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1988-2019 31 anos na vanguarda das DST
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VOLUME 31 Nº 1 2019

CONTENTS

EDITORIAL

ARTICLES

REVIEW ARTICLE

CASE REPORT

LETTER TO THE EDITOR

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The extraordinary trajectory of the Papillomavirus through human history

A extraordinária trajetória do Papilomavirus através da história da humanidade

Geilson Gomes de Oliveira[1], José Eleutério Jr[1, 2], Mauro Romero Leal Passos[3]

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 Babeș 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.

Participation of each author

The authors declare that all authors were active participants.

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Conflict of interests

Nothing to declare.

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The Th1-Th2 profile in immune responses to Human papillomavirus (HPV) in vitro in men from the city of São Paulo, Brazil

Perfil Th1-Th2 nas respostas imunes ao Papilomavírus Humano (HPV) in vitro em homens da cidade de São Paulo, Brasil

Fernando Augusto Miranda da Costa[1,2], *Karen Eliane de Oliveira Gaester*[2], *Alberto José da Silva Duarte*[2], *Jorge Casseb*[2]

ABSTRACT

Introduction: The cell-mediated immune response plays an important role in the control of HPV-induced cancers. Cytokines play an important function in host defense against HPV infection by modulating viral infection and polarizing the immune response towards Th1 or Th2 cells. **Objective:** To evaluate the specific immune response to HPV in vitro in men with and without lesions caused by HPV. **Methods:** We recruited 31 patients and 11 volunteers and divided them into the following four groups: 12 patients in Group A (HIV+/HPV+); 9 patients in Group B (HIV-/HPV+); 10 patients in Group C (HIV+/HPV-); and 11 healthy subjects in Group D (HIV-/HPV-). PBMCs culture assays were performed to measure the levels of Th1/Th2/Th17 cytokines (IFN-γ, IL-2, TNF-α, IL-4, IL-10 and IL-17) in cells from patients stimulated with a quadrivalent HPV vaccine (HPV 6, 11, 16 and 18) and the E7 protein of HPV-16. **Results:** The coinfected group A (HIV+/HPV+) showed higher levels of cytokines, especially Th2 cytokines, compared with the other study groups. The coinfected group had significantly higher levels of IL-6 and IL-10, which are Th2 cytokines, compared to the control group (HIV-/HPV-) ($p<0.0001$ and $p<0.0001$, respectively). **Conclusion:** This study reports a high production of cytokines in the coinfected group, suggesting strong immunomodulatory effects by HIV/HPV coinfection. However, further studies should be conducted to confirm these data. Because this group had high levels of Th2 cytokines, especially IL-6 and IL-10, these data suggest that these two cytokines may serve as biomarkers for viral persistence because HIV seropositive patients have a higher HPV persistence and may allow for the progression to more serious injuries to be monitored.

Keywords: human papilloma virus; cytokines; Th1-Th2 balance.

RESUMO

Introdução: A resposta imune celular exerce um importante papel no controle dos cânceres induzidos pela infecção por HPV. As citocinas desempenham um papel importante na defesa do hospedeiro contra a infecção pelo HPV pela modulação da infecção viral e a polarização da resposta imune para células Th1 ou Th2. **Objetivo:** Avaliar a resposta imune específica in vitro ao HPV em homens com e sem lesões causadas pelo HPV. **Métodos:** Foram recrutados 31 pacientes e 11 voluntários, divididos em quatro grupos: 12 pacientes no grupo A (HIV+/HPV+); 9 pacientes no grupo B (HIV-/HPV+); 10 pacientes no Grupo C (HIV+/HIV-); e 11 sujeitos saudáveis no grupo D (HIV-/HPV-). Uma cultura de PBMCs foi realizada para medir os níveis de citocinas Th1/Th2/Th17 (IFN-γ, IL-2, TNF-α, IL-4, IL-10 e IL-17) de células de pacientes estimulados com a vacina quadrivalente para HPV (HPV 6, 11, 16 e 18) e a proteína E7 de HPV-16. **Resultados:** O grupo A coinfectado (HIV+/HIV+) apresentou altos níveis de citocinas, especialmente citocinas do perfil Th2, comparados com os demais grupos estudados. O grupo coinfectado apresentou níveis significativamente mais elevados de IL-6 e IL-10, citocinas do perfil Th2, comparados ao grupo controle (HIV-/HPV-) ($p<0,0001$ e $p<0,0001$, respectivamente). **Conclusão:** Este estudo reportou uma elevada produção de citocinas no grupo de coinfectados, sugerindo um forte efeito imunomodulatório pela coinfecção HIV/HPV. Entretanto, outros estudos devem ser conduzidos para confirmar estes dados. Devido este grupo apresentar altos níveis de citocinas Th2, especialmente IL-6 e IL-10, esses dados sugerem que essas duas citocinas podem servir como biomarcadores para a persistência viral, uma vez que pacientes soropositivos para HIV apresentam níveis mais altos de persistência pelo HPV e podem permitir que a progressão para lesões mais graves possa ser monitorada.

Palavras-chave: papilomavírus humano; citocinas; equilíbrio Th1-Th2.

INTRODUCTION

Human papillomavirus (HPV) infection plays an important role in cervical cancer. HPV-infected women are 50 times more likely to develop cervical cancer than uninfected women[1]. To date, more than 200 genotypes of papillomaviruses that infect both humans and animals have been sequenced, classified as high-risk (HR) and low-risk (LR) for cancer. HR types include HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68[2,3]. Approximately one-third of these viruses infect the squamous epithelium of the genital tract, resulting in the appearance of several types of cancer, such as cervical, and anal cancer[3-12].

Currently, cervical cancer is one of the most common gynecologic malignancies in the world, accounting for approximately 15% of all cancers[10-14]. It is known that a risk factor for cervical cancer includes infection with specific types of human papillomaviruses (HPV), which is considered a necessary factor for the development of neoplastic lesions[1-17].

HPV infection in men is suggested to be as common as infection in women. Diseases associated with HPV in men also occur, such as carcinomas of the penis, anus and oropharynx. Male infection is usually subclinical, potentially resulting in a number of asymptomatic men serving as reservoirs of the virus and transmitters[18-21].

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One of the most important cofactors in HPV persistence and the development of cancer is coinfection with Human Immunodeficiency Virus (HIV). About 37 million people in the world are HIV-infected and 2 million are infected in Latin American and Caribbean[22]. Coinfection with HIV and HPV also affects the spread and persistence of human papillomavirus. In fact, it is unknown whether HIV has an additional effect on the transmission, reactivation and persistence of HPV infection[23]. Epidemiologic studies of HPV infection, HPV-associated disease, and HIV infection have helped us understand the relationship in HIV/HPV coinfection[24]. The sexual transmission of both infections leads to a high rate of coinfection in Brazil[25].

In immunocompetent patients, HPV infection is usually eliminated in seven to ten months[19]. In these patients, during the time that the HPV infection persists, immunodeficiency caused by HIV plays an important role in the pathogenesis of anal cancer. However, the exact mechanism behind the interaction between HIV and HPV is not well understood. It is thought that there is an absence of a cell-mediated immune response as a result of HIV infection[26]. Viral shedding by CD4+ T cells is important for the resolution of HPV infection, and there is a clear association between low levels of CD4+ T cells and cervical dysplasia[27].

The cell-mediated immune response plays an important role in the control of HPV-induced cancers[28-31]. The activation of CD4+ T cells results in the production and secretion of cytokines. The pattern of cytokine expression characterizes two major subsets of CD4+ T cells known as Th1, which produce IFN- γ, TNF-α and IL-2, and Th2, which produce IL-4, IL-6 and IL-10[28,32]. Cytokines play an important role in host defense against HPV infection by modulating viral infection and polarizing the immune response towards Th1 or Th2 cells[33]. The pattern of cytokines induced by T helper cells type 1 and 2 (Th1 and Th2) has been used to characterize the immune response in a number of human diseases, including diseases associated with HPV[34,35].

Some studies have demonstrated that during carcinogenesis of the cervical epithelium, there is a shift from a Th1 to a Th2 immune response[30-36]. The change from a Th1 to a Th2 immune response, which results in the down regulation of cellular immunity, might explain the loss of immune control of HPV infection and oncological complications[34-38]. The studies suggest that a Th1 response is associated with viral clearance[39,40] and a Th2 response is associated with the persistence of HPV infection and lesions progressing to cancer[34,41].

The immunosuppression observed in HIV-1-infected patients indicates that an efficient host immune response is closely linked to viral clearance or persistence. Cytokines play a very important role in host defense against HPV infection, as they affect the polarization towards either a Th1 or a Th2 response. Therefore, it is necessary to evaluate the immune profile of HPV-infected patients and correlate this profile with the progression of lesions. The results from this study provide useful information to better understand the specific host immune response to HPV infection and disease progression.

OBJECTIVE

To evaluate the immune responses to Human papillomavirus (HPV) *in vitro* in men from the city of São Paulo, Brazil.

METHODS

We recruited 31 patients and 11 healthy volunteers. The following four study groups were formed: 12 HIV-1-infected patients with lesions caused by HPV (Group A – HIV+/HPV+), 9 patients seronegative for HIV-1 with lesions from HPV (group B – HIV-/HPV+); 10 patients seropositive for HIV-1 but without HPV infection (group C – HIV+/ HPV-); and 11 healthy subjects (group D – HIV-/HPV-).

This article is part of a project approved by the Research Ethics Committee of both Clinics Hospital, School of Medicine, Universidade de São Paulo (HC/USP) and the Center for Reference and Training in HIV/AIDS in São Paulo (CRT/SP). All of the patients and volunteers received written and oral information about the project and, upon agreement with the study, signed an informed consent form.

Blood collection

The samples were collected from peripheral blood by venipuncture in three 10-mL vacutainer tubes containing sodium heparin (Becton Dickinson, NJ, USA).

Isolation of peripheral blood mononuclear cells

The PBMCs were isolated using density gradient centrifugation with Ficoll-Hypaque (GE Healthcare Life Sciences, Little Chalfont Buckinghamshire, England). The blood samples were diluted in saline at a 1:1 ratio and transferred to a 50 mL conical tube containing the Ficoll-Hypaque (1:3). The samples were centrifuged at 800xg for 20 minutes at 16ºC.

The PBMCs separated by the density gradient were removed and transferred to a 15 mL conical tube. The cells were washed with saline solution and then centrifuged at 450xg for 10 minutes at 16ºC. A second washing with saline was carried out under the same conditions. The supernatants were removed, and the cell pellets were resuspended in 1mL of RPMI 1640 culture medium to count leukocytes in a Cell-Dyn cytometer (Abbott Park, Illinois, USA). The cells were divided into single use aliquots at 2×10^6 cells/mL in RPMI 1640 supplemented with 10% AB serum. These aliquots were added to 24 well culture plates and treated with different stimuli.

Stimuli for PBMCs

We used a commercially available vaccine (Gardasil®, Merck & Co., Inc., USA) containing antigens from the L1 protein of four HPV types (HPV-6, 11, 16, 18) and E7 protein from HPV-16, which were available from the Institute of Biomedical Sciences of Universidade de São Paulo.

The PBMCs were suspended in RPMI 1640 culture medium supplemented with 10% AB serum at 2×10^6 cells/well in 24-well culture plates and stimulated separately with 2 µg/mL of Gardasil® or 15 µg/mL of the E7 protein for 72 hours at 37ºC and 5% CO2. The supernatants were then collected and frozen at -72ºC for subsequent analysis.

Cytometric bead array assay

For the analysis of cytokine expression by flow cytometry, the BD cytometric Bead Array (CBA) Human Cytokine Th1/Th2/Th17 kit (Becton Dickinson, NJ, USA) was used, according to the manufacturer's instructions. The BD CBA Th1/Th2/Th17 Human cytokine kit measures the protein levels of IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ and IL-17 in a single sample. The supernatants were analyzed on the BD LSR Fortessa cell analyzer (Becton Dickinson, NJ, USA).

