

Correlation of Human Papillomavirus types with the level of damage in women attended in a private laboratory

Correlação dos tipos de Papilomavírus Humano com o grau de lesão em mulheres atendidas em um laboratório privado

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ABSTRACT

Introduction: the Human Papillomavirus (HPV) is a virus capable of promoting various types of skin and mucosal lesions, which can be malignant or benign. Some of them can cause genital warts, and others can reach the cervix and cause a Cervical Intraepithelial Neoplasm (CIN) that can progress to a more advanced stage, which is cancer. The sexually active population is more likely to come into contact with the virus at some point in life, and as a result, the human papillomavirus is the most common sexually transmitted infection (STI) in the world. **Objective:** to evaluate the incidence of HPV in women attended at a private laboratory in Fortaleza/CE. **Method:** this was a quantitative, cross-sectional, retrospective and descriptive research, using data from the patients' medical records. **Results:** after analyzing the patient's medical records, it was observed that the predominant age group in this study was concentrated between 16 and 34 years old. Furthermore, it was evident that the vast majority of women obtained positive results for HPV, and they arrived with observations in the search for

screening. Furthermore, the most prevalent intraepithelial lesions were LSIL and ASC-US. Conclusion: HPV was more associated with young women, and screening is of paramount importance since HPV is asymptomatic, and this factor can lead to women's erroneous thinking that without evident symptoms there is no lesion.

Keywords: Cancer, HPV, cervical intraepithelial neoplasia, sexually transmitted infection, women, health, cytology.

RESUMO

Introdução: o Papilomavírus Humano (HPV) é um vírus capaz de promover vários tipos de lesões de pele e mucosas, que podem ser malignas ou benignas. Alguns deles podem causar verrugas genitais, outros podem alcançar o colo uterino e causar uma Neoplasia Intraepitelial Cervical (NIC) que pode progredir para um estágio mais avançado, que é o câncer. A população sexualmente ativa tem maior probabilidade de entrar em contato com o vírus em algum momento da vida e, como resultado, o papilomavírus humano é a infecção sexualmente transmissível (DST) mais comum no mundo. **Objetivo:** avaliar a incidência do HPV nas mulheres atendidas em um laboratório privado em Fortaleza/CE. **Método:** esta foi uma pesquisa quantitativa, transversal, retrospectiva e descritiva, utilizando dados dos prontuários médicos dos pacientes. **Resultados:** após análise dos prontuários médicos do paciente, observou-se que a faixa etária predominante neste estudo se concentrava entre 16 e 34 anos de idade. Além disso, ficou evidente que a grande maioria das mulheres obteve resultados positivos para o HPV, e elas chegaram com observações na busca de triagem. Além disso, as lesões intra-epiteliais mais prevalentes foram o LSIL e o ASC-US. **Conclusão:** O HPV estava mais associado a mulheres jovens, e o rastreamento é de suma importância, uma vez que o HPV é assintomático, e este fator pode levar as mulheres a pensar erroneamente que, sem sintomas evidentes, não há lesão.

Palavras-chave: Câncer, HPV, neoplasia intra-epitelial cervical, infecção sexualmente transmissível, mulheres, saúde, citologia.

1 INTRODUCTION

The Human Papillomavirus (HPV) is a virus capable of promoting various types of lesions on the skin and mucous membranes, which can be malignant or benign. The sexually active population is more likely to come into contact with the virus at some point in their lives, and as a result, the human papillomavirus is the most common sexually transmitted infection (STI) in the world¹.

There are more than 100 types of HPV, and those that most affect the cervix and are considered carcinogenic are: (16, 18, 31, 33, 39, 45, 51, 52, 56, 58 and 59). The HPVs that are considered low risk, such as 6 and 11, are associated with 90% of cases of anogenital warts, and the high-risk ones comprise 99% of cervical cancers.²

The infection caused by HPV can be asymptomatic and naturally regress in women with integrated immune functions, so they will be more resistant and have a spontaneous regression of up to 1 year and 6 months. However, in cases where HPV is persistent, it has a high oncogenic

risk, especially for HPV types 16 and 18, which are strong predictors of the development of cervical cancer (CC), comprising a percentage of 70% of all neoplasms¹.

