The Brazilian Journal of
INFECTIOUS DISEASES
www.elsevier.com/locate/bjid

Original article

Uncommon non-oncogenic HPV genotypes, *TP53* and *MDM2* genes polymorphisms in HIV-infected women in Southern Brazil

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ARTICLE INFO

Article history:
Received 11 November 2013
Accepted 11 July 2014
Available online 1 September 2014

Keywords:
HPV infection
HIV infection
Cervical cancer
Epidemiology

ABSTRACT

Background: It is believed that Human Papillomavirus (HPV) and Human Immunodeficiency Virus coinfection contributes to increase the risk for cervical intraepithelial injuries. Several factors may contribute to cervical cancer (CC) development, including genetic variants such as *TP53* and *MDM2* gene polymorphisms.

Materials and methods: A hundred HIV-infected women were examined for HPV detection and its genotypes, as well as the frequencies of the SNPs Arg72Pro and SNP309 and their associations with CC risk factors. Nested Polymerase Chain Reaction (nPCR) was used for HPV detection and PCR-RFLP for *TP53* and MDM2 SNP309 genotyping.

Results: HPV DNA was detected in 68% of samples. A higher frequency of low-risk HPV genotypes (66.7%) was observed when compared to high-risk genotypes (33.3%). Nine different HPV genotypes were identified, with the highest prevalence of HPV-6, followed by HPV-16 and 31. p53 Arg72Arg and SNP309 TG genotype were the most prevalent. HPV genotyping was performed by sequencing.

Conclusion: The data obtained suggest that HIV-infected women are more susceptible to be infected by low-risk HPV (LR-HPV) genotypes than by high-risk (HR-HPV), and Pro72Pro of *TP53* gene and TG of MDM2 SNP309 genotypes apparently seem to be protective factors among HIV-infected women for HPV acquisition and HR-HPV infection, respectively, in a sample of Southern Brazilian woman. Future investigations in larger populations are necessary to better understand the potential roles of these SNPs and the behavior of non-oncogenic HPV genotypes in HIV-mediated immunosuppression cases.

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http://dx.doi.org/10.1016/j.bjid.2014.07.005
1413-8670/

Introduction

It is estimated that cervical cancer (CC) is the second most common type of cancer in the Brazilian female population and the third cause of death in women.[1] Today, it is possible to confirm that CC development is closely associated with the Human Papillomavirus (HPV) presence and persistence,[2] with HPV DNA detected in up to 99.7% of invasive CC cases across the world.[3] The World Health Organization attributes to cervical infection with HPV-16 or -18 about 70% of invasive CC cases in Brazil.[4] Cervical intraepithelial neoplasia (CIN) development and cervical cancer in HPV-infected women are associated with risk factors, such as young age, high number of sexual partners throughout life, early sexual debut, smoking, genetics variants, among others.[5,6] Moreover, co-infections, such as those with Human Immunodeficiency Virus (HIV) and *Chlamydia trachomatis* bacterium, may be involved as co-factors to CC development, acting as adjuvants of the neoplastic process.[7,8]

The polymorphism on codon 72 (Arg72Pro) of *TP53* tumor suppressor gene has been extensively investigated regarding association with a wide range of cancers worldwide. In HPV-infected cells, the E6 oncoprotein binds to p53 protein and promotes its degradation through an ubiquitin proteolytic system altering the p53 activity in some processes, such as tumorigenesis, transcription regulation, telomerase activation, and apoptosis, thus resulting in deregulation of the cell cycle.[9] Previous studies have shown that the Arg72Arg genotype is related to a higher risk for CC development when compared to the Pro72Pro genotype.[10]

Studies have shown that a Single Nucleotide Polymorphism (SNP) on promoter region of MDM2 gene, the SNP309 (T to G change on nucleotide 309 of the first intron), results in a higher level of MDM2 mRNA and MDM2 protein, and consequent reduction of the p53 pathway.[11] The SNP309 occurs at a relatively high frequency in the general population, and it was shown that it presents a strong association with HPV-mediated cervical carcinogenesis.[12]

Based on the presented data, this study aimed to investigate the HPV infection spectrum, to identify the most prevalent genotypes, to determine the frequencies of SNPs Arg72Pro and MDM2 SNP309, and their association with possible risk factors for viral persistence and for the development of pre-neoplastic and neoplastic lesions of the uterine cervix in HIV-infected women. The study was conducted in patients of the Service of Specialized Care for HIV/AIDS, College of Medicine, Federal University of Pelotas (*Serviço de Assistência Especializada em HIV/SIDA* – SAE-UFPel).

Materials and methods

Study type, characterization and sample size

This was a cross-sectional study consisting of 100 HIV-infected women, which were sequentially randomly invited to participate. The eligibility criteria were: not being in the menstrual period, aged between 18 and 45 years, and not being pregnant. Women who had undergone cervical conization and/or hysterectomy were excluded. The Epi-Info 6.0 software was used to calculate the required sample size for the prevalence study, using an estimated HPV prevalence of 80%, with a 95% confidence level and 80% testing power.

Logistics

All participants completed a standardized questionnaire with the purpose of obtaining information about the patient and other relevant epidemiological and socio-demographic variables. An adapted questionnaire from an intervention study with HIV-infected (HIV+) women conducted at SAE-UFPel was used.[13] The Research Ethics Committee of the College of Medicine of the Federal University of Pelotas approved the present study by June 2009, and informed consent was obtained from all participants. All procedures were conducted according to the Helsinki Declaration guidelines.

Sample collection and DNA extraction

Cytobrushes were collected containing cervical secretion samples from the endocervical region. The collected samples were stored in eppendorf tubes containing 300 μL of Cellular Lysis Solution (PUREGENE^{TM}Gentra Systems Kit) and routed in an appropriated container to the Laboratory of Functional Genomics (Center for Technology Development – CDTec-UFPel) for viral molecular detection by Polymerase Chain Reaction (PCR). Before DNA extraction, the samples were subjected to enzymatic digestion with 1.5 μL of Proteinase K (10 mg/mL) and incubated for 16 h at room temperature. The extracted DNA was performed according to manufacturer's specifications (PUREGENE^{TM}Gentra Systems Kit).

TP53 gene polymorphism analysis

The Arg72Pro polymorphism of *TP53* gene was analyzed by PCR using the 72A and 72S primers described by Lin et al.[14] The amplification conditions consisted of an initial denaturation at 94 °C for 3 min followed by 40 cycles at 94 °C for 30 s, 57 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 3 min.

The PCR product obtained was subjected to enzymatic digestion by RFLP (Restriction Fragment Length Polymorphism) using the *Bst* UI restriction enzyme for restriction fragments analysis. The fragments obtained were analyzed by agarose gel electrophoresis (2.5% agarose in TBE buffer), stained with GelRed^{TM} (Biotium Inc., CA) and observed under ultraviolet light (UV). The p53 genotypes were characterized according to Lin et al.[14]

MDM2 SNP309 analysis

The MDM2 T/G SNP309 polymorphism was analyzed by PCR using the primers described by Sotomaa et al.[15] The reaction was performed with initial denaturing at 94 °C for 10 min followed by 30 subsequent cycles at 94 °C for 30 s, 60 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR products were analyzed by agarose gel electrophoresis (2%