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Human Papiloma Virus Detection in Various Cervical Lesions by Molecular Methods

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BRIEF TEXT

Background
Human papillomavirus (HPV) is recognized as one of the causes of anogenital cancers, including uterine cancer.
Previous Studies
About 30-40% of the different types of the virus cause mucosal contamination, especially in the anogenital areas. These viruses are divided into two types of high risk and low risk for cancer. Of course, in some sources, they are categorized into three types of low-risk, medium and high risk. Most papillomavirus infections of the cervix and anogenital areas are of high risk type and can cause cervical cancer (uterine carcinoma). Virusologically, the papillomavirus belongs to the group of naked viruses with 20-fold cadmium, and has eight primary and regulatory genes,

two L1 and L2 genes, and delayed expression of the capsid. The gene and capsid protein are common in almost all types of the virus, and in most molecular methods, they are the basis for isolating the virus from the patient's specimen. Of the eight regulatory genes, e6 and e7 genes determine the ability of the virus to convert the lesion to uterine carcinoma. Therefore, the first essential step in detecting the presence of the virus in the cervical sample and other samples obtained from the patient is the isolation of the DNA of the virus. Viral infection is responsible for about one-fifth of human cancers, and HPV (human papillomavirus) is one of the most important oncoviruses, that not only causes damage in the anogenital area, but also in the respiratory tract, lung, mucosa, scalp and neck, and its type 16 and 18 cause cancer. Anogenital infections include warts, condyloma acuminates, cervical mucous membrane lesions (CIN) [1]. Uterine lesions may be present in mild cervical lesions, mild LSIL epileptic lesions, severe HSIL, and uterine carcinoma due to HPV infection [2, 3]. These lesions are classified according to the Bethseda classification as CINII, CINI, CINIII, or CIS. The most common types of HPV are types 6 and 11, but infection with types 16 and 18 is also common [4, 5]. Anogenital warts are an infectious and sexually transmitted disease that occurs in both sexes and is a serious clinical problem for women [6]. The relationship between the papillomavirus virus and malignant and progressive lesions, as well as the cancer of the uterus, has been well identified. Today, the virus is accurately detected by various methods such as DNA replication of the target with PCR and the Hybrid capture method [2, 5].

Aim(s)

The aim of this study was to investigate the presence of human papillomavirus in samples of Iranian patients and the relationship between the type of virus (high risk and low risk) and cervical lesions by molecular methods based on PCR virus samples.

Research type

Research society, place and time

Sampling method and number

The present study was performed on 67 cervical cytology samples (LBC) and cervical tissue samples were evaluated using PCR and molecular methods in Sarem Hospital.

Used devices&materials

With an extraction kit (QIAGEN), DNA was extracted from samples of cervical, condyloma and uterine cancer. To determine the presence of HPV in a patient's sample, the MY09 / MY11 primer for the L1 capsid gene was used with the following sequences: Forward: 5’-CGTCCMANNGGASACTGATC-3’ Reverse: 5’-GCMCAGGGSCATAKAATG-3’ C/A=M G/A=N T/A=S T/C=K With the aid of a specific primer of L1 capsid virus, and with observing temperature of different stages of PCR in thermocyler, the Denaturation temperature of 95 ° C, 56 ° C primer annealing and multiplication temperature of 72 ° C, during 35 cycles, followed by electrophoresis in 2% agarose gel was run. After this step, DNA

samples from HPV virus were selected based on PCR for typing with a special kit (DNA-Technology JSC, 115478; Moscow, Russia). The reaction mixture was prepared in separate micro tubes, with 10 µl buffer and the same amount of reaction mixture and 5 µL of the sample, or positive or internal control. Based on the order of the kit in 45 cycles with a denaturation temperature of 94 ° C, a primer annealing of 94 ° C and a reproduction temperature of 70 ° C, the PCR reaction was performed and finally, the product was observed with 2% agarose gel, stained with ethidium bromide and interpreted according to the instructions of the kit. Subsequently, positive samples with polyacrylamide gel were isolated for type 18 and some were separated by Real time PCR. Finally, their type was determined and classified according to the type of lesion.

Findings by Text

The existence of a 450-bp band signaled the presence of HPV in the sample (Fig. 1). The appearance of the 570 bp band, indicated the presence of HPV type 33, 58 or 67, and the presence of a 642 bp band, signaled the presence of types 16, 31, 35 or H 35 or 52. Also, bands with sizes 285, 291, 294 and 297 bp were HPV types 18, 45, 39, and 59 respectively (Fig. 2). Of the samples with lesion, the virus was not found in 2 samples (0.3%) despite the clinical signs of endothelium lesion and the effects of alteration of epithelial cells. Thus, out of 67 cases, 65 (97%) of samples were positive in terms of HPV. Two patients had uterine carcinoma with two commonly high risk infected types and seven cases of severe epithelial lesions were infected with high-risk types. Of the patients with epithelial lesion, only 2 were infected with low-risk types and the rest were risky. 30 (44.8%) patients had cellular symptoms, of which half of them were classified into high-risk virus types and half of them were infected with low-risk species. From the cytology point of view, 7 samples (10.4%) had no clinical signs and 44 (65.7%) cases were infected with high-risk virus types (Table 1; Chart 1). Most cases of infection were related to virus type 16. Among the samples, a significant number of HPV types 18 and then 52, 39, 33, and 31 were observed. Also, in 2 samples of the uterine carcinoma, the infection was accompanied by two types of viruses, one with type 16 and 31, and another with type 18 and 33.

Main comparison to the similar studies

In similar studies, an examination for intra-epithelial uterine neoplasia (CIN III) with on-site hybridization was found on a case of simultaneous infection with types 16 and 18, which suggested that HPV infection was the most important responsible for the cause and effect of malignancy changes, and these two types simultaneously Infect the squamous epithelial cells of the cervix [7]. As the persistent infection with viral carcinogenesis leads to neoplasia or HSIL lesions, determining HPV-DNA can be used as a marker for existing infection or future developmental lesions [8]. In the present study, similar to the similar studies by Garland and Tabrizi [8], it was observed that the HPV-DNA test had a higher sensitivity to detect the virus for the prognosis of precancerous lesions (LSIL, HSIL) than cytology. In the present study, 5 out of 125 patients with cervical lesions were positive for HPV-DNA, therefore, cervical ulcer cannot be said to be a specific sign for HPV infection. Ultimately, the combination of Pap smear test with HPV-DNA testing can reduce

the intervals of Pap smear testing in a group where HPV-DNA is negative and the result of a cystic Pap smear test is normal and therefore more cost-effective [8]. Based on comparative studies for propagation and hybridization methods, PCR was found to be more sensitive for detecting CIN by 83.33% than Tur e Hybrid Cap with 66.67% [9, 10]. In a sensitivity analysis for the determination of severe lesions, it was found that the Hybrid Capture method was incapable of detecting a large number of cases compared to the PCR method [11, 12]. In 12 cases of CIN, 10 cases (83.33%) were positive with PCR. In 7 cases of CIN II and CINIII, all were positive. Six cases of 16 and 18 type papilloma virus and one type 16 case were obtained [11]. The results of that study are consistent with the present study.

Suggestions

Repeated studies with more samples and molecular methods are recommended for more accurate diagnosis.

Limitations

Conclusions

97% of intraepithelial symptoms in cytology of the cervix are associated with the presence of human papillomavirus, and the highest number of viral cervical mucus is of high risk. The most common type of epithelial-infectious cervical virus type is the type 16 human papillomavirus.

Acknowledgments

Conflict of interest

Ethical Permissions

Funding Sources

TABLES and CHARTS

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