It is known that HPV is one of the infections that most affects the epithelium in the world due to its tropism for epithelial cells, and that is directly related to cervical cancer. According to the Revista de Saúde Pública, in Brazil, it is estimated that 15590 women are targets for this cancer, showing that prevention and control must be a priority in the management of women's health³.

Regarding the infections caused by HPV, it is known that there is a worldwide prevalence of 12% with an intercurrency among regions. In Brazil, it was presented a prevalence of infections caused by cervical HPV alternating between 13.7% to 54.3% in the general population and 10% to 24.5% among women who presented results for normal cytology².

Epidemiological research shows that there are other factors besides HPV that increase the risk of cervical intraepithelial neoplasia (CIN), such as immunodeficiency, multiple sexual partners, early sexual debut, and no use of contraceptive methods. Another recurrent factor is that the disease usually affects people with little or no education. There is a lack of information about the seriousness of this disease since the individual can acquire it and spend several years with the virus because when the symptoms appear, it may have already advanced to cancer⁴.

In the ranking of cancers in Brazil, cervical cancer is one of the most common types of cancer, ranking third in Brazil. In 2016, about 16340 cases with 15.8 estimated risk per 100,000 women were confirmed.⁵

Frequently, in the age group of 50 to 70 years, men are affected with cancer of the penis, accounting for an estimated 22,000 cases per year. Although this type of cancer is still considered rare, the incidence rates are higher in underdeveloped countries, accounting for 10% of male tumors in some parts of Asia, Africa, and South America. In Brazil, in the North and Northeast regions, penile cancer represents 2% of cancers in men⁶.

When HPV infections damage the cervix region, they are classified and characterized into three grades before they become cancer, CIN (I, II, and III). CIN I characterizes a low-grade squamous intraepithelial lesion, and CIN II and III characterize a high-grade squamous intraepithelial lesion. The higher the degree of an epithelial lesion, the greater its thickness and proportion of undifferentiated cells since grades II and III may replace part or all of the thickness of the normal cervical epithelium and should be seen as premalignant⁴.

The HPV virus in its genome has three regions: the regulatory region, the early region, and the late region. It is in these regions where the oncogenic genes are found, which exert different functions. In the early region, genes *E1* and *E2* encode non-structural proteins that are

involved in the process of DNA replication and transcription, and genes *E5*, *E6*, and *E7* are involved in cell transformation. In the late region, genes *L1* and *L2* encode structural proteins of the viral capsid.⁷

The transmission of HPV can be sexual, but it can also be vertical, with transmission occurring hematogenously through the placenta or during delivery of the neonate. Research has reported that HPV can infect the umbilical cord, the human placenta, and breast milk. However, there is still no statement in the literature about this type of mother-to-child transmission of HPV⁸. Other forms of transmission have also been reported, such as autoinoculation, from hands to genitalia, and by fomites, which are associated with the non-sexual transmission, since research shows that there is a possibility that the infectivity is maintained at room temperature⁹.

The vaccine has the ability to increase the immune response by up to 10 times compared to an infection that remains for several months, thus making this vaccine effective and necessary. The vaccine helps prevent the progression of cervical cancer if people come into contact with types 16 and 18 in particular. The protection the vaccine provides against HPV types usually lasts for 8 to 9 years. In view of this, the full vaccination scheme aims at remission in cervical intraepithelial neoplasia (CIN) in CIN 1 at 29%, CIN 2 at 50%, CIN 3 at 55%.¹⁰

Among the vaccines available so far, the quadrivalent vaccine protects against HPV types 6, 11, 16, and 18 and is taken in three separate doses over a period of months. It is indicated for ages 9 to 26; moreover, it is the only one indicated for men and women. Its preventive potential reaches 70% for cervical cancers and precancerous lesions, 90% for anus cancers and precancerous lesions, 50% for vulvar cancers and precancerous lesions, and 60% for vaginal cancers and precancerous lesions. The bivalent vaccine offers protection against HPV types 16 and 18 and is taken in three doses. It is indicated for women and girls over 9 years old because there is no age restriction for taking the vaccine, and this is due to the coverage for the prevention of 70% of cancers and precancerous lesions of the cervix⁷. Besides these, there is a nonavalent vaccine that protects against more types of the Human Papillomavirus such as the 6, 11, 16, 18, 31, 33, 45, 52, and 58¹¹.