Statistical analysis

The statistical analysis was performed using the GraphPad Prism version 4.03 software (GraphPad Software, San Diego, CA, USA). An analysis of variance test ANOVA was used. $P<0.05$ was considered significant.

RESULTS

Flow cytometry and the measurement of cytokines

Table 1 shows the median and range of cytokine production in the cells stimulated with the E7 protein from HPV-16. The patients coinfected with HIV and HPV had high levels of Th2 cytokines and low levels of Th1 cytokines.

Table 2 shows the cytokine profile of the cells from the different groups stimulated with the Gardasil vaccine. In all groups, we observed high levels of IL-6 under vaccine stimulation. The cytokine TNF-α was highly produced in the HIV+/HPV+ and HIV-/HPV+ groups, but there was no statistical significance. The cells from the HIV+/HPV+ group produced high levels of all the cytokines studied, except for IL-17.

IL-17

Low levels of IL-17 were produced in the cells from all of the study groups. Upon stimulation with the E7 protein from HPV-16, the cells from group D (HIV-/HPV-) produced the highest levels of IL-17 when compared to the other study groups, but there was no statistical significance (p=0.64).

In the cells stimulated with the vaccine, there were higher levels of IL-17 in the group only infected with HPV (HIV-/HPV+) compared to the other groups, where the levels remained the same. However, there was no statistical significance (p=0.27).

IFN- γ

The levels of IFN-γ produced in the CD4+ T cell supernatants were similar in both treatments. We observed a slight increase in the level of this cytokine in the cells stimulated with the Gardasil vaccine, but this was not statistically significant [p=0.4636 (E7) and p=0.4344 (vaccine).

TNF-α

High levels of TNF-α were produced in the cells from the coinfected group (HIV+/HPV+) under both stimuli conditions. However, the HIV-/HPV+ and HIV+/HPV- groups had a slight increase in this cytokine in response to the E7 protein, but not the vaccine. However,

Table 1 – Median and Range of cytokine production (pg/mL) in the cells from the all study groups stimulated with the protein E7.

Groups	IFN-y	IL-2	TNF-⟨	IL-10	IL-6	IL-17
Group A HIV+/HPV+	5.28 (0-4,226.75)	0.00 (0-2.96)	504.72 (23.58-41,445.14)	180.99 (57.72-2,075.28)	40,199.13 (34,323.02-45,953.69)	0 (0-11.43)
Group B HIV-/HPV+	28.77 (0-137.5)	1.01 (0-8.44)	1,167.88 (4.96-1,921.8)	121.61 (0-917,46)	33,186.65 (594.84-39,591.79)	0 (0-2.9)
Group C HIV+/HPV-	0 (0-5.63)	0 (0-8)	29,89 (0-5.63)	1.34 (0-620.73)	2,266.85 (198.66-40,735.43)	0
Group D HIV-/HPV-	6.98 (0-436.16)	1.14 (0-7.05)	1,123,94 (0-14,339.14)	36.75 (0-686.31)	29,026.88 (240.45-39,703.58)	0 (0-22.8)

HIV: human immunodeficiency virus; HPV: human papillomavirus.

Table 2 – Median and range of cytokine production (pg/mL) in the cells from all study groups stimulated with the Gardasil Vaccine.

Groups	IFN-y	IL-2	TNF-⟨	IL-10	IL-6	IL-17
Group A HIV+/HPV+	6.60 (0-4,790.41)	1.87 (0-5.94)	296.29 (14.05-40,404.95)	159.96 (40.86-1,619.07)	39,281.56 (33,469.65-42,096.16)	0 (0-11.43)
Group B HIV-/HPV+	10.84 (0-38.97)	3.71 (0-13.37)	306.12 (0-840.97)	37.11 (0-620.73)	21,010.87 (158.13-39,513.6)	0 (0-35.6)
Group C HIV+/HPV-	0 (0-0.32)	0.05 (0-126.1)	0 (0-18.19)	0 (0-2.6)	25.07 (2.4-7,557.75)	0 (0-7.58)
Group D HIV-/HPV-	1.27 (0-529.44)	4.47 (0-59.77)	0 (0-92.24)	0 (0-16.45)	132.97 (54.62-30,058.3)	0 (0-15.34)

HIV: human immunodeficiency virus; HPV: human papillomavirus.

this difference was not statistically significant. There was a slight increase in the production of TNF-α in the cells from the HIV-/HPV- group upon E7 stimulation.

IL-2

In the CD4+ T cells from the coinfected group (HIV+/HPV+) that were treated with both stimuli, IL-2, which is a Th1 cytokine, was produced at low levels. Cells from the HIV+/HPV- and HIV-/HPV- groups that were stimulated with the vaccine produced high levels of IL-2, but this was not statistically significant.

IL-10

The cells from the HIV+/ HPV+ and HIV-/HPV groups produced high levels of IL-10, a Th2 cytokine, in both culture conditions. The cells stimulated with the E7 protein produced higher IL-10 levels compared to cells stimulated with the vaccine, but this was not statistically significant.

In the cells stimulated with the vaccine, there was a statistically significant difference in IL-10 production between the cells from the coinfected group (HIV+/HPV+) and the group only infected with HIV (HIV+/HPV-) [$p<0.0001$] as well as and the coinfected (HIV+/HPV+) group and control group (HIV-/HPV-) [$p<0.0001$].

IL-6

The cytokine IL-6, a Th2 cytokine, was the most highly produced out of all the cytokines tested in all of the groups and culture conditions. The highest levels were produced in the cells from the HIV+/HPV+ group that were treated with both stimuli. Upon treatment with the E7 protein, there was significantly more IL-6 produced in the cells from the HIV+/HPV+ group compared to the cells from the HIV-/HPV+, HIV+/HPV- and HIV-/HPV- groups ($p=0.0005$, $p=0.0001$ and $p=0.0436$, respectively). IL-10 production was significantly higher in group A than in group B [$p<0.0241$].

DISCUSSION

Th1/Th2 immune responses

Persistent infection with HPV is a requirement for the cancer progression, and failure of viral clearance has been attributed to a deficiency in the immune response[41]. Diseases caused by HPV are characterized by the absence of cytotoxic responses specific and the absence of Type 1 CD4+ T cells that secrete IFN-γ, IL-2 and TNF-α. These cell types are critical in the generation of adaptive immunity[42,43].

It has been reported that a Th1 response is associated with the clearance of HPV infection and cervical lesion regression, while immunosuppressive cytokines of the Th2 profile are associated with viral persistence and the progression of cervical lesions[35-45].

Our data indicate a high production of both Th1 (IFN-γ and TNF-α) and Th2 (IL-10 and IL-6) cytokines in patients co-infected with HIV-1 and HPV, suggesting a strong immunomodulatory effect in HIV and HPV coinfection. HIV infection results in a dysregulation of cytokine production. In addition, little is known about HIV/HPV coinfection and the production of Th1 and Th2 cytokines. HIV-1-infected subjects have a high prevalence of HPV, a higher persistence of oncogenic viruses and a faster progression to cancer[46].

The data corroborate the results from other studies that showed high levels of IL-6 and IL-10 in women coinfected with HIV and HPV. The coinfection can cause an imbalance in the levels of cytokines, which can facilitate opportunistic infections[47-49]. We observed that in the cells stimulated with the E7 protein and Gardasil, the median levels of Th1 cytokines (IFN-γ, TNF-α and IL-2) in the cells from the HIV+/HPV+ group were lower compared to the levels in the cells from the other groups. However, there were higher levels of Th2 cytokines (IL-6 and IL-10) in the coinfected group (HIV+/HPV+) compared to the other groups.

Thus, we report that the HIV+/HPV+ group produced the highest levels of Th2 cytokines, corroborating other studies that indicate that this imbalance, especially in patients with HPV-associated lesions, promotes a faster progression to precursor lesions and cancer. A study carried out in 2007 indicated that Th2 cytokines were produced at higher levels in the blood of women with cervical dysplasia and increased with the degree of lesion, CIN II and CIN III[50]. A study published in 1999 reported the production of IL-6 (a Th2 cytokine) in cervicovaginal secretions. Increased levels were present in women with lesions compared to the control group, which correlates the production of IL-6 to the severity of cervical neoplasia[51].

Th-17 immune response

In 2005, a third CD4 cell type was identified: Th17. Some studies demonstrate that IL-17 cytokine promotes tumor growth and these effects are stronger than the anti-tumor T cell effects. In ovarian cancer, Th17 cells are elevated and have a pathogenic role in cancer development[52]. There are no data in the literature correlating the levels of IL-17 with lesions or cancer related to HPV.

We report a small increase in the production of IL-17 in the cells from patients infected with HPV-16 with lesions in response to the vaccine Gardasil, but no statistical significance was found. However, these data may be important in understanding the mechanisms as to why some patients progress more rapidly to cancer than others.

IFN-γ

Our data indicate that the cells from the co-infected group (HIV+/HPV+), with both stimuli had higher levels of IFN-γ. These data support an earlier study by Hong et al., where patients with a CD4+ T cell count >500 cells/mm^3 had increased levels of IL-2 and IFN-γ, suggesting that the cytokine profile can be influenced by HIV infection in the pre-HAART era[53]. Patients undergoing HAART have increased numbers of CD4+ T cells and an increase in the levels of IL-2 and IFN-γ[54].

TNF-α

The TNF-α controls HPV infection by inducing apoptosis in the infected cells and cervical cancer cells. An excess of TNF-α can result in a damaging inflammatory response that contributes to persistent infection and may also affect antigen presentation.

Insufficient antigen presentation to T lymphocytes may contribute to the persistence of HPV-16 and progression to cervical cancer[55].

Our data reveal an increased production of TNF-α in the HIV+/HPV+ patients. Azar et al. in 2004, reported high levels of TNF-α in patients with high-grade lesions[36]. These patients have lesions induced by HPV-16 and an inflammatory response, which may influence the persistence of infection. However, these data were not statistically significant.

IL-2

IL-2, a Th1 cytokine, is an important cytokine produced by activated T cells and is responsible for the clonal proliferation of T cells[56]. The cells from the coinfected (HIV+/HPV+) and HPV infected (HIV-/HPV+) groups with lesions produced lower levels of IL-2 than the cells from the HIV+/HPV- and HIV-/HPV- groups without lesions. These data support the work of Tsukui et al., who were the first to demonstrate that lymphocytes from women with cervical intraepithelial neoplasia produced lower levels of Th1 cytokines, especially IL-2, in response to HPV peptides[46]. In 2004, Lee *et al*. reported a lower proportion of CD4+ T cells producing IL-2, IFN-γ and TNF-α in patients with high-grade lesions compared to controls[48].

However, Garcia-Pineres et al. reported a significant increase in Th1 cytokines, particularly IL-2 and IFN-γ, in CD4+ T cells incubated with L1 VLPs[57]. The cells stimulated with the Gardasil in this study also produced higher levels of IL-2, which is similar to the previous study. However, our data were not statistically significant. Our data suggests the potential of the vaccine to induce a Th1 response, which is associated with viral clearance.

IL-10

The IL-10 results are similar to the results obtained by Bhairavabhotla et al. In cervical tumors, the presence of IL-10 mRNA was revealed, which can explain the immunosuppressive status of the patients with cervical cancer[58]. Park et al. in 2013 reported high levels of CD3/IL-10 T cells in patients with warts, suggesting that changing from the Th2 response and increasing IL-10 can prevent the clearance of infection[59].

Furthermore, high levels of IL-10 were found in patients with low-grade lesions, suggesting that IL-10 may inhibit the immune response against HPV infection. Azar et al. reported high levels of IL-10 in low-grade cervical lesions, making IL-10 a useful indicator for HPV-induced cervical lesions[36]. Bais et al. reported increased expression of IL-4 and IL-10 in the CIN III stage. The results obtained in our study confirm the results found by Bais et al. for IL-10 that indicate a shift from the Th1 to Th2 response[50].

