The extraordinary trajectory of the Papillomavirus through human history

A extraordinária trajetória do Papilomavirus através da história da humanidade

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ABSTRACT
Papillomaviruses have been infecting people since the beginning of human life on earth. The most relevant chapters of this story were written by the brilliant, diverse, and prominent scientists of their respective times. However, an important part of this story is the Papillomavirus victims and their adverse situations. There have also been disputes over intellectual primacy of the discoveries and the collaborators who were not given the recognition according to the role they played. This article will guide the reader through the remarkable facts of this conflicting and interesting relationship between humans and the Papillomavirus.
Keywords: Papillomaviridae; papillomavirus infections; history of medicine; heLa cells; Papanicolaou test; papillomavirus vaccines.

RESUMO
O papilomavírus tem infectado pessoas desde o começo da vida humana na Terra. Os capítulos mais relevantes dessa história foram escritos por mãos brilhantes de diversos e proeminentes cientistas em seus respectivos tempos. No entanto, parte importante dessa história também foi construída por vítimas do papilomavírus e suas situações adversas. Houve também disputas sobre a primazia intelectual das descobertas e os colaboradores cujo reconhecimento é menor que o papel que desempenhavam. Este artigo levará o leitor por um breve passeio pelos fatos marcantes dessa conflituosa e interessante relação entre o homem e o papilomavírus.
Palavras-chave: Papillomaviridae; infecções por papillomavirus; história da medicina; células heLa; teste de Papanicolau; vacinas contra papilomavirus.

INTRODUCTION

Papillomavirus (PV) is a deoxyribonucleic acid (DNA)-virus that belongs to the Papillomaviridae family, and it is found exclusively in vertebrates. It is found widely and most commonly among mammals, but it has also been described in birds and reptiles. More than 200 types of the human variants of PV (HPV) have already been described[1].

According to phylogeny studies, the most variable parts of the genomes of PV are divergent at a rate of approximately 1% each 40,000-80,000 years[2]. This suggests that the common ancestor of PVs dates back to the Paleozoic era, which was about 330 million years ago. PV may have arisen in Africa and has been genetically modifying as its DNA was integrated into the hosts' DNA, evolving and accompanying them in their migrations, and helping to spread PVs worldwide[3].

Most individuals become infected in childhood by direct contact, and the virus can continue to be part of the healthy skin microbiota. Virtually all humans are simultaneously colonized by different HPV types, causing mainly asymptomatic skin and mucosal infections. However, the carcinogenic potential of these viruses is well-established, giving PV a reputation as a bad guest[4].

To date, a query of the term *HPV* in the PubMed digital database resulted in 50,000 citations, 98% of which have been published in last 40 years. HPV has caused considerable debate in the scientific community, and the reader may be more interested in knowing about new discoveries than reading about the past. However, the history that accompanies HPV can be as rich and exciting as its pathophysiological mechanisms or its treatment.

THE RELATIONSHIP BETWEEN SEXUAL ACTIVITY AND BOTH GENITAL WARTS AND CERVICAL CANCER: A BRIEF RETURN TO THE PAST

HPV has closely followed the progress of mankind. HPV infection has been following humans on their journey for at least 500,000 years, according to genetic studies developed with HPV16 in Barcelona[5]. Possibly the earliest physical evidence of an infection was identified in 1974 when a plantar wart was found during an autopsy that was performed on the embalmed body of Nakht, a former Egyptian worker who lived in the 12th century before Christ (BC)[6].

People of ancient Greece and Rome already associated condylomas with sexual contact, but cataloging and describing these lesions as well as skin warts and cervical cancers is attributed to Hippocrates, who lived between 460 and 360 BC[7]. Even in the Roman period, Celsus and Galen reported Papilloma treatment techniques. Contribution of these ancient cultures went beyond their description and treatment and included etymology, where *condyloma* derives from ancient Greek and means "a round tumor" and *acuminata* comes from Latin meaning "sharp tips"[8].