For the diagnosis of HPV, the Pap smear is used, which is a cytopathological exam, along with colposcopy, which is more accurate and locates the lesion, being used as a preventive exam for the detection and tracking of cervical cancer, thus influencing the reduction of cancer confluence and its morbidity and mortality. The pap smear is performed by means of a cervicovaginal smear, in which the cervix is scraped with a spatula, and the collected material (cells) is inserted in a glass slide where it will later be analyzed by the clinical cytologist⁴.

Molecular diagnosis can be cytopathological, colposcopic, and histopathological, and by molecular tests. The main molecular tests performed are hybrid capture and real-time PCR since they are cheaper and simpler tests. Hybrid capture is based on molecular hybridization of DNA and detects any type of high-risk HPV, with a sensitivity of 95% to 97%. Real-time PCR is based on the emission of fluorescence as the viral DNA is amplified. In this method, it is possible to detect a low viral load.¹²

There is no specific treatment for the human papillomavirus since it can cause many warts, such as intraepithelial lesions. So, it is considered for warts the use of chemical substances, such as trichloroacetic acid and podophyllin, which are applied directly to warts. But they can also be removed by cryotherapy, laser, or surgical removal using anesthetics since this causes pain. When it is an intraepithelial lesion, depending on the degree, electrocauterization of the lesion site or surgical removal can be performed. Immunotherapy can also be a solution with the use of *imiquimod*, which enhances the immune system and the effectiveness of other treatments¹³.

This study aimed to evaluate the correlation of human papillomavirus (HPV) types with the degree of the lesion in patients of a private laboratory in Fortaleza.

2 METHODS

The study has a quantitative, cross-sectional, retrospective, and descriptive nature. The study was conducted by analyzing the degrees of HPV present in the medical records of patients seen in 2017 in the private Laboratory of Fortaleza, which serves the entire city of Fortaleza for 20 years, performing cytology tests, biopsy, molecular biology, and genetics. The medical records of patients aged 13 to 59 years assisted by the clinic were evaluated in order to analyze the incidence of human papillomavirus.

The inclusion criteria for the study were: female patients with the human papillomavirus (HPV), patients with some type of lesion associated with the virus, and patients between the ages of 13 and 59.

Excluded from the study were: male and female patients who do not have HPV; patients who have another sexually transmitted infection (STI); patients older than 59 years or younger than 13 years.

For data collection, the information present in the medical records of patients of the Private Laboratory of Fortaleza/CE was used. The data were collected with the help of a structured form.

The instrument used for reporting results for HC2 assays, including the *digene* HC2 high-risk HPV DNA test was the DML 2000, which is a luminometer that aids in the detection of the amplified chemiluminescent signal. Specimens were considered positive for one or more HR-HPV types if the value of the relative light units / cut-off ratio (RLU / CO) was ≥ 1 (QIAGEN).

The data were processed and tabulated using the Excel program and later consolidated in the form of tables and graphs for analysis and discussion. The data were analyzed using a statistical program.

As for the ethical aspects, the principles of bioethics were respected, based on Resolution 466/12 of the National Health Council¹⁴, which governs research involving human beings. The project was sent to the Ethics and Research Committee designated by Platform Brazil and was approved at the end of May according to Opinion No. 2,686,034, initiating the study. The research was carried out with total confidentiality, ensuring the anonymity of the individuals during the study.