IL-6

IL-6 is a proinflammatory cytokine that has been implicated in cervical cancer but also in many functions in normal conditions[60]. Our study revealed high levels of IL-6 in vaccine-treated cells from coinfected (HIV+/HPV+) and HPV infected (HIV-/HPV+) patients.

Tjiong et al. reported that IL-6 was present in higher concentrations in patients with cervical cancer. These data corroborate the results obtained in our study. The high levels of IL-6 may berelated to the severity of the lesions[51]. Wei et al. reported high levels of IL-6 in cancer biopsy tissues. These results suggested that a microenvironment containing high levels of IL-6 can promote angiogenesis and cancer development[60]. Another study conducted in 2010 reported high levels of IL-6 in HPV+ women compared with the healthy group[61].

There are few studies in the literature correlating the levels of different cytokines with the natural history of HPV infection[40] and coinfection with HIV in men. Further studies are necessary to understand the antiviral immune response mediated by T lymphocytes to develop new strategies that change the balance of Th1 cytokines in HPV-associated cancers[62].

CONCLUSION

We report increased cytokine production in patients coinfected with HIV+/HPV+, suggesting a strong immunomodulatory effect of HIV and HPV. The coinfected group (HIV+/HPV+) presented a Th2 cytokine response, which was characterized by the high production of IL-6 and IL-10 ($p<0.0001$, both cytokines) and low production of Th1 cytokines. It has been suggested that IL-6 and IL-10 may serve as biomarkers for viral persistence; monitoring the progression to more severe lesions resulting from virus infection. The quadrivalent vaccine was a good inducer of the Th1 response in patients not infected with HPV. Higher levels of IFN-γ and IL-2 were produced in the vaccine treated cells compared to the E7 protein treated cells. However, this increase was not observed in the patients already infected with HPV. The vaccine more effectively protected the patients that were naïve to the virus.

Our data on IL-17 was also intriguing. Several studies have reported that IL-17 promotes cancer development. Our study reported an increase in IL-17 in some patients infected with HPV with lesions. Therefore, it is important to understand the role of this cytokine in the progression/induction of lesions and cancer associated with HPV. Finally, it is important to conduct further research on the cytokine profiles of the Th1/Th2/Th17 responses and the relationship between cytokine levels and the degree of injury and HPV-associated cancer. This will aid in the understanding of these proteins in the antitumor immune response, especially in men and the HIV positive population, where there is very little information.

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Participation of each author

This work is the result of a doctoral thesis of the main author in Department of Dermatology of School of Medicine of São Paulo University. All steps of the study were performed by the author. Karen Eliane de Oliveira Gaester was the master student that helped in all the techniques of this work. Jorge Casseb was the study design, discussion and supervisor of this work. Alberto José da Silva Duarte was the study supervisor of this work.

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Conflict of interests

There is no conflict of interest to be reported.

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Clinical and epidemiological profile of patients who seek post-exposure prophylaxis after sexual exposure to HIV and their adherence to the medication regimen in a specialized center in Cascavel, Paraná, Brazil

Perfil clínico e epidemiológico de pacientes que buscam profilaxia pós-exposição sexual ao HIV e sua adesão à medicação em Cascavel, Paraná, Brasil

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ABSTRACT

Introduction: Post-Exposure Prophylaxis (PEP) is part of a new strategy for the prevention of Human Immunodeficiency Virus transmission adopted by the Brazilian Ministry of Health. The approach involves the use of antiretroviral medication for 28 days after potential exposure to HIV in order to prevent the establishment of infection. **Objective:** To evaluate the epidemiological profile of patients who seek PEP after consensual sexual activity in a specialized center for infectious diseases in Cascavel, Paraná, Brazil. **Methods:** This study involved retrospectively evaluating a cohort based on the medical records of patients who received PEP between November 2011 and July 2016. **Results:** A total of 153 medical records were analyzed and it was observed that more men (77.12%) than women (22.9%) sought PEP. The average age of women and men was 30.05 years and 29.06 years, respectively. Since the implementation of PEP in 2001, the annual demand for the treatment has steadily increased. The majority of patients (96.76%) sought care within the 72-hour deadline for the start of prophylaxis. Although 85.62% of total cases received recommendations for the use of prophylaxis, it was possible to verify adherence to the medication regimen for the recommended time in only 45.90% of cases. Among the patients who adhered to treatment, no cases of seroconversion were observed. A gradual decrease in attendance of follow-up appointments was noted, with approximately 45% of patients abstaining after 30 days of initial care, increasing to nearly 80% after 12 weeks. **Conclusion:** Despite the apparent efficacy of prophylaxis, keeping track of patients undergoing prophylactic treatment remains difficult. Improved knowledge on the epidemiological profile of the population in question may be expected to guide public policies aimed at the prevention of Acquired Immunodeficiency Syndrome.

Keywords: AIDS; post-exposure prophylaxis; HIV.

RESUMO

Introdução: A profilaxia pós-exposição (PPE) faz parte das novas estratégias de prevenção da transmissão do vírus da imunodeficiência humana adotadas pelo Ministério da Saúde do Brasil. A abordagem constitui-se do uso de medicação antirretroviral por 28 dias após potencial exposição ao vírus, impedindo que o mesmo se estabeleça no organismo. **Objetivo:** Avaliar o perfil epidemiológico de pacientes que buscam PPE por atividade sexual consensual em um centro especializado em doenças infecciosas de Cascavel, Paraná, Brasil. **Métodos:** O trabalho consistiu na avaliação de uma coorte retrospectiva baseada na coleta de dados de prontuários de atendimentos para PPE sexual de novembro de 2011 a julho de 2016. **Resultados:** Foram analisados 153 prontuários e observou-se procura superior por PPE por indivíduos do sexo masculino (77,12%) em relação ao feminino (22,9%). A média de idade foi de 30,05 anos entre as mulheres e 29,06 entre os homens. Observou-se tendência de demanda anual ascendente de procura pelo serviço desde a implantação da PPE em 2011. A grande maioria dos pacientes (96,76%) buscou atendimento dentro do prazo limite de 72 horas para o início da profilaxia. Do total de casos, 85,62% recebeu recomendação para o uso da medicação profilática, em apenas 45,90% desses foi possível verificar a aderência à medicação pelo tempo recomendado. Entre os pacientes que aderiram à profilaxia não foram registrados casos de soroconversão. Verificou-se redução gradativa do comparecimento às consultas de acompanhamento, houve abstenção de aproximadamente 45% após 30 dias do atendimento inicial, chegando a quase 80% passadas 12 semanas. **Conclusão:** Apesar da aparente eficácia da profilaxia, ainda existe dificuldade em manter o acompanhamento dos pacientes para os quais o tratamento foi instituído. Espera-se que o melhor conhecimento das informações acerca do perfil da população em questão possa contribuir para o direcionamento de políticas públicas voltadas à prevenção da síndrome da imunodeficiência adquirida.

Palavras-chave: AIDS; profilaxia pós-exposição; HIV.

INTRODUCTION

The epidemic of acquired immunodeficiency syndrome (AIDS) has caused worldwide concern since the first confirmed case. In Brazil, where universal access to treatment and prevention is available, the disease presents a chronic character, with a reduction in mortality and an increase in life expectancy[1]. Currently, 827,000 people are infected with the Human Immunodeficiency Virus (HIV) in Brazil; however, 112,000 of these people are still unaware of their disease status[2], indicating the need for improved infection prevention strategies . In 2014, during the International AIDS Conference, the 90-90-90 target was presented as a global goal to end the epidemic by 2030[3]. As no single prevention method is able to contain the AIDS epidemic[4], the use of combined prevention strategies has

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become essential[5]. In order to be part of the global trend to end the epidemic, these new strategies were adopted by the Brazilian Ministry of Health to form part of the HIV transmission prevention policy. The use of antiretroviral medications, in the form of Post-Exposure Prophylaxis (PEP) after possible contact with seropositive patients is part of this prevention approach.

PEP is the last available prevention resource to avoid the establishment of HIV infection after failure or non-use of other preventive methods, and thus, it plays a strategic role in fighting the increase in the number of HIV cases[2]. PEP is based on the daily use of antiretroviral medications for 28 days after HIV exposure[6]. Addressing potential HIV transmission after sexual contact involves accepting the demand for PEP, evaluating the exposure circumstance, and characterizing the risk of transmission to consider the indication of chemoprophylaxis[1]. Therefore, sexual PEP has been applied as an emergency precaution in specific situations, to complement, but not replace, other preventive methods[7]. Access to prophylaxis is of major importance in a post-exposure treatment strategy, since antiretroviral prophylaxis should be administered as soon as possible after exposure and the period to start treatment is limited to 72 hours following the occurrence[8]. Prophylaxis is indicated in cases of exposure with a significant risk of HIV transmission, such as unprotective sex with partners with HIV or unknown serology, and considers factors that increase transmissivity, such as rupture of the mucosal barrier and the presence of bleeding or sexually transmitted infections (STIs)[6,8]. At present, the preferred and universally indicated antiretroviral regimen for PEP, independent of the type of exposure and biological material involved, is combined therapy composed of Tenofovir (TDF), Lamivudine (3TC), and Atazanavir/Ritonavir (ATV/r)[6,9].

Patient adherence to the 28-day antiretroviral therapy period is essential to ensure effective prophylaxis. However, published studies have shown that a low proportion of patients complete the entire course of PEP[10]. Limited research has been done to determine ideal rates of PEP adherence and on ways to encourage patient participation. In a study on more than 3,500 participants in the United States, 70% of patients completed the entire course[11]. A recent review of studies on PEP adherence carried out across several continents indicated that adherence rates varied between 49% and 92%[10]. Thus, the World Health Organization considers that improved adherence support could increase PEP completion rates and recommends that enhanced support for adherence with specific interventions be provided as part of PEP treatment[8].

Studies that evaluate the AIDS epidemic at a local level are rare and it is recommended that local policies be reduced to allow cities to take responsibility for planning and organizing health care according to their context[12]. The use of PEP for consensual sexual relations is a recent development in Brazil. The approach was first indicated in 2010[1], but until now assessment of it has mainly been at a local or regional level.

OBJECTIVE

The aims of this study were to characterize the clinical and epidemiological profile of patients who seek PEP after high-risk sexual exposure, to verify the outpatient follow-up, as well as to evaluate adherence to the proposed drug treatment as a form of prevention of HIV infection.

METHODS

Patients

Data for this retrospective cohort study were taken from the medical records of patients undergoing PEP treatment at the Specialized Center for Infectious-Parasitic Diseases (CEDIP) in the city of Cascavel, Paraná, Brazil.

Data collection occurred for nearly 5 years, from November 2011 to July 2016. Medical records of all male and female patients who underwent medical evaluations for consensual sexual PEP and their HIV infection risk were analyzed and included in the study. Patients were excluded if they were undergoing treatment for non-sexual exposure to HIV such as work accidents involving sharp objects, if they formed part of a serodiscordant couple in search of natural conception, and if they received relief from respiratory arrest without contagion risk.

The medical records of included cases were collected using a standardized protocol to build the research database, which included patient identification data (initials, gender, age, and marital status), factors related to exposure, indication of prophylaxis, follow-up data, and information on adherence to treatment.

This work was approved by CEP under opinion number: 1.295.811.

Data analysis

After collecting the information using a form, data compilation was carried out in Microsoft Excel (2016), followed by a descriptive analysis. Quantitative variables were represented by their measures of central position, and variability was represented by median and standard deviation (SD). Categorical variables were characterized by their absolute and relative/percentage values. The continuity of outpatient follow-up was determined by analyzing the rate of attendance of previously scheduled medical appointments. Variable analysis corresponding to the characteristics of patients was indicated by the total number, averages, and percentages. The use of prescribed drugs was verified by means of a voluntary verbal response recorded in the patient's medical record.

