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Short Comunications

Molecular Identification of Human Papilloma Virus Genotypes in Patients With Cervical Cancer

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ABSTRACT

Introduction: The principal cause of cervical cancer is human papillomavirus. In Camaguey population, molecular study about human papillomavirus does not exist. The Polimerase Chain Reaction is a molecular procedure often used for medical diagnostic; it allow to confirm the human papillomavirus DNA in the total DNA purified of cervical cancer patients sample.

Objetive: The first time demonstration the human papillomavirus circulating in the cancer cervical patients of Camaguey population, Cuban.

Methods: The analytic study was carried out with 22 patients of the pathology consult of Genecology hospital of Camaguey. The identification and characterization of human papillomavirus genotype was carried out through restriction fragment lenght polymorphic procedure.

Results: The mean age was 52.3 years and the more frequency was the 50-59 age group in 50% of the women. Were studied by cytology only 10 women and the false negative of the cytology was of 80%. The 63.3 percent of patients showed exophytic lesion, 4.5 percent endophytic lesion and 3.8 percent another lesion. Were studied by cytology only 10 women of the total participants and the false negatives were of 80%. The 16 and 31 was the circulation genotypes in the Camaguey population and the 16 genotype was the most frequently.

Conclusions: This is the first report of molecular study of human papillomavirus in cervical cancer patients on Camaguey, Cuban. Present research and the other research of Cuban authors is an important contribution to therapeutic and prevent vaccine more effective in the solution anticipate for the cervical cancer in Cuban.

Keywords: Human Papillomavirus, PCR, RFLP, Cervical Cancer.

METHODS

The study was carried out with 22 patients of the pathology consult of Genecology hospital of Camaguey.

The sample was taken from cervical lesions at the time of physical examination and processed according to the Wizard SV Genomic DNA Purification System protocol of Promega Corporation.[1]The identification and characterization of human papillomavirus genotype was carried out through restriction fragment lenght polymorphic procedure (RFLP), using Polymerase Chain Reaction (PCR) and restriction enzymes. The MY09 / 11 consensus primers used in PCR, recognize a complementary nucleotide sequence in the L1 gene of several High-risk oncogenic HPV HPV-HRs and low-risk oncogenic HPV (HPV-LR), generating a DNA segment of 450 base pairs (bp). As a negative control, we used human genomic DNA from a patient not infected with HPV and, as an internal amplification control, we used the PC04 / GH20 primers, which generate a 268 bp DNA segment from the gene coding for the human β globin protein.[2] For the PCR, the following parameters were set in the thermocycler (MultiGene TC050-18, Sweden): initial denaturation 5 min. at 95 ° C, and then 40 cycles were performed, consisting of denaturation 1 min at 95 ° C, hybridization 1 min at 55 ° C, extension 2 min at 72 ºC. After the cycles, a long extension of 15 min was performed at 72 ºC. For HPV typing, a diagnostic algorithm was first designed and the restriction enzymes used were: Pst I (Applichem, Germany), Dra I (New England BioLab, United Kingdom), EcoRI

(Applichem, Germany), Msp I (New England BioLab, United Kingdom), according to the diagnostic algorithm.

RESULTS

The mean age was 52.3 years with a SD of 10.3, and the more frequency was the 50-59 age group in 50% of the women. (Table 1)

Table 1. Distribution of the age in the 22 patients under study

Age groups	Frequency	(%)
30 a 39	4	18,2
40 a 49	3	13,6
50 a 59	11	50,0
60 a 69	4	18,2

Were studied by cytology only 10 women of the total participants (45.5%). From these data, the false negative of the cytology was of 80% and only 2 patients were positive in 20%. (Table 2)

Tabla 2. Distribution of the cytologic findings in the 45.5% of women

Cytologic findings	Frequency	(%)
Positive	2	20
Negative	8	80
Total	10	100

The 22 patients who entered the study belong to the province of Camagüey. In 14 patients, exophytic type lesions were found for 63.6%, endophytic type lesions were found in 4.5% in one patient, and other types of injuries in 31.8% in 7 patients. (Figure 1)