There are few reports of HPV during the middle age, except in the writings of Lanfranchi and Guglielmo da Saliceto in thirteenth century. A syphilis outbreak affected Europe in late fifteenth century,

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which gave rise to the myth that all diseases of the genital organs had the same cause, which was called venereal poison. This theory suggests that condyloma would be a manifestation of syphilis. Centuries later, the Scottish surgeon Benjamin Bell (1749-1806) suggested different causes for condyloma and syphilis. He also described the emergence of anal cancer in a preexisting condylomatous lesion[8].

In the mid-nineteenth century, cervical cancer was probably the leading cause of cancer deaths in Western Europe. In 1842, the Italian physician Rigoni-Stern published an 80-year analysis of deaths of women in Verona city, where he observed a marked frequency of cervical cancer in women who had an active sexual life and a rare occurrence in virgins and nuns, and he concluded that there was a relationship between sexual activity and cancer[7]. He noted that the risk of uterine cancer was higher in women with an earlier sexual debut and those with more partners, and he also described the greater frequency of this cancer among women in their fourth and fifth decade of life, which is similar to current knowledge[9].

In addition to nuns, another subpopulation with relative infrequency of cervical cancer was Jewish women. Thus, researchers correlated the role of the husbands' circumcision in preventing the spread of a causal agent in the following century[7].

Years later, the contagious nature of warts was established by Joseph Payne, in a very peculiar way. He inoculated his thumb with a scraping from a boy's verrucous lesion, and he subsequently developed a similar lesion[6]. Parisian pediatrician Gaston Variot preferred to inoculate his assistant's finger and obtained same result years later. Since then, histological studies have established that oral and genital condylomas and skin warts have same etiology[8]. In 1907, Giuseppe Ciuffo, in Italy, demonstrated the transmission of human warts through the inoculum of a cell-free filtrate, thus showing its viral nature[7].

PVs were determined to be a cause of warts on rabbits' tails in 1933 and interest in these viruses in humans further increased when similar experiments in cows identified bovine PV (BPV). Francis Peyton Rous, the Nobel Prize winner in Medicine in 1966, conducted studies showing that chemical carcinogens induced malignant transformation in benign papillomas in rabbits, thereby associating PV with cancer. In 1949, Maurice Strauss used electron microscopy to demonstrate the existence of HPV in human papillomas[6,7].

Until very recently, the scientific community believed that the herpes simplex virus (HSV) was the etiologic agent for cervical cancer[7]. In the 1970s, German researcher Harald zur Hausen defended the hypothesis, which is now accepted, of a correlation between HPV infection and cervical cancer. Similarly, Meisels and Fortin correlated the cytological finding of koilocytosis with HPV infection, which also suggested the possibility of differentiating benign and condylomatous lesions that do not progress to cervical cancer from precursor lesions that may evolve into cancer. The link between HPV and cervical cancer and studies of the natural history of the infection provided an understanding of the cervical carcinogenesis stages[7,10].

In 1980, Lutz Gissmann, in the Harald zur Hausen laboratory, reported a novel HPV DNA called HPV6. Over 4 years, DNA sequences from HPV11, HPV16, and HPV18 were also described by the Gissmann and zur Hausen group. Years later, these authors won a Nobel Prize in Medicine for isolating and characterizing HPV 16 and 18 viruses, and for showing their involvement in the etiology of cervical cancer. Emergence of new methods of cancer prevention and detection, such as HPV vaccine and biomolecular tests, was made possible because of this finding[7,10].

THE ERA OF GYNECOLOGICAL CANCER PREVENTION: PAPANIKOLAOU AND BABES

Millions of women died of cervical cancer until scientists discovered potential preventive measures. Among them was the actress and politician from Argentina, Eva Perón, who died of this kind of cancer a few years after the first experimental cervical cytology in her country. The trajectory of the cervical cancer's high incidence and mortality changed after adoption of cervical cytology by the pioneer Papanikolaou in the second half of the last century. Still widely used worldwide, this method allows prevention at a relatively low cost and it is widely accepted as the most important public health strategy to reduce incidence and mortality of cervical cancer[11].