The research project was previously submitted to and approved by the Research Ethics Committee (REC) of the University Center Assis Gurgacz Foundation (FAG) (Approval Opinion No. 1.295.811).

RESULTS

During the study period, we identified 168 individuals who sought medical appointments for PEP. Of these, 15 were excluded because they did not fulfill the inclusion criteria of exposure by sexual contact; the remaining 153 patients were analyzed. Women accounted for 22.9% (35/153) of cases and men for 77.12% (118/153). The average age of female patients was 30.05 years (SD: 9.988) and 29.06 years (SD: 8.802) for male patients. Among the total number of patients, 58.82% (90/153) were single; 26.80% (41/153) married;

9.15% (14/153) separated, widowed, or divorced; and 5.23% (8/153) did not supply this information.

When we evaluated the recurrent demand for PEP, we found that only 4.57% (7/153) of visits were from patients who sought prophylaxis for more than one occurrence of sexual exposure to HIV.

Regarding the number of medical appointments for PEP during the study period, an increase was observed in the annual average demand since the implementation of the service in 2011 (Figure 1). Concerning the time elapsed after exposure, 96.73% (148/153) of the patients sought PEP within the maximum recommended interval (up to 72 hours after contact), and only 3.27% (5/153) sought PEP after this period. When questioned about the HIV status of their partners, 50.32% (77/153) of patients were unaware of the serology of their partner, 35.95% (55/153) were aware that their partner had positive HIV serology, 4.57% (7/153) requested that their partner undergo testing, and 9.15% (14/153) did not supply this information.

It was not possible to obtain clear data on sexual orientation because patients were questioned on the sexual behavior of their partners and not their own behavior. As for the partners, it was observed that 31.37% (48/153) were men who had sex with men, 56.21% (86/153) were heterosexual, and in 12.42% (19/153) of the cases, no information was available. Furthermore, it was found that only 4.57% (7/153) of the interviewees declared themselves to be sex workers (SW), but 22.22% (34/153) stated that their partners were SW. When habitual sexual behavior was evaluated, 65.36% (100/153) of patients indicated that they use a barrier method (condom), 18.95% (29/153) did not frequently use it, and in the remaining 15.68% (24/153) it was not possible to assess this information.

By using the information recorded in the medical records, it was possible to analyze the type of sexual contact that motivated patients to seek PEP **(Table 1)**. Among women, the great majority (80.55% [29/36]) reported receptive vaginal penetration as the type of sexual contact that motivated them to seek prophylaxis. Among men, the type of contact was mainly reported to be insertive vaginal penetration (47.06% [56/119]), insertive anal penetration (25.21% [30/119]), and receptive anal penetration (16.80% [20/119]).

Since routine testing for other STIs was not established initially, not all patients were tested. However, using the 153 analyzed records we were able to verify that 126 patients were tested for syphilis, with 6.78% (8/126) being positive for the disease. For Hepatitis C, only 0.88% (1/114) of patients presented positive serology, and none presented positive HIV serology, allowing all at-risk patients to receive prophylaxis.

When the need for PEP was analyzed, it was observed that 85.62% (131/153) of medical evaluations indicated the use of PEP, whereas PEP was discouraged in 14.38% (22/153) of cases. The main clinical reason for the non-recommendation of PEP was the low risk of infection and exceeding 72 hours after contact.

The rates of outpatient follow-up after the initial appointment, determined by the attendance of previously scheduled appointments, were also evaluated **(Table 2)**. The CEDIP service initially required patients to return after 30 days, 12 weeks, and 24 weeks following the start of PEP treatment in order to follow up and repeat serological tests. However, after changing the clinical protocol in July 2015, the Ministry of Health no longer recommended the 24-week return appointment, and the returns were subsequently analyzed up to the 12-week appointment only.

Upon evaluation of the collected data, it was observed that 54.20% of patients returned for the appointment scheduled for 30 days after receiving initial treatment, and 20.61% returned for the 12-week appointment, indicating a gradual abandonment of follow-up.

When adherence to the PEP regimen was assessed, 83.78% of the 131 patients for whom prophylaxis was recommended and who returned for the follow-up appointment at 30 days or more after treatment initiation adhered to the treatment for the prescribed time; 16.21% (12/74) reported discontinuation of PEP. The remaining 43.51% (57/131) did not return to CEDIP after the initial appointment, and it was not possible to verify whether these patients adhered to the treatment schedule, if subsequent serological tests were performed, or even if follow-up was done at another center.

HIV serological monitoring of patients using a rapid test was mainly performed during the first appointment, after 30 days, and after 12 or 24 weeks. No patients tested positive at their initial appointment, and no instances of seroconversion were observed among any of the patients who attended the subsequent appointment.

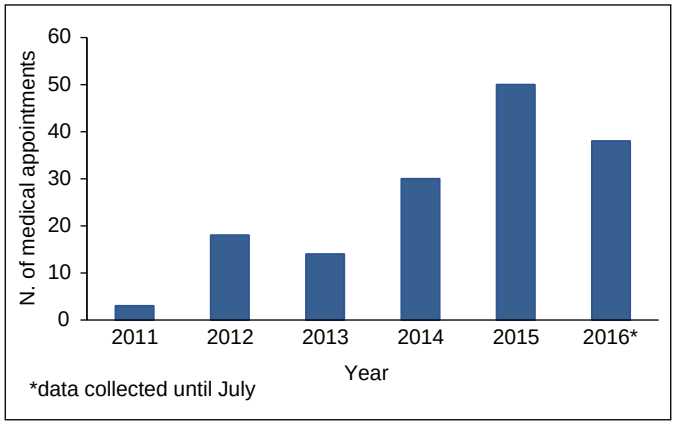

Figure 1 – Annual distribution of the number of medical appointments for PEP in the Center for Infectious-Parasitic Diseases (CEDIP) unit, Cascavel, PR, Brazil, from November 2011 to July 2016.

Table 1 – Type of sexual contact during exposure according to gender.

Type of sexual contact	Female	Male	General
Receptive anal penetration	4	20	24
Receptive vaginal penetration	29	-	29
Insertive vaginal penetration	-	56	56
Insertive anal penetration	-	30	30
Receptive oral sex	3	12	15
Unavailable information	5	13	18

Table 2 – Percentage of attendance of follow-up appointments.

	Number of patients who attended follow-up	Percentage of patients who attended follow-up
30-day return	71	54.20%
12-week return	27	20.61%

DISCUSSION

This study focused on the characterization of the clinical and epidemiological profile of patients treated with PEP due to sexual exposure to HIV, their attendance of outpatient follow-up appointments, as well as the evaluation of adherence to the treatment regimen when established.

It was found that our study population consisted mainly of young adults, represented predominantly by men. This profile was also observed in another Brazilian study, in which only 15.9% of patients seeking PEP after sexual contact were represented by women[13]. In addition, a North American study of more than 3,500 patients found that 92% were men[11]. Approximately 95% of patients sought PEP for the first time; therefore, recurrent use was low.

Since the beginning of prophylactic care in 2011, an increasing number of people have been seeking PEP, indicating that the population may be more knowledgeable on the treatment and that the number of professionals who recommend the specialized centers to patients is increasing.

In the present study, it was observed that the majority of patients who sought PEP did so within the maximum allowed time frame to start prophylaxis. Although the knowledge and use of PEP by the public are described as low[14,15], the studied population was shown to be aware of the recommended time limit for beginning treatment.

Nearly 35% of the patients were aware of the seropositivity of their sexual partner; however, more than half of the patients were unaware of their partner's serology. This result is similar to that of an Australian study where 32% of patients were unaware of their partner's serological status[16].

It was observed that among women, receptive vaginal penetration prevailed as the most common form of sexual contact responsible for the search for PEP. Among men, the most commonly reported sexual contact was insertive vaginal penetration; however, when grouping insertive and receptive anal intercourse together, the incidence of anal intercourse approximates that of insertive vaginal intercourse. Considering that PEP has been suggested as a cost-effective prophylactic method for all types of sexual contact between men[17], finding vaginal and anal intercourse to be the main types of contact motivating the search for PEP justifies promoting access to prophylaxis in this population.

The results show that PEP was recommended to a high proportion of patients in the studied population. It was noted that 85.62% of patients were at a high risk of HIV transmission and PEP treatment was therefore recommended. A similar result was found in a study on a North American cohort, where PEP was recommended for 78% of patients[11].

Although interventions to increase treatment adherence are still controversial[7], the Brazilian Ministry of Health recommended the use of strategies to improve follow-up and adherence, such as cell phone messages (SMS) and telephone calls[6]. Based on the progressive withdrawal from follow-up, some strategies may need to be rethought by local policymakers in order to intensify the monitoring of patients receiving prophylaxis.

When considering the patients who maintained follow-up, adherence to the treatment regimen was 83.78%, a slightly improved result compared to other studies where adherence was approximately 80%[10]. It is unlikely that all participants who abandoned follow-up discontinued the use of their medication. However, assuming all patients lost to follow-up discontinued treatment as scheduled, an adherence rate of 43.51% can be expected. This is slightly lower than the rates observed in a review of 17 studies that analyzed adherence to PEP where rates varied between 49% and 92%[10]. Even considering an adherence rate of 43.51%, this is still higher than the nearly 40% adherence rate observed by studies that evaluated PEP use after experiencing sexual aggression[8,18].

Although more than 54% of the followed-up patients underwent testing within 30 days after exposure and no seroconversion was detected among them, it was possible to confirm the absence of HIV infection after 12 weeks in only 20.61% of patients. Whether the prescribed medication was used or not could only be confirmed in patients who returned after 30 days; therefore, this assessment was not possible in more than 40% of cases. The high rate of patient evasion impaired the analysis of data on seroconversion, even though it was part of the CEDIP service's protocol to guide patients on the importance of clinical and laboratory monitoring during and after the prophylaxis period.

Globally, significant progress has been made to eliminate new HIV infections among children; however, the number of new HIV infections among adults remains stable, and our results demonstrate the need to expand prevention measures against HIV infection in this age group[19].

This study presented limitations concerning the difficulties in characterizing the levels of education and socioeconomic status of the participants, the inability to characterize patient adherence to the medication regimen, and the lack of serology of patients who did not return to the CEDIP service for follow-up. Even withthese difficulties, it is expected that the epidemiologic profile of the public who seek PEP may be useful in determining protection measures against HIV infection within a local context. Furthermore, this is the only study on the use of PEP in this region of Paraná State.

CONCLUSIONS

The profile of patients who seek PEP was characterized as being mainly young men who are unaware of the serological status of their sexual partner. The search for prophylaxis was mainly motivated by insertive anal and vaginal intercourse. Additionally, the study demonstrated a low rate of outpatient follow-up. Nevertheless, it was found that among the patients who maintained follow-up, adherence to the treatment regimen was good and no instances of seroconversion were observed in patients, regardless of their adherence to the entire treatment period of PEP. Based on our results, it is clear that strategies aimed at increasing patient adherence to PEP treatment need to be reevaluated and restructured in order to improve prophylaxis monitoring.

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Participation of each author

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Conflict of interests

Nothing to declare.