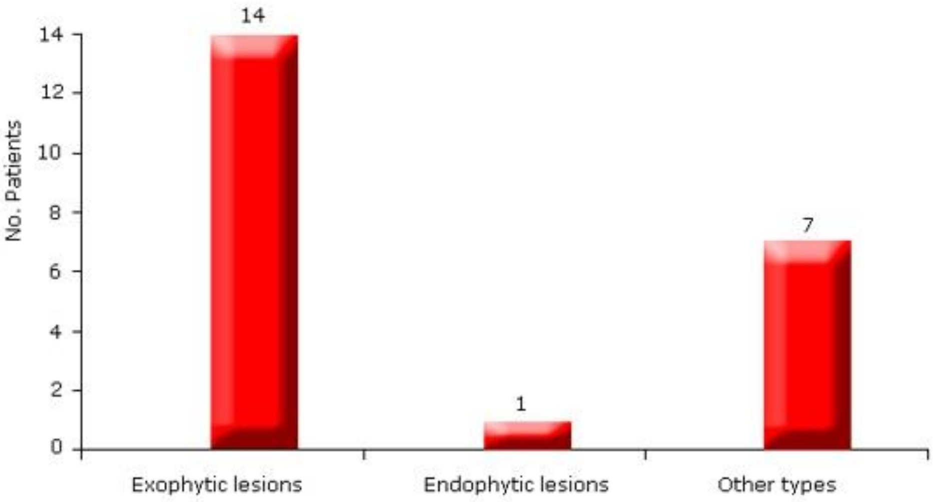

Fig. 1. Distribution of lesions found in the 22 patients under study.

Genomic DNA was purified from the cervical samples obtained from each patient and its quality was verified by DNA electrophoresis in 0.8% agarose gel.

A segment of 450 bp was amplified by PCR from the L1 gene of the HPV genome, from the DNA extracted from each sample of the patients with a positive diagnosis of CCU, for a 100% positivity.

Through RFLP, the HPV-HR 16 genotype was identified in 21 patients for 95.4% and only the genotype PHV-HR 31 was identified in a single patient for 5.6%. In none of the patients studied was the presence of multiple infections with several HPV-HR genotypes. (Figure 2)

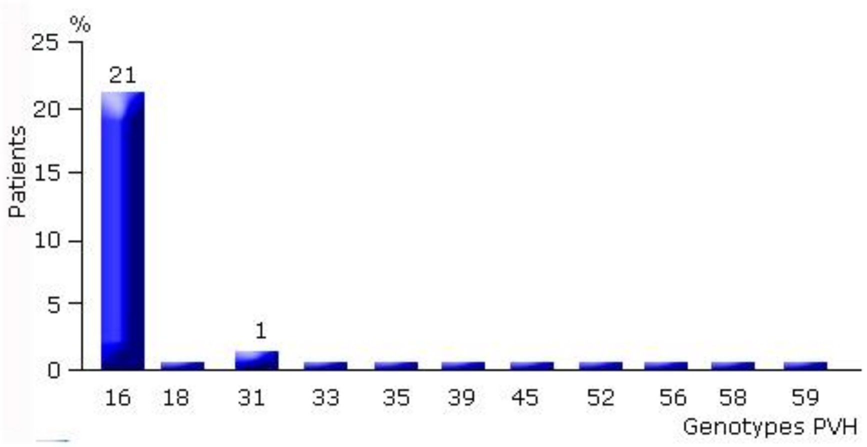

Fig. 2. Distribution of PVH-HR genotypes identified in the 22 patients under study, using the RFLP procedure.