The evaluation methods that were performed presented minimal risks to the participants since they were not disturbed by answering questionnaires, as the data were collected from medical records in the private laboratory archive. We guaranteed complete confidentiality and anonymity of the information provided by the patients in the study.

3 RESULTS

After analyzing the medical records of 470 women patients from a private laboratory in Fortaleza; of these, 361 (76.8%) were between 13-59 years old. It was observed that 101 (21.5%) women had no reported age. Of the 470 patients included, 84 (17.9%) were positive for HPV, and of these, 83 (23%) were within the age range, 224 (47.6%) were negative, and 219 (60.7%) were within the age range.

According to the women included by age group, 228 (63%) were observed to be screened; they arrived without any specific symptoms, and of these, 172 (75%) were negative, 47 (21%) were positive, and 9 (4%) had no reported result.

Of the 361 women, 24 (6.6%) presented as a result of liquid medium cytology, varied lesions such as low-grade cervical intraepithelial lesion (LSIL), high-grade intraepithelial lesion (HSIL), atypical squamous cells of undetermined significance (ASC-US) and atypical squamous cells (ASC-H). Of these, 12 (50%) had LSIL, 8 (33.3%) ASC-US, 1 (4.1%) ASC-H, and 1 (4.1%) HSIL. The patients who were negative for HPV and positive for the lesion were only two: 1 presenting LSIL and the other presenting ASC-H. In the literature, it is evident the

growing worldwide concern with screening for Human Papillomavirus (HPV) associated or not with lesions.

The viral load presented in the data collection was based on the parameter established by the device, above 1 is considered positive for HPV. Of the 84 (23%) women positive for HPV, all showed viral load compatible with the high oncogenic risk types, and 9 (10%) showed viral load compatible with low and high-grade types.

Among the 361 women's records according to age group, 22 patients had a mild inflammatory condition, 16 (72.7%) were negative for HPV, 6 (22.3%) were positive, already 17 patients were related to an inflammatory condition that was moderate, 7 (41.2%) were positive, and 10 (58.8%) were negative, and when we started to observe the 27 patients with the marked inflammatory condition, 9 (33.3%) patients were positive for HPV, and 18 (66.6%) were negative.

4 DISCUSSION

Data were collected from 470 female patients of a private laboratory in Fortaleza, and of these, 361 (76.8%) were between 13-59 years old, which is the age range proposed by the study. It was observed that 101 (21.5%) women had no reported age. Of the 470 patients included, 84 (17.9%) were positive for HPV, and of these, 83 (23%) were within the age range, 224 (47.6%) were negative, and 219 (60.7%) were included in the age range. According to one study, most women were older than 34 years which corresponded to 71% (4282/6058) of the study population, and confluent to this a positivity for HPV of 45%, data that are close to those present in this study¹⁵. However, in other studies, the women were older compared to the data from this study since they were 44-84 years old and in the menopausal period¹⁵. Based on a work performed in the Department of Pathology at Hvidrove University Hospital, located in Hvidrove in Denmark, the age range among women was 18-74 years, resembling in parts the age range proposed by the study¹⁶.

A study conducted in Brazil showed that age may be related to HPV evolution; moreover, women over 40 years of age who present some sexual infection exhibit a higher risk of the advancement of cervical cancer compared to young women¹⁷.

Of the 361 women, 24 (6.6%) presented as a result of liquid medium cytology, varied lesions such as a low-grade cervical intraepithelial lesion (LSIL), high-grade intraepithelial lesion (HSIL), atypical squamous cells of undetermined significance (ASC-US) and atypical squamous cells (ASC-H). Of these, 12 (50%) had LSIL, 8 (33.3%) ASC-US, 1 (4.1%) ASC-H, and 1 (4.1%) HSIL. There were only two patients who were negative for HPV and positive for