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Prevalence of hepatitis B vaccination and serological immunity of women in prenatal care at an university outpatient clinic in Southern Brazil

Prevalência da vacinação contra hepatite b e imunidade sorológica em mulheres que realizaram o pré-natal em um ambulatório universitário no Sul do Brasil

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ABSTRACT

Introduction: Viral Hepatitis B is an infection with a high transferability, and delivery is the main form of transmission to newborns. Investigating HBV infection in pregnant women should be undertaken through research on the surface antigen of Hepatitis B virus (HBsAg), and the immunity to the virus should be assessed by the presence of the antibody against the surface antigen of the Hepatitis B virus (anti-HBs). Vaccination is recommended during pregnancy to all patients with non-reactive HBsAg and anti-HBs results. **Objective:** To analyze data related to the preventive measures of HBV infection in pregnant women who underwent prenatal follow-up at the Gynecology and Obstetrics outpatient clinic of the Medical School of Universidade Federal de Pelotas (Rio Grande do Sul State). **Methods:** A cross-sectional study was carried out by analyzing the medical records of patients from the Obstetrics and Gynecology outpatient clinic. Data related to the preventive measures of HBV infection, the socioeconomic profile and ethnicity of these patients were analyzed and described. **Results:** The total number of pregnant women studied was 121, aged between 15 and 46 years old, mostly white, who had a partner, and earned more than two minimum wages. Seventy-one patients presented non-reactive HBsAg, and no patient showed a reactive test. Roughly 40% of pregnant women had not undertaken the HBsAg test. Among the 121 analyzed pregnant women, 74 (61%) performed the anti-HBs test, and only 14 (19%) showed they were reactive. Approximately 15% showed complete vaccination before prenatal care, but only 9 of them had taken the reactive anti-HBs test. **Conclusion:** A lower expected number of pregnant women who underwent screening for infection (HBsAg), and immunity (anti-HBs) from Hepatitis B, and there was a strong incomplete adhesion to vaccination. It is necessary to encourage the request and performance of the tests and adequate medical records filling out, as well as confirmation on the adhesion to the three doses of the vaccination schedule recommended by the Brazilian Ministry of Health.

Keywords: hepatitis B; seroconversion; vaccination; prenatal care.

RESUMO

Introdução: A hepatite viral B (HVB) é uma infecção com elevada transmissibilidade, sendo o parto a principal forma de transmissão para recém-nascidos. A investigação da infecção por HBV na gestante deve ser realizada com pesquisa do antígeno de superfície do vírus da hepatite B (HBsAg); já a imunidade ao vírus deve ser avaliada pela presença do anticorpo contra o antígeno de superfície do vírus da hepatite B (anti-HBs). A vacina é recomendada durante a gestação para todas as pacientes com resultado HbsAg e anti-HBs não reagentes. **Objetivo:** Analisar dados relacionados às medidas preventivas da infecção por HBV em gestantes que realizaram o acompanhamento pré-natal no ambulatório de Ginecologia e Obstetrícia da Faculdade de Medicina da Universidade Federal de Pelotas (Rio Grande do Sul). **Métodos:** Estudo transversal por análise dos prontuários das pacientes do ambulatório de Ginecologia e Obstetrícia, analisando e descrevendo dados relacionados às medidas preventivas da infecção por HBV, perfil socioeconômico e etnia dessas pacientes. **Resultados:** O número total de gestantes analisadas foi de 121, de idades entre 15 e 46 anos, maioria branca e com companheiro, e com renda maior que dois salários mínimos. Setenta e um pacientes apresentaram HBsAg não reagente e nenhuma paciente apresentou o exame reagente. Pouco mais de 40% das gestantes não havia realizado o exame HBsAg. Entre as 121 gestantes analisadas, 74 (61%) realizaram o teste de anti-HBs, e apenas 14 (19%) tiveram o teste reagente. Cerca de 15% comprovaram vacinação completa antes do acompanhamento pré-natal, porém apenas nove delas apresentaram o exame anti-HBs reagente. **Conclusão:** Foi encontrado um número inferior ao desejado de gestantes que realizaram os exames de triagem de infecção (HBsAg) e imunidade (anti-HBs) da hepatite B, e expressiva adesão incompleta à vacinação. É necessário incentivo à solicitação e realização de exames, e um preenchimento adequado do prontuário médico, além de confirmação da adesão das três doses do esquema vacinal recomendado pelo Ministério da Saúde.

Palavras-chave: hepatite B; soroconversão; vacinação; cuidado pré-natal.

INTRODUCTION

Viral Hepatitis B is an acute infection that can develop into chronicity with high transferability and impact on public health[(1)]. It can be transmitted by parenteral, sexual, and vertical transmission, and delivery is the main form of transmission to newborns[(2)]. Hepatitis B virus (HBV) infection does not interfere in the course of pregnancy, nor does pregnancy worsen the progression of Hepatitis B, which is not related to an increase in maternal mortality or to a teratogenic effect on the fetus[(1)]. However, infection of the newborn shows a chronic percentage far superior when compared to infection of adults, with about 90% of neonates evolving into the disease's chronic form, with higher rates of morbidity and mortality, especially related to cirrhosis and hepatocellular carcinoma[(2)].

The investigation of HBV infection in pregnant women should be conducted through research on the surface antigen of

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Hepatitis B virus (HBsAg) during the 1st trimester of pregnancy, or when prenatal care is started. The test should be repeated in the 3rd trimester of pregnancy to detect possible infections occurring during pregnancy[3]. Pregnant women with reactive HBsAg test, an indicative of active infection, should be guided to early administration of immunoglobulin to Hepatitis B virus (HBsAg) specific vaccine to the newborn, as well as later, at the time of delivery, to services that provide such care[2]. Pregnant women not evaluated during prenatal care for the infection should undergo a search for HBsAg at the time of hospital admission for delivery[3].

Despite the introduction of the vaccine, the vertical transmission of Hepatitis B is still a reality[2]. Immunization against Hepatitis B is performed in three doses, with a month interval between the first and second doses, and of six months between the first and the third dose, and can be performed at any age[3]. The vaccine for Hepatitis B during pregnancy is recommended for all patients with no reactive HBsAg results, and administered at any quarter[3]. This is an opportunity to vaccinate those women who had not been immunized for some reason.

After administering three doses of vaccine against Hepatitis B, about 90% of adults and 95% of children and adolescents are expected to develop immunity against Hepatitis B. This rate may be reduced in premature neonates, individuals over 40 years old, immunocompromised, obese, smokers, drinkers, or patients with other chronic diseases, like cirrhosis[4].

Testing for the development of immunity by detecting antibody (anti-HBS) after vaccination is not routinely recommended by the Brazilian Ministry of Health, only in specific cases, such as when it is done by health professionals or people who work in risk environments of an accident with instruments contaminated with the Hepatitis B virus, dialysis, HIV-positive patients, people who have sex with partners who are HBsAg positive, and children of HBsAg positive mothers[5].

The American College of Obstetricians and Gynecologists (ACOG), as well as the Centers for Disease Control and Prevention (CDC) recommend the prenatal screening of all HBsAg pregnant women[6]. The recommendation by the Advisory Committee on Immunization Practices (ACIP) is to perform the Anti-HBs test on all pregnant women. There is no evident risk to the fetus when taking Hepatitis B vaccine during pregnancy. Pregnancy itself is not a contraindication for vaccination if it is formally indicated[7].

Research has been approved by the Research Ethics Committee (REC) of the Medical School of Universidade Federal de Pelotas, Rio Grande do Sul State.

OBJECTIVE

To analyze adhesion to the recommended Hepatitis B vaccination schedule, by the Ministry of Health, and to conduct a research test for Hepatitis B virus (HBsAg) infection, in addition to the serological immunity survey (anti-HBS) after the vaccine is performed in pregnant women who attended prenatal care at the Gynecology and Obstetrics outpatient clinic of the Medical School of Universidade Federal de Pelotas (Rio Grande do Sul State).

METHODS

A cross-sectional study was carried out through the analysis of the medical records of patients from the Gynecology and Obstetrics outpatient clinic of Universidade Federal de Pelotas, in Rio Grande do Sul State, Brazil. Data collection was performed by the co-authors of this study, who are academics at the Medical School of Universidade Federal de Pelotas. The students, who performed the curricular internship in Gynecology and Obstetrics at the outpatient clinic, voluntarily initiated the review of the medical records and the accounting of the number of pregnant women in prenatal care, accessing the results of serological tests for Hepatitis B.

The criterion used in the selection of the analyzed medical records was pregnant women in prenatal care from November 2017 to May 2018, regardless of their gestational age. Thus, all medical records of pregnant women who were on that day in prenatal consultations were daily evaluated by the students. The review of the medical records included data related to preventive measures of HBV infection, such as rapid tests and vaccination, socioeconomic profile and ethnicity, complete age in years, skin color, marital status, and family income. All pregnant women in prenatal follow-up during the study period were included, and the data were updated in the subsequent antenatal consultations, as serological tests were performed.

RESULTS

The total number of pregnant women analyzed was 121, aged between 15 and 46 years old, and the mean age was 26.7 years. For mothers, white skin color was predominant (66.1%), most of the pregnant women had a partner (66.7%), and a monthly income more than 2 minimum wages (59.5%) **(Table 1)**.

Table 1 – Sociodemographic characteristics of pregnant women who received prenatal care at the Medical School outpatient clinic of Universidade Federal de Pelotas, Rio Grande do Sul State, from November 2017 to May 2018.

Variable	N	%
Age		
15 to 20	19	15.70
21 to 25	31	25.62
26 to 30	34	28.10
31 to 35	22	18.18
36 to 40	7	5.79
Over 40	3	2.48
Not notified	5	4.13
Partner		
With partner	66	54.54
No partner	13	10.74
Not notified	42	34.72
Color		
White	80	66.12
Not White	32	26.44
Not notified	9	7.44
Income		
Less than 2 minimum wages	10	8.26
More than 2 minimum wages	72	59.51
Not notified	39	32.23

Regarding the laboratory HBsAg test, 71 patients (58.7%) showed non-reactive results, indicating the absence of acute infection, and no patient showed reactive tests. As opposed to the recommendations by the Ministry of Health, 50 pregnant women (41.3%) had not undergone the HBsAg exam until data collection time. When analyzing the immunity of patients, only 14 (11.6%) showed the reactive anti-HBS test, representing immunity to Hepatitis B virus. However, 47 pregnant women (38.8%) had not undergone the anti-HBS examination during data collection. Only one patient (0.8%) showed an inconclusive result, but there was no information on the vaccination status of this patient in medical records. Concerning the vaccination status, 16 patients (13.2%) confirmed complete vaccination before prenatal care, and 24 pregnant women (19.8%) did not present information on vaccination in medical records **(Table 2)**.

Of the 30 patients with complete vaccination status, only 9 (30%) presented the anti-HBS reactive test, proving immunity to Hepatitis B virus, and eight patients (26.7%) did not undergo the examination. However, 13 patients with pre or peri-gestation with complete vaccination schedule had non-reactive anti-HBS (43.3%) and could be interpreted as a failure in the serological shift after vaccination. Among the 24 patients who did not have information on their vaccination status, 3 (12.5%) showed reactive anti-HBS exams, immunized against Hepatitis B. This may have happened because of previous vaccination, or also immunization after contact with Hepatitis B virus **(Table 3)**.

Table 2 – Immunological, Hepatitis B infection virus, and vaccine characteristics of pregnant women served at the Medical School prenatal outpatient clinic of Universidade Federal de Pelotas, Rio Grande do Sul State, from November 2017 to May 2018.

Vaccination status	N	%
Hepatitis B Vaccine		
Pre-gestation complete vaccination schedule	16	13.22
Peri-gestation complete vaccination schedule	14	11.57
Only first dose	56	46.29
Only two doses	11	9.09
Not notified	24	19.83
Anti-HBs		
Reactive	14	11.57
Non reactive	59	48.76
Not performed	47	38.84
Inconclusive	1	0.83
HBsAg		
Reactive	0	0.00
Non reactive	71	58.68
Not performed	50	41.32

Anti-HBs: hepatitis B virus surface anticorp; HbsAg: hepatitis B virus surface antigen.

DISCUSSION

The vaccine against Hepatitis is recommended by the WHO (World Health Organization) since 1991, and was only included in the Brazilian National Program of Immunizations in 2009. In 2013, the Brazilian Ministry of Health included pregnant women as a priority group for vaccination[8]. Prenatal follow-up is the ideal time to trace Hepatitis B virus infection, and also to indicate the vaccination to patients who are not immune[8].

Evidence shows that the vaccination schedule in three doses is highly effective in preventing the disease and creating antibodies, with a response rate higher than 90%[7].