The results of the sequencing of the segments amplified by PCR from each sample of patients were subjected to the analysis of sequence alignment, using the BLAST tool of the NCBI. This analysis confirmed that the sequences subjected belonged to the L1 gene of the HPV-HR 16 and HPV-HR genomes and from the HPV-HR 31.

DISCUSSION

There have been numerous molecular investigations in the world on the subject of HPV in cervical cancer,[3] however, today there is very little information about the Cuban population in relation to the circulating genotypes in the different provinces of Cuba.

As background, the work of Río Hernández M. and collaborators was reported, who conducted an investigation with 45 patients, 44 patients from Havana, the HPV-HR genotypes detected were 16,18,31,39,45,51; where the most frequent was the HPV-HR 16 genotype.[4] On the other hand, the works of Soto Brito Y. and collaborators are also reported. This has carried out very important molecular studies of HPV, where the fundamental purpose has been to determine the sensitivity, feasibility and normalization of the qualitative PCR technique [5] and more recently quantitative PCR,[6] and in last case, all samples were examined by Real Time PCR and the HPV-HR genotypes 16, 18, 31, 33 and 58 were identified. In the present investigation, the molecular studies were done in the basic sciences laboratories of the Medical Sciences University, Camagüey, Cuba. The mean age was 52.3 years with a SD of 10.3, and the more frequency was the 50-59 age group in 50% of the women. The prevalence of HPV infection decreases as the age of the patients increases, this fact corresponds to several studies and suggest that HPV infection is associated with younger women, because at this stage of life, women have more sexual activity.[7]

However, the results of the present investigation do not match those studies. One possible explanation for this is that the development of CCU is also associated, as other authors have suggested, with the number of sexual partners during a woman's life; that should be greater while older. As a result, the prevalence of infection decreases with age only, if the number of sexual partners is less than five and tends to increase if this number is greater than five, reaching 87.2% in older women.[8]

Of the total of the participants, only 10 women were studied by cytology for 45.5% and the percentage of false negatives was 80%, which is somewhat higher than that reported by several authors for cytology studies, which can reach up to 60%; [9,10] perhaps by number of patients who participated in the present investigation.

The HPV genotypes detected were, HPV-HR 16 and 31, not so for the HPV-HR genotypes 18, 39, 45 and 51, which were not found. So far there are no or at least we have not found other molecular studies of HPV in cervical cancer that have been carried out in other provinces of Cuba, being studied only the province of Havana and this time a study is reported for the first time of HPV in the province of Camagüey.

It is clear from these studies that the HPV-HR 16 genotype was the most frequent in the provinces of Havana and Camagüey, highlighting the HPV-HR 16 and 31 genotypes that are the most widely distributed possibly in Cuba, especially if the province of Havana and the province of Camagüey are geographically distant. While other investigations are not carried out in the other provinces of Cuba, which contribute to reinforce these conclusions or add other genotypes besides the two reported in frequency and distribution, the results obtained, compared and discussed in the present investigation should be considered as the molecular causes, determinants and predictions of the CCU in Cuba.

Finally, these results, together with those obtained by other authors, is an important contribution in the design of preventive or therapeutic vaccinal preparations that are increasingly effective in an early solution for cervical cancer in Cuba.

CONCLUSIONS

Cervical cancer is the second cause of cancer death in women worldwide. In Cuba and specifically in the province of Camagüey, it has been observed over the years that the number of patients with cervical cancer has had a visible tendency to increase. This research is the first report of a molecular study of HPV from samples of patients with cervical cancer in the province of Camagüey, Cuba. 63.6% of the patients presented exophytic type lesions, 4.5% endophytic and 31.8% of other types. This PLFR study confirmed that the circulating HPV-HR genotypes in the province of Camagüey are genotypes 16 and 31, where the most frequent is HPV-AR 16.

These results, together with those obtained by other authors, is an important contribution in the design of preventive or therapeutic vaccinal preparations that are increasingly effective in an early solution for cervical cancer in Cuba.

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