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How to differentially detect potentially risky human papilloma virus strains in the human population?

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Abstract

The human papillomavirus (HPV) represents one of the most common sexually transmitted infections, knowing more than 100 viral types that, in relation to their oncological pathogenesis, are classified into types of high and low oncological risk. Cervical cancer is the second leading cause of death from malignant neoplasms in women.

HPV can often cause warts. Most types of this virus are harmless, but a percentage of them are associated with an increased risk of cancer. These types are born from the genitals and are acquired through sexual contact with an engaged partner. Cervical cancer is the second leading cause of death from malignant neoplasms in women. HPV infection is responsible for various lesions in different areas of the body. Common warts are the most frequent. They consist of whitish papillomatous lesions that can be in any area, oral mucosa, genital mucosa, etc.

Most people infected with the genital human papillomavirus are only carriers. It usually has no symptoms and goes away on its own, without causing serious health problems.

There is no cure for HPV, but there are treatments for the health problems that some types of HPV can cause, such as genital warts and cervical cancer.

Thanks to cytology and histopathology, it can be detected and treated promptly, reducing the impact of this disease. In addition, there are vaccines that promise to reduce this cancer, especially in countries with the highest number of cases.

The virus can remain in the body, even after receiving treatment for genital warts. This means that HPV can still be transmitted to sexual partners, despite not having physical manifestations of it.

This work will present the bases for the differential detection between the riskiest HPV strains: HPV-18 and HPV-16 through their detection using the amazing idea of Kary Mullis: Polymerase Chain Reaction.

Keywords: HPV infection; Cancer; HPV-16; HPV-18; Differential detection

1. Introduction

The *Human Papilloma Virus* (HPV) is a virus belonging to the *Papillomaviridae* family, which are grouped under this name to the extent that they share DNA and their ability to generate tumors. Generically, they are characterized by not being encapsulated, being epitheliotropic, with a double strand of DNA that infects mucosal and cutaneous epithelia, inducing cell multiplication. Papillomaviruses are species-specific –that is, they only evolve into a particular species-

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and can be found in a large part of the world, infecting both birds and mammals in general; In most cases, the infection manifests itself with the presence of warts or papillomas that can develop anywhere on the body [1, 2].

The incubation period once the virus takes hold is highly variable, usually within three months, but can last for years in the subclinical stage. In persistent cases, approximately 25% develop low-grade lesions, although most lesions pass without being apparent and disappear without leaving evidence of infection [3].

Both women and men can be asymptomatic carriers and vehicles of genital HPV infection. Transmission occurs through sexual contact and the organs most susceptible to infection with the potential to initiate a neoplastic transformation are the cervix (transition zone) and the pectineal line of the anal canal. HPV infections are frequently sheet-fed, in which cases viral DNA can be recovered from the cervix, vulva, vagina, anal canal, penis, and scrotum [4].

Due to the difficulty in identifying and demonstrating HPV, data regarding prevalence and incidence are variable depending on which studies are referred to. Despite this, all agree on the high prevalence of the infection and on the impossibility of accurately detecting the magnitude of the number of people infected, since in most cases they do not present symptoms, and improve without the need for a treatment after a period, so it goes unnoticed [1].

1.1. Structure and classification

HPV is a small, non-encapsulated virus with an icosahedral structure and a circular double-stranded DNA of 7,500 to 8,000 bp. This virus belongs to the *Papillomaviridae* family, included in the *Papillomavirus* genus. They are species-specific parasites, widely distributed in nature, infecting both birds and mammals. Usually, the result of the infection is the formation of a benign growth, wart, or papilloma, located anywhere on the body. There is great interest in HPV as a cause of malignancy, particularly in cervical cancer. At least 58 different HPVs have been identified using molecular techniques, making their relationship with types of tumors [5].

HPV represents one of the most common sexually transmitted infections, knowing more than 100 viral types that, in relation to their oncological pathogenesis, are classified into types of high and low oncological risk [4].

There are more than a hundred HPV identified, of which over 30 types are transmitted sexually. HPV is the most prevalent Sexually Transmitted Infection -STI- in the world population and it is estimated that 75% of the population of childbearing age will be infected with HPV at some point in their lives; although it has been established that the highest prevalence of HPV is found in women between 15 and 25 years of age. The International Agency for Research on Cancer (IARC) considers HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66 to be high-risk types to humans, and those other types, including HPV 6 and HPV 11, are possible types of low cancer risk for humans [4].

The main risk factor associated with infection with the virus is related to the number of sexual partners a person has, which ultimately is a matter of probability: the greater the number of sexual partners, the exposure increases and with it the probability of acquiring the infection [1].

The prevalence of HPV infection is associated with age, being higher in the immediate ages at the beginning of sexual relations related to the pattern of sexual behavior in the community; subsequently there is a very marked decrease, between 25-40 years to stabilize from this age. In some populations, a second prevalence peak has been observed in postmenopausal women, the interpretation of which is still under investigation [4].

The technology to detect markers of exposure to HPV and the description of new HPV families has made it possible to study the viral presence in samples of neoplastic tissue from multiple locations [4].