This study, however, showed an adhesion less than the ideal; approximately 20% of the patients did not take any doses of the vaccine. Among the non-vaccinated pregnant women, 54% did not have any test performed, which corresponds to 11% of the total number of pregnant women under prenatal care during the study period. It means that one-tenth of pregnant women are at high risk of acquiring or even carry the HBV virus, which may contribute to a more significant number of newborns who acquire Hepatitis B by vertical transmission.

According to the Clinical Protocol and Therapeutic Guidelines for Vertical Prevention transmission of HIV, syphilis, and viral hepatitis, congenital Hepatitis B corresponds to 5-10% of cases of the disease in Brazil, and the risk of transmission to the fetus increases from 10%, in the first quarter of pregnancy, to 60% in the third quarter[1]. Therefore, virus research in non-vaccinated pregnant women, as well as the antigen that provides immunity against HBV, is of utmost importance. Furthermore, the vaccination schedule control is necessary to protect these patients throughout pregnancy, especially in their third quarter, when the risk of transmission to the fetus increases. Another study held in the Teresina City, Piauí State, in 2012, evaluated the vaccine coverage and showed that 77.5% of women did not receive any doses of Hepatitis B vaccine, a higher percentage than the one found in this study[8]. Therefore, it can be concluded that monitoring and caring for pregnant women served and evaluated, both in this study and in the aforementioned study, was not carried out respecting all requirements for Hepatitis B control, according to the Protocol by the Ministry of Health[1].

A survey conducted in 1993 assessed both pregnant women and non-pregnant women, and reported that after 6 months of the 3-dose-vaccination schedule application, 25% of pregnant women showed non-reactive anti-HBS[9]. This result resembles the one found in our study, where 43% of pregnant women with the full vaccination

Table 3 – Correlation between vaccination status and immunogenic response to Hepatitis B of pregnant women served at the prenatal outpatient clinic of Universidade Federal de Pelotas, Pelotas City, Rio Grande do Sul State, from November 2017 to May 2018.

Vaccination Status	N	Anti-HBs reactive	Nonreactive	Not performed	Inconclusive
Complete pre-gestation	16	9 (56.25%)	4 (25%)	3 (18.75%)	0
Complete peri-gestation	14	0	9 (64.29%)	5 (35.71%)	0
Only 1 dose	56	1 (1.79%)	26 (46.42%)	29 (51.79%)	0
Only 2 doses	11	1 (9.09%)	9 (81.82%)	1 (9.09%)	0
Not notified	24	2 (8.33%)	8 (33.33%)	13 (54.17%)	1 (4.17%)

Anti-HBs: hepatitis B virus surface anticorp.

schedule had no reactive anti-HBS. Furthermore, among the 30 pregnant women with fully completed vaccination schedule, eight did not undergo antigen research, which can result in a higher number of patients with anti-HBS negative tests.

Another study evaluating the prevalence of Hepatitis B vaccination in health care workers, in the state of Bahia, showed 59.9% of adhesion to the full vaccination schedule, more than four times the percentage found in this study[10]. Only 61.7% of workers who have completed the three dose-scheme underwent an exam to check immunity after vaccination, and in 13.4% of patients there was no seroconversion, represented by the negative anti-HBS smaller number than the one found in this study, which revealed 46.7% of patients with full vaccination schedule, with negative anti-HBS[10].

Although vaccination against Hepatitis B is highly successful, from 5% to 10% of individuals did not complete the serological turning[11]. Some causes that contribute to the non-response to the vaccine are the following: genetic predisposition, immunosuppression, and chronic diseases[11].

It is crucial to distinguish non-response after full vaccination schedule from declining levels of anti-HBs[11]. Individuals with decreasing levels of anti-HBS may still be protected by immunologic memory in acute HBV infections, or can prevent chronic infections[11].

According to clinical data published by Gilbert Greub et al. in 2001, care should be provided when considering patients vaccinated with non-reactive anti-HBS as non-responders, and those vaccinated whose anti-HBS showed falling titles as not being better protected, due to their immunological memory[12]. Therefore, patients should not necessarily receive additional doses or be revaccinated[12].

When performing anti-HBs testing, it is necessary to identify those patients who have not reacted to the vaccination schedule and should be revaccinated. It is not required to revaccinate patients whose vaccination schedule is complete and has lower levels of anti-HBs. However, adhering to the three doses of the vaccination schedule recommended by the Ministry of Health must be confirmed.

CONCLUSION

A lower expected number was observed in pregnant women who underwent Hepatitis B infection screening tests (HBsAg) and immunity (anti-HBS), and a strong incomplete adhesion to vaccination. Besides that, incomplete medical records were found, whereas almost one-third of patients did not present information on vaccination. Probably, more accurate and systematic supervision were missed during prenatal medical records, not only concerning Hepatitis B serological data, but also of all the elements involved in an appropriate prenatal. Our results are in agreement with those of another study conducted in the same city among puerperal women[13].

It is necessary, therefore, to encourage the testing request and accomplishment, as well as a proper medical report. According to WHO, less than 5% of people in the world living with chronic hepatitis are aware of their serological status[6].

It is of the utmost importance that vaccination against Hepatitis B is encouraged by health professionals to reduce Hepatitis B infection and mortality rates. In view of the above, further guidance and supervision must be carried out in a more systematic and frequent way, and an audit system to be held periodically to verify the correct medical records filling out must be created. Moreover, promoting training is essential to raise the awareness of professionals who will serve pregnant women on the importance of vaccination against Hepatitis B, and also about the correct medical records filling out.

Participation of each author

Carolina Silveira da Silva and Mariangela Freitas Silveira created and developed research. All authors participated in the data collection and analysis, and assisted in the completion of the article.

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Conflict of interests

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INFERTILITY AND VAGINAL MICROBIOME: REVIEW STUDY

INFERTILIDADE E MICROBIOMA VAGINAL: ESTUDO DE REVISÃO

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ABSTRACT

Introduction: Infertility is an important public health problem and has many causal factors. Previous findings, based mainly on culture techniques, suggest an association between infertility and changes in the vaginal microbiome. The metagenomic approach allowed the discovery of new bacterial species, previously unidentified in the female genital tract, permitting a deeper knowledge of the role of vaginal microbiome in female reproductive health. **Objective:** To evaluate the association of changes in the vaginal microbiota with infertility, and its repercussions on the outcome of assisted reproduction techniques after a decade of the Human Microbiome Project. **Methods:** A systematic search was carried out in the MEDLINE database between September and November 2018, by selecting 14 studies, associating vaginal microbiome with infertility, or with results of assisted reproduction techniques. **Results:** The findings showed a higher prevalence of bacterial vaginosis, and increased microbial diversity in the vagina of infertile women. Regarding the success of assisted reproduction techniques, most studies did not show any significant association between bacterial vaginosis and reduction in pregnancy rates. **Conclusion:** Further studies are needed to better understand the influence of the balance of vaginal microorganism species on female reproductive health, addressing the microbiome composition in contexts beyond in vitro fertilization techniques.

Keywords: infertility; microbiota; vaginosis, bacterial; Gardnerella vaginalis; in vitro fertilization; reproductive techniques, assisted.

RESUMO

Introdução: A infertilidade é um importante problema de saúde pública e possui muitos fatores causais. Achados prévios, baseados principalmente em técnicas de cultura, sugerem uma associação entre infertilidade e alterações do microbioma vaginal. A abordagem metagenômica permitiu a descoberta de novas espécies bacterianas, anteriormente não identificadas, no trato genital feminino, possibilitando um conhecimento mais aprofundado do papel do microbioma vaginal na saúde reprodutiva feminina. **Objetivo:** Avaliar a associação das alterações da microbiota vaginal com a infertilidade e suas repercussões no resultado das técnicas de reprodução assistida após uma década do Projeto Microbioma Humano. **Métodos:** Foi realizada uma busca sistemática na base de dados MEDLINE, entre setembro e novembro de 2018, e selecionados 14 estudos que associavam o microbioma vaginal com a infertilidade ou com resultados de técnicas de reprodução assistida. **Resultados:** Os achados evidenciaram uma maior prevalência de vaginose bacteriana e um aumento da diversidade microbiana na vagina de mulheres inférteis. Com relação ao sucesso das técnicas de reprodução assistida, a maioria dos estudos não evidenciou uma associação significante entre a vaginose bacteriana e a redução nas taxas de gravidez. **Conclusão:** São necessários novos estudos para melhor compreender a influência do equilíbrio das espécies de microrganismos vaginais na saúde reprodutiva feminina, abordando a composição do microbioma em contextos além das técnicas de fertilização in vitro.

Palavras-chave: infertilidade; microbiota; vaginose bacteriana; Gardnerella vaginalis; fertilização in vitro; técnicas de reprodução assistida.

INTRODUCTION

The vaginal microenvironment and a brief history of taxonomy

Since the times of Aristotle, and later on, of Lineu, living organisms were classified into two kingdoms: the Kingdom Animalia, which included heterotrophic organisms that, in general, move in the environment, capture and ingest food, including the protozoan, considered unicellular animals, and metazoans, or multicellular animals; and the Kingdom Plantae (Vegetal Kingdom) which included the photosynthesizing autotrophs, prokaryotes or eukaryotes. In the latter, there were non-photosynthesizing bacteria and fungi, considered achlorophyllous plants[1]. Until then, the microorganisms that inhabited the vagina were in their set called vaginal flora.

With the advances in Biology, new proposals for classification emerged, and a system of four kingdoms was created. Bacteria were then included in the Moneran Kingdom, which represented all prokaryotes organisms[2]. In 1959, Whittaker presented a new proposal for classifying living organisms into five kingdoms, including the Fungi Kingdom[3].

So far, all classifications characterized bacteria based on phenotypic markers, such as morphology, growth or pathogenic potential, as well as physiological and biochemical properties[4].

In 1990, a new classification was proposed based on the analysis of rRNA (Ribosomal Ribonucleic Acid), dividing living organisms into three domains (a taxonomic category superior to kingdom): Archea, Bacteria, and Eucaria, based on phenotypic data, chemotaxonomic, genotypic, and phylogenetic evolution[4,5].

Microbiota was then defined as the "set of microorganisms that exist within a given environment, and are revealed through molecular techniques"[6], and the consequent denomination of vaginal microbiota.

The Human Microbiome Project, initiated in 2007 by the National Institutes of Health (NHI), performed metagenomic studies through sequencing and analysis of high-performance DNA, which helped to characterize the bacterial population of various sites of the human body[7]. These molecular techniques take advantage of the 16S rRNA gene, which is unique to bacteria and contains several hypervariable regions that serve as identifiers for a genus or bacterial species[8]. Human microbiome was then defined as "the total of commensal,

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symbiotic and pathogenic microorganisms, and their genetic material existing in the human body"[9].

Thus, the vaginal microbiome was also characterized, and areas previously considered sterile, such as the uterine cavity and the placenta, were evidenced a microbiome themselves[10,11].

With the advances in molecular biology, microbial taxonomy and phylogenetic experienced rapid changes, making the microbial classification process quite complex[12], bringing us the following question: What will be the proper term to define the vaginal microenvironment in one or two decades when new genomic technologies will have surely emerged?

Vaginal microbiota and infertility

Historically, bacteria are identified using Gram staining or culture-based techniques. Only 20% of the bacteria in the human body, however, can be cultivated, and culture methods can therefore underestimate the diversity of such microbiome[13].

Information obtained from the combination of molecular biology methods with culture-based methods can clarify not only the role of bacteria in gynecological health, but also how the shift of vaginal microbiota affects the susceptibility to diseases.

Infertility, in turn, is defined as the inability to conceive after 12 months of regular sexual activity, without the use of contraceptive methods[14]. It is an important public health problem, globally affecting around 9% of women in the menacme, and approximately 1.5 million women in the United States[15]. It is estimated that one in every six couples will present problems with fertility during their reproductive life[16].