The young Greek physician Geórgios Nikolaou Papanikolaou, as his name was originally written, followed in the footsteps of his father and graduated with a medical degree in 1904, and after completing his doctorate in zoology in Munich (1910), he emigrated in 1913 to the USA. Without a job in his area, and until being accepted following year at University of New York and Cornell University Medical School, he was a carpet seller, a violinist in a restaurant, and an employee at a Greek newspaper. In 1928, he published his findings on the cytopathology of the human female genital tract, describing changes that differentiated between normal and malignant cells in a cervical smear. Years later, he became an American citizen, published four books and more than 100 articles, and was awarded medical and scientific honors. His face was on the 10,000-drachma banknote, which was an old Greek coin[11,12].

The reader may not know all this history, including the challenges and achievements of Papanikolaou, but they may know that he created an examination that bears his name. The problem is that, depending on the point of view, the primacy of the gynecological prevention technique would belong to another researcher.

In 1927, and thus a year before Papanikolaou, Romanian physician Aurel Babeş presented his cytology technique to Romanian Gynecology Society in Bucharest and published it in the following year in the French journal *La Presse Médicale*. Using a platinum loop to collect cells from cervix, a smear was made to detect presence of cancer. Cells were spread on glass slides, fixed in methanol, and stained using the Giemsa method. His studies described preinvasive diagnostic aspects and early invasive cancer[13].

Why did Babes not name the exam technique, which would have been a simpler name? Papanikolaou, although Greek, conducted his research at an American university and continued his research, while Babes, who wrote his article in French, did not publish further on this subject in subsequent years, although he published many articles on other pathologies[13]. In addition, the techniques are substantially different.

Some authors argue that the technique used by Babes in his study was not purely cytological but, rather, it was mainly histological because the platinum loop obtained tissue blocks instead of free cells. Babes suggested that his technique could be used as an alternative method for diagnostic confirmation of different tissue

that was obtained using biopsies, while Papanikolaou specifically mentioned using his technique as a method to prevent cervical cancer[14]. His phrase about it became famous: "The first observation of cancer cells in the smear of the uterine cervix was one of the most thrilling experiences of my scientific career"[12].

Long years passed until the Pap smear technique to detect cancer could be recognized. This was only possible thanks to two Papanikolaou publications. In 1941, together with Herbert Traut, he published "The Diagnostic Value of Vaginal Smears in Carcinoma of the Uterus"[15], and 2 years later, they published "Diagnosis of Uterine Cancer by the Vaginal Smear". These papers are the basis of modern cytopathology and these results supported the use of Papanikolaou's method[11].

Papanikolaou's name was submitted twice to the Nobel committee as a candidate for Nobel Prize in Medicine, but he was never chosen. Koss and Melamed[16] wrote in his book that he had heard from a member of the Swedish committee that because Papanikolaou had never acknowledged the previous contributions of Babes, his name was rejected to receive this unique distinction. Anyway, both were important for the early detection of genital cancer, and their findings, which were almost simultaneous, helped to save the lives of many women.

IMMORTALIZING HENRIETTA LACKS

Many people have heard of or even had the opportunity to work in the laboratory with HeLa cells. This reliable and resistant immortal cell line has been used in diverse research in different fields of medicine, and they were even used in research that took place in space[17]. Derived from samples of an aggressive cervical adenocarcinoma, recent studies have shown that HPV 18 DNA is involved in HeLa cells[18]. The history behind the origin of this cell line has generated important bioethical debates.

When Henrietta Lacks died on October 4, 1951, she could never imagine how valuable would be her unintentional contribution to science. She was a poor, black immigrant who worked in the tobacco fields and a mother. This simple American, who experienced a common end that was met by many other equally simple women, would remain unknown if her story was not also immortalized in a 1976 issue of Rolling Stone magazine and a book that was later published by Rebecca Skloot, titled "The Immortal Life of Henrietta Lacks". George Otto Gey, the researcher who generated the immortal HeLa cell line, had also never imagined how controversial his discovery would become in subsequent decades[19].

She was diagnosed with a cervical tumor at Johns Hopkins Gynecology Clinic in Baltimore, which was later confirmed in a biopsy that was performed a few months before her death, and the results of the biopsy erroneously revealed "epidermoid carcinoma, cervix uteri, spinal cell type". Ms. Lacks then underwent intense radiotherapy in the following months, which had no effect on the tumor. The cancer rapidly spread to her pelvis, and Mrs. Lacks progressively developed severe and intractable pain and bleeding, and she remained hospitalized with intense pain at Johns Hopkins Hospital from August 1951 until her death[18].