It should be noted that relationships have also been established between HPV and other types of anogenital and oropharyngeal cancer: there are studies that assert that 40% of cancers of the vulva, vagina, and penis could be triggered by infection with the virus; 90% of anal and 12% of oropharyngeal cancers would also be related to HPV [1].

Approximately 70% of cervical cancer cases in the world are caused by HPV types 16 or 18. The low-risk genotypes, HPV6 and 11, cause a high percentage of mild cervical dysplasias and more than 90% of genital warts or condylomas [4].

The task associated with the diagnosis of genital warts or common warts is quite simple, since the diagnosis is based on clinical evidence, so it is not necessary to send samples to laboratories, due to their anatomical location and histology [1].

For a woman to perform the HPV test, she must first undergo a cytology, then a colposcopy, that is, she must present abnormalities or show the presence of lesions [1].

The PAP is an exam, whose purpose is to detect early abnormalities of the cervix, which can later evolve to cancer. Its effectiveness depends on it being carried out regularly and within the recommended periods. Its easy and quick performance, as well as its high diagnostic value, the default in the main method of early detection of cervical-uterine cancer, is part of the routine health care of women, since, like other types of cancer, the uterine cervix can be treated more successfully when it is detected in early stages and consequently prolong life and its quality [6].

If the cytology presents irregularities, and once these irregularities have been confirmed either through new cytologies, and/or the performance of a colposcopy, it is necessary to determine if one is facing cancer, precancerous lesions or abnormalities associated with other Factors, such as hormones [1].

The PAP is not 100% infallible, which is why in this work the fantastic idea of Kary Mullis was developed to detect the virus in the sample that is taken at the same time for the PAP test and thus complement the test original [7].

1.2. HPV prophylaxis

It is widely accepted in the scientific community that latex preservatives are not 100% effective in preventing HPV infection, since penetration is not required for infection, but rather contact alone of genitals, mano-genitals, etc. infection could be achieved; Although the viral particles cannot pass through the latex of the condom, the uncovered genital area could harbor HPV or be infected with the virus. Even with this background, it has been possible to determine that its use lowers the chances of contagion, presumably because the exposure is lowered and with it the viral load. [1].

There are two vaccines against HPV: Gardasil, a recombinant quadrivalent vaccine, which includes types 6,11,16 and 18 and which has obtained European marketing authorization, and Cervarix, a recombinant bivalent vaccine that includes types 16 and 18, which is in evaluation process [4].

Since 2008, the vaccine has been integrated into the vaccination schedule of Spanish adolescents, who are recommended to be vaccinated between 11 and 14 years of age, while in Chile it has not yet been integrated into preventive health public policies [1].

2. Materials and methods:

In this work, the option of using both the swab or the spatula used when performing the Papanicolaou (PAP) exam on patients, which are discarded in a normal sample collection, is proposed.

This swab or spatula are identified in their respective original containers and are used in the laboratory.

Once in the laboratory, the swab will be placed in a 2 mL Eppendorf tube and 100uL of nuclease-free water will be added. The spatula will be treated in a similar way. Subsequently, each Eppendorf tube will be vortexed for 30 seconds twice. This mixture will constitute the sample to be used in the PCR reaction.

2.1. Differential amplification of HPV16 and HPV18 by PCR.

The PCR reaction contemplates the design of *in silico* primers, which will be achieved through the online and free use of the Oligoperfect Program [8] incorporating as input the nucleotide sequences of viruses according to the official GenBank® database [9] and indicating the generation of one amplified between 300 and 500 bp.

Once the sequence of the primers is known, its synthesis will be entrusted to a commercial company. The PCR protocol to be used will be determined by using a temperature gradient thermocycler.

2.2. Visualization

Visualization of the amplified product is performed by electrophoresis in 2% agarose gel (Winkler®) in Trisacetate EDTA (TAE) buffer (Fermentas®) and subsequent staining with GelRed TM (Fermelo®). 5 µL of the PCR product will be taken and mixed with 1 µL of a commercial loading product, 6X Mass Ruler Loading Dye Solution (Fermentas®), which has glycerol to provide density to the sample and bromophenol blue to verify the progress of migration of DNA bands. Electrophoresis will be carried out at 90V for 90 minutes. As molecular size marker, Hyperladder IV (Fermentas®) was secured, which contains DNA fragments between 100 and 1000 base pairs. At the end of the

electrophoresis, the bands will be visualized in a UV light transilluminator (UVP® Transilluminator) and will be photographed with a digital camera and appropriate filter.

3. Results

The application of free access biotools: CLUSTAL Omega [10], BLAST [11], Oligoperfect design® and others would allow the correct detection of high-risk HPV-16 or HPV-18 types in samples from PAP-positive patients and thus complement the proposed screening study.

4. Conclusion

The bases for the differential detection of HPV genotypes have been considered. Of course there may be changes, but one cannot fail to establish that today, with the existing biotools and the brilliant idea of Kary Mullis, one of the most dangerous pathologies for women can be dealt with in a better way and perhaps on time. The PAP test is not infallible, and we have presented a complementary technique.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Authors declare no conflict of interest.

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