the lesion: 1 (one) had LSIL, and the other had ASC-H. In the literature, it is evident the growing worldwide concern with human papillomavirus (HPV) screening, associated or not with lesions. It was observed in a study of Norwegian women presenting LSIL (low-grade cervical intraepithelial lesion) and ASC-US (atypical squamous cells of undetermined significance) that 63% of the tests were positive for HPV associated with LSIL and 39% positive and associated with ASC-US. This is in agreement with our study in which the lesion that had more incidence in women with positive HPV was LSIL and ASC-US, lesions considered low-grade, thus demonstrating that the need for health care is essential to prevent the evolution of this lesion¹⁵. A study showed that women with normal cytology and who did not present any type of lesion had high-risk HPV, and these women corresponded to almost 75%¹⁹, making clear the importance of periodic screening. In the literature, a study reported that of the 269 women included for analysis, 68 (25%) had normal cytology, 31 (12%) LSIL, 33 (12%) ASC-US, 9 (3%) ASC-H, 124 (46%) HSIL, 2 (0.7%) with cytological characteristics of cancer and 2 (0.7%) with inadequate cytological sampling, observing with these data compared to those already existing in the study a higher incidence of lesions in the population studied.¹⁷

According to the women included in the age group, 228 (63%) had as an observation the performance of screening, being possible to conclude that they arrived without any specific symptoms, and of these, 172 (75%) were negative, and 47 (21%) were positive, and 9 (4%) had no reported result. According to a study, the women who had irregular cytology and/or positive HPV were included, and of these, more than half had associated lesions, showing the importance of this periodic screening, preventing the evolution of a possible lesion to a more critical stage that is configured as cervical cancer¹⁷.

The viral load presented in the data collection was based on the parameter established by the device; above 1 is considered positive for HPV. Of the 84 (23%) women positive for HPV, all showed viral load compatible with the high oncogenic risk types, and 9 (10%) showed viral load compatible with low and high-grade types. According to the literature, of 665 women patients analyzed, 323 (48.6%) had positivity for HPV and, of these, it was observed that more than 50% had HR-HPV, according to which types 16 and 56 were the most prevalent, indirectly correlated with the data collected in this study since the types were not specifically reported¹⁸.

In accordance with a study, among 127 women, 52 (40.9%) presenting HR-HPV were associated with ASC-US lesions, and 81 out of 118 (68.6%) were related to LSIL lesions. It was observed that younger women or those aged 29 years who had ASC-US lesion had a higher periodicity of HR-HPV and those who had LSIL was weakly observed²⁰.

It was analyzed that of the 361 women within the age range, 22 patients had a mild inflammatory condition, 16 (72.7%) were negative for HPV, and 6 (22.3%) were positive, already 17 patients were related to a moderate inflammatory condition, 7 (41.2%) were positive, and 10 (58.8%) were negative, and when the 27 patients with a severe inflammatory condition were observed, 9 (33.3%) patients were positive for HPV, and 18 (66.6%) were negative. These data concluded that the inflammatory condition revealed by liquid-based cytology was more associated with a negative situation for HPV since more than 60% of patients in whom the inflammatory condition was compared to HPV positivity were negative. According to Okuyama et al²¹, the inflammatory microenvironment caused by the Human Papillomavirus, whether explicit or not, will lead to the development of preneoplastic conditions, thus certifying how important is annual screening.

5 CONCLUSION

It was observed that more than half of the women who were analyzed were positive for the HPV virus and confluent to this, most went in search of the health system to perform screening without necessarily having a characteristic symptom. Most women in our study were aged between 16 and 34 years, data that conflict with other studies. HPV in our study was related more to younger women. The most prevalent lesions among patients were low-grade intraepithelial lesions (LSIL) and atypical squamous cells of undetermined significance (ASC-US), which are similar to data from other studies. Furthermore, we conclude that the inflammatory condition may provide a favorable environment for the development and progression of premalignant lesions and that prevention is the most prudent thing to do.

It is clear that the Human Papillomavirus (HPV) is a virus that can be transient, not causing serious changes, as well as, can be a virus that causes cervical cancer. So, it is necessary that efforts are effective regarding the control of this dysplasia, aiming to reduce the annual cases that worry public health.

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