Several decades ago, studies showed that some microorganisms, such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, have a well-established association with infertility[15-18].

The association between bacterial vaginosis (BV) and infertility has also been studied, with findings of BV prevalence up to three times higher among infertile women compared to fertile women[19].

Moreover, with the development of molecular biology techniques, new microorganisms associated with infertility were identified[20,21].

Disorders in the composition of bacterial communities seem to contribute to disease conditions, and there are growing evidences that vaginal microbiota, which is unique for each woman and presents variations associated with the menstrual cycle, sexual activity, stage of reproductive life, habits and external factors, plays an important role in determining the multiple facets of reproductive health[6,22].

OBJECTIVE

The objective of this review is to evaluate the association of vaginal microbiota alterations with female infertility, and its repercussions on the results of assisted reproduction techniques, addressing the findings of the last decade after the advent of the Human Microbiome Project.

METHODS

Research was carried out from September to November 2018. We sought to identify studies addressing associations between vaginal microbiome and women with infertility diagnosis. Studies in English, published over the last ten years (January 2008 to November 2018), such as meta-analyses, original cross-sectional, cohort and case-control studies were evaluated.

The levels of evidence of the mentioned studies were evaluated according to the Oxford Centre for evidence-based Medicine levels (March 2009 – www.cebm.net)[23].

A systematic search in the MEDLINE database was conducted using the following terms from the Medical Subject Headings (MeSH) Dictionary: ("Infertility") AND ("Microbiota" OR "Vaginosis, Bacterial" OR "Gardnerella vaginalis"). Complementary search was performed using the terms of the MeSH: ("Fertilization in Vitro" OR "Reproductive Techniques, Assisted") AND ("Microbiota" OR "Vaginosis, Bacterial" OR "Gardnerella vaginalis").

RESULTS

Thirty studies were initially identified, fourteen of which were selected after reading the abstract. Those that evaluated the microbiome of the male reproductive tract and studies not performed in humans were excluded, according to the flowchart shown in **Figure 1**.

Studies were divided into two groups: those comparing the vaginal microbiota of fertile and infertile women; and those evaluating the association of vaginal microbiome with the results from assisted reproduction techniques.

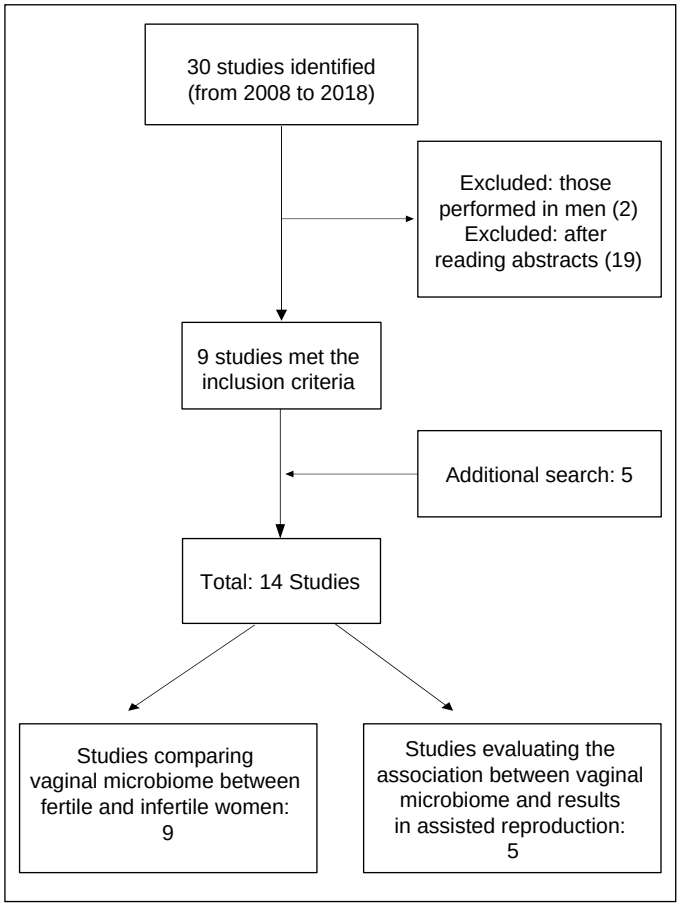

Figure 1 – Flowchart of the selected studies.

Vaginal microbiome and infertility

Eight studies[17-30] were selected by comparing the vaginal microbiome of fertile and infertile women, totaling 3,611 patients, in addition to a systematic review with meta-analysis[19], which evaluated twelve articles, and a total of 3,229 patients. The origin of each study and the diagnostic methods used to characterize the microbiota are described in **Table 1**.

Mania-Pramanik et al.[24] evaluated a group of 510 women: 112 (21.96%) had a diagnosis of infertility; 115 (22.5%) a history of repeated miscarriage; 100 (19.6%) women presented signs and symptoms of lower genital tract infections; 102 (20%) were healthy pregnant women (gestational age from 2 to 3 months); and 81 (15.9%) were asymptomatic women. Reproductive tract infections, such as bacterial vaginosis (BV), *Candida sp.*, *Trichomonas vaginalis*, *Chlamydia trachomatis*, and *Human papillomavirus* (HPV) were investigated with the Nugent score, fresh examination, and Polymerase Chain Reaction (PCR). The BV rate was higher (25.9%; 29/112) among infertile women when related to the other groups, evidencing a statistically significant association (p=0.0001).

A study[25] evaluated the vaginal microbiota, with Gram scoring, culture and molecular biology (BD Affirm™ VPIII [Becton, Dickinson and Company, Franklin Lakes, NJ, USA], and COBAS Amplicor™ [Roche, Milan, Italy]), of 952 women divided into two groups: fertile women with vaginal discharge (N=556); and asymptomatic infertile women who would undergo assisted reproduction procedures (N=396). Statistical analysis showed a significant association (p≤0.001) between the presence of *Ureaplasma urealyticum*, *Streptococcus agalactiae*, *Gardnerella vaginalis*, and *Enterobacteriaceae*, or *Enterococci* in the vaginal microbiota with the decrease in *Lactobacilli* species. The study stated that the reduction of *Lactobacilli*, and the presence of a high number of polymorphonuclear in the vaginal content are important parameters to be considered when analyzing the health status of the female urogenital tract.

In a prospective cohort study[26], evaluating 874 infertile women, and 382 fertile controls, the prevalence of BV evaluated with the modified Spiegel method was also significantly higher among infertile women. The prevalence of BV was of 45.5% (398/874) in infertile women, compared to 15.4% (59/382) in fertile women (p<0.001). The highest prevalence of BV in infertile patients was found in women with Polycystic Ovary Syndrome (PCOS) (60.1%), and Infertility Without Apparent Cause (IWAC) (37.4%).

Van Oostrum et al.[19], in a systematic review with meta-analysis, evaluated 12 Nugent scores studies, and demonstrated that BV is significantly more prevalent in infertile women (OR=3.32; 95%CI 1.53-7.20). On the other hand, in this study, infertile women for tubal factor had a significantly higher prevalence of BV (OR=2.77; 95%CI 1.62-4.75), compared to women with other infertility causes.

The prevalence of BV diagnosed with the Amsel criteria among patients with infertility for tubal factor was evaluated in a study with 356 women (178 fertile; 178 with tubal infertility)[27]. Bacterial vaginosis was observed in 50 women (28.1%) with tubal infertility, compared to 14 (7.9%) fertile women (p<0.001). Infertile women showed a higher risk of bacterial vaginosis when belonging to a lower socioeconomic level (OR=11.89; 95%CI 5.20-27.69), using vaginal showers (OR=19.15; 95%CI 7.2-47.75), using agents that dry out the vagina (OR=17.04; 95%CI 6.91-43.24), initiating their sexual activity early (OR=32.08; 95%CI 12.02-88.89), or having a history of sexually transmitted infections (OR=12.42; 95%CI 5.36-29.35).

The prevalence of asymptomatic BV diagnosed with the Nugent score in fertile women (N=84), and interfile women (N=116) was also evaluated in another study[28]. It was observed that the vaginal microbiota of healthy women was dominated by *Lactobacillus* (40, 27.8%), whereas the percentage of microbiota with predominance of *Lactobacillus* in the group of infertile women was relatively low (4, 3.5%). Asymptomatic bacterial vaginosis was present in 27.6% (32/116) of infertile women, while in the fertile women group only 7.1% (6/84) had asymptomatic BV (p≤0.05).

Wee et al.[29] examined the vaginal, cervical and endometrial microbiota through 16S rRNA sequencing of 15 women with a history of infertility, compared with 16 fertile women (controls), and observed that infertile women were more likely to present more often two most prevalent microorganisms: *Ureaplasma* in the vagina (p=0.042), and *Gardnerella* in the cervix (p=0.044). Four out of five women with infertility colonized by *Ureaplasma* also had vaginal microbiota dominated by *L. inners* (p=0.015). There was no statistically significant difference in the expression of genes selected in the endometrium and microbiome composition between cases and controls.

Table 1 – Comparison studies of reproductive tract microbiome among fertile and infertile women.

Study/Year	Country	Population	Diagnosis Method	Microbiome Site
Graspeuntner et al. (2018)(17)	Germany	N=210	Culture, PCR, 16S rRNA	Cervical
Van Oostrum et al. (2013)(19)	Belgium	-------	Nugent score	Vaginal
Mania-Pramanik et al. (2009)(24)	India	N=510	Nugent score, PCR and fresh examination	Vaginal
Casari et al. (2010)(25)	Italy	N=952	Gram, culture, molecular biology	Vaginal
Salah et al. (2013)(26)	Egypt	N=1,256	Spiegel modified	Vaginal
Durugbo et al. (2015)(27)	Nigeria	N=356	Amsel	Vaginal
Babu et al. (2017)(28)	India	N=200	Fresh examination, Gram and Nugent score	Vaginal
Wee et al. (2017)(29)	Australia	N=31	16S rRNA	Vaginal, cervical, and endometrial
Campisciano et al. (2017)(30)	Italy	N=96	rDNA V3-16S	Vaginal and cervical

PCR: polymerase chain reaction.

The vaginal and cervical microbiota were characterized by sequencing the rDNA V3-16S gene in a study[30] that evaluated 14 women with idiopathic infertility, 13 women with non-idiopathic infertility, 39 fertile women with BV and 30 healthy women (controls). The affected groups (idiopathic, non-idiopathic infertility, and BV) had α-diversity (diversity of microorganisms within the same sample) greater than that of the control group. The controls were significantly different from the group with idiopathic infertility ($p<0.05$), non-idiopathic infertility ($p<0.01$), and fertility with vaginosis ($p<0.01$). An unequal distribution of *Lactobacilli* was observed among the studied groups. *L. inners* acted as a marker of microbiome health based on its prevalence in each group: controls (51%), compared to idiopathic infertility (29%), non-idiopathic infertility (18%), and vaginosis (15%). There was a decrease in the prevalence of *L. crispatus* in infertile women, and with BV (25 and 6%, respectively) in relation to women with idiopathic infertility (31%), which, in turn, was lower than in controls (36%). There was higher prevalence of *L. gasseri* in the group of women with idiopathic infertility.

In order to evaluate the bacterial composition and other microorganisms of the reproductive tract of infertile women with infectious cause, a study[17] recruited a group of 210 women, as follows: 26 women with non-infectious infertility; 21 women with infectious infertility, 89 fertile women; and 54 sex workers. Three cervical samples were collected: the first, for conventional culture of commensal and pathogenic bacteria of the urogenital tract; the second, for PCR test for *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, *M. hominis* and *U. urealyticum*; and, the third, for 16S rRNA gene sequencing. Women with infectious infertility differed significantly in the frequency of *C. trachomatis* infections compared to fertile controls ($p<0.01$) and women with non-infectious infertility ($p<0.05$). No differences were observed between groups with HPV, HSV, *Treponema pallidum*, HIV, or hepatitis B and C infections. Despite the increase in the rate of positive tests for *U. urealyticum*/*parvum* (41.30%), *N. gonorrhoeae* (7.90%), *M. genitalium* (9.50%), and *M. hominis* (34.90%) among sex workers, no significant differences were observed between the other groups.