The controversial point is that Dr. Gey's research with Lacks' tumor cells occurred without her consent, which was a standard procedure at the time. Gey researched tissue culture surveys long before Mrs. Lacks' fatal illness, and for his research, he used biopsy specimens from surgical procedures that were performed at the hospital. However, the sample obtained from Lacks' biopsy presented extraordinarily successful growth, becoming the first cell line of human cancer cells that were immortalized in tissue culture. The cells were named "HeLa", which represents the first two letters of Henrietta Lacks' first and last names[18,20].

With the success of Gey's experiments, he distributed free samples of these cells to many research projects around the world, including cancer research and testing for the new polio vaccine by Jonas Salk. Samples are currently marketed and are an important part of the lucrative tissue and cell banking industry[21].

Without engaging in deeper issues on the subject, the case of Henrietta Lacks is a landmark example in the bioethical debate and has served as basis for the rules of conduct for research around the world when using human material for research and for the rights of donors.

THE HPV VACCINE: FRAZER AND ZHOU

A major step towards effective prevention of HPV-related diseases has been the development of the HPV vaccine. In November 2005, scientists announced the creation of world's first cervical cancer vaccine. The virus-like particle (VLP) method provided technical feasibility and excellent clinical results, and it was first reported by a team of immunologists including Dr. Ian Frazer in the early 1990s[22].

Several researchers concurrently studied this technique at different centers, and an inevitable patent dispute in courts took 16 years and involved Frazer (University of Queensland), and also John Schiller and Doug Lowy (National Cancer Institute), Richard Schlegal (Georgetown University), and Robert Rose (University of Rochester). US entities have negotiated their rights with GlaxoSmithKline while Australia has done so with Merck and both have developed almost identical vaccines[22,23].

In a very brief and sequential way, the following were the litigants' contributions: Frazer produced small incorrectly assembled VLPs; Schlegal did not produce VLPs but produced proteins with conformational epitopes that were identical to the HPV L1 protein; Schiller and Lowy produced morphologically correct VLPs but with L1 of the BPV, and Rose obtained VLP from HPV 11 L1, which is common to condylomas. Schiller and Lowy later produced the first VLP of HPV 16, the subtype that is most frequently implicated in cervical cancer[24]. In August 2007, the US Court of Appeals granted the definitive patent to Frazer[22].

Thus, Ian Frazer is the father of cervical cancer vaccine. However, at the commemorative event for the first vaccine implementation, Frazer made a point of praising the role of virologist Dr. Jian Zhou for his work[25]. Who was Zhou?

Dr. Zhou excelled at viral oncology studies at Beijing universities and then at Cambridge, where he met Frazer and collaborated with him in 1990 to research the HPV vaccine in Australia. Two years earlier, Zhou had developed the method of using the vaccinia virus as a vector to express specific proteins *in vitro*. Using the same recombinant DNA technique with a viral vector, Zhou produced HPV,

L1, and L2 capsid proteins in mammalian cells. Expressed capsid protein could be self-assembled in the VLP format[25]. The system assembled by Zhou was effective, but it had a low yield. To efficiently produce VLP with HPV capsid proteins, the contribution of other subsequent studies was necessary[9].

There was no dispute over the HPV vaccine paternity, as occurred in US courts. Frazer recognizes Zhou's co-authorship, and both authored pioneering article on the topic. Both applied for a provisional patent in June 1991. Frazer was established as having a major role for later leading the development team, and although Zhou's death in 1999, years before Frazer announced the results of his research to world. Zhou's previous studies were important in developing the vaccine[25].

CONCLUSION

Science and medicine have a history that is full of transformative and controversial events; and with HPV, it would not be any different. Knowing about the past and its characteristics, the achievements – despite difficulties, and the solutions that are found inspire and help us to understand the course that these events have taken and how we got here. This may also help us to have a future where HPV, at least as a pathogen, ceases to accompany us on this journey through the centuries.

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Conflict of interests

Nothing to declare.

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