - 3’ / R: 5’ - GAA AAA TAA ACT GTA AAT CAT ATT C – 3’) were used, that amplifies the fragment of 170 bp of the viral DNA. For the positive HPV genome samples was done a PCR aiming to genotype the virus, using type-specific primers forHPVs 6, 11, 16 and 18.

PCR Fragments Analysis

For the analysis of the PCR products, the DNA was submitted to electrophoresis in a 10 V/cm constant electric camp in 8% Polyacrylamide gel in TBE 1X. To visualize the DNA, the gel was stained with silver nitrate solution (5μg/mL) and the images captured and analyzed in a video documentation system Image Master (VDS Amersham Pharmacia Biotech, EUA)®.
Results

In the 45 pregnant women group the age average was 21 years old, being the youngest 14 and the oldest 33 years old. The molecular analyses results showed a 4.44% (2/45) positive result for the viral genome and both individuals were 19 years old.

For the samples amplified with GP5+/6+ (Figure 1), we tried to genotype with type-specific primers for HPV 6, 11, 16 and 18. The observed amplifications (Figure 2) show that none of the pregnant women presented an amplification for the type-specific primers used, suggesting a different viral type. Therefore, it was impossible to genotype the participants HPV through the intended proposal.

Figure 2: Genotyping of HPVs 6, 11, 16 and 18: in C+, using as positive controls HPV 11 – 90pb. The 50bp ladder (Ld) was used.
DISCUSSION

The proposal of this pilot study was to optimize the detection of the HPV in women in the pregnant state, using cervical washed samples. The samples showed degraded DNA, therefore, primers MY9/11 were not used, even being considered sensible and of initial triage in relation to primers GP5+/GP6+ (COUTLÉE et al., 2002; FERNÁNDEZ-CONTRERA et al., 2000).

A number of molecular methods were utilized, such as: in situ hybridization, amplification by PCR and DNA sequencing, the most used one among them is the PCR with consists in the use of the universal consensus primers that amplify reserved regions of the virus. In this context, only two analyzed participants showed amplification for the HPV genome, since the samples showed a degraded DNA. Specific primers for viral subtypes 6, 11, 16 and 18 were used in the PCR to genotype the virus. Genotyping the viral type was not possible, suggesting a different viral type, that could be identified using other type specific primer or trough amplified DNA sequencing. The difficulty in genotyping the viral genome found in the samples was a consequence of the great amount of existing viral types, exceeding the hundreds (FERNÁNDEZ-CONTRERA et al., 2000).

The influence of the pregnancy in the detection of HPV is controversial; however the pregnancy period might interferer in the evaluation. PENG et al. 2000, showed that an average of 34 analyzed women in the pregnancy period using the same specific primers as our study, seven women presented infection by viral types 16 and 18. They also demonstrated that the infectivity rate for HPV is significative in the third month, but there is no significative difference between the first the second and the third trimester. Worda et al. (2005), found 36,6% (56/153) of HPV in pregnant women who were analyzed using the PCR technic and hydride capture and the statistics analysis showed a regression of the HPV infection with the increase of the maternal age (P = 02).

Related studies showed that the rate of HPV DNA detection of the mother and the new-born varies from 4% to 87%. Studies confirmed that vertical transmitted HPV can cause high airways infection or genital infections in children. However the HPV infection can probably be attained during the baby passage trough
the birth canal, the transmission in the uterus or in the postnatal position also seems possible (CASON, MANT, 2005; SINAL, WOODS, 2005; NETO et al. 2002; CZEGLEDY, 2001; CASON et al., 1998).

In the present study, the HPV genome was identified in 4.44% (2/45) of the cases. Additionally, the number of molecular studies being developed with the objective of understanding the vertical transmission and the possibility of the newborn contamination leading to a laryngeal papillomatosis and recurrent respiratory papillomatosis (CONEJO et al., 2001), in a result of the HPV potential oncogenic risk (DRAGANOV et al., 2006; FREGA et al., 2006; TORRENTE et al., 2005). The exact path from where the children papillomatosis origins is of crucial importance for the prevention of the birth canal contamination. It has to be evident that all vaccination programs could decrease the incidence of HPV infections by introducing the HPV vaccine.

HUMAN PAPILLOMAVIRUS IN PREGNANT WOMEN CERVICAL WASHED

Abstract: more than 130 types of HPV have been identified to date. Only one third of these tend to cause anogenital, oral or laryngeal infections, with types 6, 11, and 16 predominating among these. Perinatal infection may occur via amniotic fluid during gestation and delivery and through direct exposure to cervical and genital lesions during birth. This study aimed to detect the HPV genome in pregnant women.

Keywords: HPV. Pregnancy. PCR.

References


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LEONARDO BARCELOS DE PAULA
Biólogo da Universidade Católica de Goiás, Núcleo de Pesquisas Replicon. Goiânia, Goiás, Brasil.

ANGELA ADAMSKI DA SILVA REIS
Doutoranda em Biologia Celular e Molecular do Instituto de Ciências Biológicas da Universidade Federal de Goiás Biológicas. Goiânia, Goiás, Brasil.

CAROLINE DIAS MONTEIRO
Iniciação Científica – PIBIC-CNPQ, Núcleo de Pesquisas Replicon, Departamento de Biologia – Universidade Católica de Goiás, Goiânia, Goiás, Brasil.
APARECIDO DIVINO DA CRUZ
PhD em Biologia Molecular pela University of Victoria, Canadá, Professor Titular no Departamento de Biologia da Universidade Católica de Goiás, Coordenador do Núcleo de Pesquisas Replicon da Universidade Católica de Goiás e Biomédico Geneticista da Superintendência de Ciência e Tecnologia em Saúde - Leide das Neves Ferreira, LaGene Laboratório de Citogenética Humana e Genética Molecular do Estado de Goiás. Goiânia, Goiás, Brasil.