The analysis of the microbiota using amplification of the 16S sequence of cervical smears revealed significant differences between the group of women with infectious infertility and the fertile controls' as to the prevalence of *Gardnerella* (10.08% *vs*. 5.43%). The α-diversity varied between groups: among fertile women, communities dominated by *Lactobacillus* prevailed; among women with infectious infertility, communities dominated by *Gardnerella* occurred more often; and a diversity of communities in other groups was observed.

Results in assisted reproduction

Five studies[19-33] evaluated the association between vaginal microbiota and the results of assisted reproduction techniques, as shown in **Table 2**.

Selim et al.[31] investigated with culture and Nugent score the impact of vaginal bacterial microbiota on the rates of live births during Intracytoplasmatic Sperm Injection (ICSI). In women with bacterial vaginosis, intermediate microbiota, and normal microbiota, the conception rates were of 35 (9/26), 42 (14/33), and 58% (7/12), respectively, with no statistically significant difference between groups ($p=0.06$). The conception rate was of 29% (2/7) in those with *S. viridians*, and 22% (2/9) with *Staphylococcus aureus* isolated from the embryonic transfer catheter tip, 39% (18/46) when no bacteria was isolated from the catheter tip, and 80% (8/10) when the *Lactobacilli* H2O2 producers were recovered ($p<0.001$).

With 16S rRNA sequencing techniques, a study[21] evaluated the composition of the vaginal microbiota on the day of embryo transfer in women undergoing *in vitro* fertilization (IVF). Through a sophisticated calculation of the diversity index (Shannon Diversity Index), comparing vaginal fluid swabs of women who had a live newborn to those who did not succeed, it was demonstrated that a lower rate of diversity of vaginal microbiota correlated with the highest rate of live births ($p=0.01$).

With the Nugent score and PCR, a study[32] evaluated the prevalence of BV in 307 infertile women submitted to *in vitro* fertilization, and the impact of BV on the pregnancy rate after IVF. The embryo implantation rate did not decrease significantly, when comparing the normal vaginal microbiota women group to the BV women group (36.3 *vs.* 27.6%, respectively; p=0418), nor the rate of clinical pregnancy (33.1 *vs.* 27.6%, respectively; p=0.68). Obstetric results (frequency of early miscarriage, premature rupture of membranes, gestational age at delivery and delivery or birth weight) also showed no statistically significant differences.

In the systematic review by Van Oostrum et al.[19], it was demonstrated that BV was not associated with decreased conception rates (OR=1.03; 95%CI 0.79-1.33), nor with increased risk of abortion in the first trimester (OR=1.20; 95%CI 0.53-2.75), but associated with a significantly elevated risk for pre-clinical gestational loss (OR=2.36; 95%CI 1.24-4.51). None of the studies evaluated in the review found an association between abnormal microbiota and conception rates after IVF.

Another study[33] evaluated the diagnostic performance of PCR tests, compared to the Nugent score for abnormal vaginal microbiota, and to predict the success rate of the treatment of women submitted to IVF. The prevalence of BV by the score was of 21% (27/130), whereas the prevalence of abnormal vaginal microbiota defined by

Table 2 – Studies evaluating the association between the reproductive tract microbiota and the results of assisted reproductive techniques.

Study/Year	Country	Population	Diagnosis Method	Microbiome Site
Van Oostrum et al. (2013)(19)	Belgium	-------	Nugent score	Vaginal
Hyman et al. (2012)(21)	USA	N=30	16S rRNA	Vaginal
Selim et al. (2011)(31)	Egypt	N=71	Culture and Nugent score	Vaginal
Mangot-Bertrand et al. (2013)(32)	India	N= 307	PCR and Nugent score	Vaginal
Haahr et al. (2016)(33)	Denmark	N= 130	PCR and Nugent score	Vaginal

PCR: polymerase chain reaction.

PCR was of 28% (36/130), with high concentrations of *Gardnerella vaginalis,* and/or *Atopobium vaginae*. The PCR approach showed sensitivity and specificity, respectively, of 93 and 93% for BV defined with the Nugent score. In addition, PCR allowed the stratification of Nugent's intermediate microbiota. A total of 84 patients completed IVF treatment. The overall rate of clinical pregnancies was of 35% (29/84). Curiously, only 9% (2/22) with abnormal microbiota defined by PCR obtained a clinical gestation (p=0.004).

Table 3 summarizes selected studies with their respective levels of evidence and results.

CONCLUSION

Studies suggest that bacterial vaginosis and abnormal vaginal microbiota are significantly more prevalent in infertile women, compared to fertile patients, and that the healthy vaginal environment has lower microbial diversity.

The role of vaginal microbiome in the success or failure of assisted reproduction techniques (ART) is still unclear, and most of the selected studies did not reveal a significant association between bacterial vaginosis and reduction in pregnancy rates. Hence, further studies addressing the microbiome composition in contexts beyond the *in vitro* fertilization techniques are needed.

Participation of each author

Dr. Muse Santiago de Oliveira did the conception of research, conducted research of articles in MEDLINE, and wrote this article. Dr. Francisco das Chagas Medeiros conducted research of articles and helped to write this article. Dr. José Eleutério Jr. helped in the conception of the idea and reviewed this article.

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Conflict of interests

There is no conflict of interest to be reported.

Table 3 – Summary of selected studies results.

Reference	Type of study	No. Patients	Results (P value)
Graspeuntner et al. (2018) (B3B)(17)	Case-control study	N=210	Frequency of *C. trachomatis* greater in women with infectious infertility compared to fertile controls (p<0.01), and women with non-infectious infertility (p<0.05).
Van Oostrum et al. (2013) (B2A)(19)	Systematic review with meta-analysis	N=3,229	BV more prevalent in infertile women, compared to women in prenatal monitoring (OR=3.32; 95%CI 1.53-7.20), and associated with an increased risk of early gestational loss (OR=2.36; 95%CI 1.24-4.51). BV was not associated with decreased rates of conception (OR=1.03; 95%CI 0.79-1.33) nor with increased risk of abortion in the first semester (OR=1.20; 95%CI 0.53-2.75).
Hyman et al. (2012) (B2B)(21)	Cohort study	N=30	The diversity of species varied in different hormonal environments, and on the day of embryo transfer correlated with the outcome (live births/no live births) (p=0.01).
Mania-Pramanik et al. (2009) (B3B)(24)	Cross-sectional study	N=510	Statistical analysis between negative and positive women for BV revealed a statistically significant association (p=0.0001) with infertility.
Casari et al. 2010 (B3B)(25)	Case-control study	N=952	Significant association (p≤0.001) between the decrease in *Lactobacilli* and the increased prevalence in *Ureaplasma urealyticum*, *Streptococcus agalactiae*, *Gardnerella vaginalis*. *Enterobacteriaceae,* or *Enterococci* in the vaginal flora.
Salah et al. (2013) (B2B)(26)	Cohort study	N=1,256	BV higher prevalence in infertile women than in fertile women (45.5% *vs.* 15.4%). The highest prevalence was observed in PCOS (60.1%), and IWAC (37.4%) patients. Cumulative pregnancy rates among patients with PCOS and IWAC were significantly higher among patients who were treated for BV.
Durugbo et al. (2015) (B3B)(27)	Cross-sectional study	N=356	Prevalence of higher BV among women with tubal infertility compared to fertile women (p<0.001).
Babu et al. (2017) (B3B)(28)	Case-control study	N=200	Asymptomatic BV present in 27.6% of infertile women, and in 7.1% of fertile women (p≤0.05).
Wee et al. (2017) (B3B)(29)	Case-control study	N=31	Infertile women showed two predominant microorganisms: *Ureaplasma* in vagina (p=0.042), and *Gardnerella* in cervix (p=0.044); not adjusted.
Campisciano et al. (2017) (B3B)(30)	Case-control study	N=96	The α-greater diversity in patients with idiopathic infertility (p<0.05), non-idiopathic (p<0.01), and BV (p<0.01) compared to the control group.
Selim et al. (2011) (B2B)(31)	Cohort study	N=71	In women with bacterial vaginosis, intermediate flora and normal flora, the conception rates were of 35 (9/26), 42 (14/33), and 58% (7/12), respectively (p=0.06).
Mangot-Bertrand et al. (2013) (B2B)(32)	Cohort study	N=307	There was no significant decrease in the rates of embryo implantation by comparing the groups with normal vaginal flora and BV (p=0.418), nor in the clinical pregnancy rates between the two groups (p=0.68).
Haahr et al. (2016) (B2B)(33)	Case study	N=130	BV prevalence with Nugent and PCR were highly correlated; women with abnormal microbiota defined by PCR were significantly less likely to obtain a clinical pregnancy (9%), compared to the overall rate of 35% (p=0.004).

p≤0.05 (statistically significant); BV: bacterial vaginoses; OD: odds ratio; 95%CI: confidence interval of 95%; PCOS: polycystic ovary syndrome; IWAC: infertility without apparent cause; PCR: polymerase chain reaction.

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Inflammatory and linear verrucous epidermal nevus in vulvar region: a differential diagnosis with vulvar condyloma

Nevo epidérmico verrucoso inflamatório e linear na região vulvar: um diagnóstico diferencial com condiloma vulvar

José Humberto Belmino Chaves[1], Julia Espíndola Guimarães[1], José Eleutério Junior[2]

ABSTRACT

Introduction: Inflammatory linear verrucous epidermal nevus (ILVEN) is a variant of verrucous epidermal nevus. It has a psoriasiform or eczematous and itchy aspect, and has differential diagnosis compared to other more common dermatoses; thus, histological studies are often necessary. It mainly affects women of early age and must be differentiated from condyloma acuminatum. Interestingly, the lower left limb is often involved, but the genital region is rarely affected. Treatment is refractory and the best method is not yet established. **Objective:** We present a case of unusual vulvar involvement known as inflammatory linear verrucous epidermal nevus. **Methods:** This was a clinical case report of a child diagnosed with ILVEN in the vulvar region. **Case report:** An 11-year-old female presented to the gynecology department of the Universidade Federal de Alagoas complaining of pruritic lesions on the large left vulvar lip, perianal and anal regions, and vaginal introitus. The lesions were hypochromic, eroded, and covered by scabs along the Blaschko line with verrucous lesions in the abdomen and upper and lower limbs. These characteristics fit the clinical criteria of Altman and Mehregan, and the histological criteria of Dupre and Christol for diagnosis of ILVEN. The treatment was performed with Vitanol A® and Epidrat Ultra® with partial improvement of the lesions. We chose to excise the lesions to control the condition. **Conclusion:** These lesions are characterized by recurrent inflammatory phenomena including psoriasiform or eczematous aspects in the extremities with genital involvement being rare. Other common dermatoses are often confused with ILVEN and make anatomically pathological analysis extremely important for diagnosis. Despite details on several types of treatment for ILVEN, there are no studies on relative advantages because this lesion is very refractory to the treatment.

Keywords: nevus, pigmented; neoplasms; vulva.

RESUMO

Introdução: Nevo epidérmico verrucoso inflamatório linear (NEVIL) é uma variante do nevo epidérmico verrucoso. Tem aspecto psoriasiforme ou eczematoso e pruriginoso, sendo o diagnóstico diferencial com outras dermatoses mais comuns e o estudo histológico é necessário para diferenciá-las. Afeta, principalmente, mulheres em idade precoce e deve ser diferenciado do condiloma acuminado. Curiosamente, o membro inferior esquerdo está envolvido, sendo a região genital raramente afetada. O tratamento é refratário e ainda não está estabelecido qual o melhor método. **Objetivo:** Apresentar um caso de incomum acometimento vulvar conhecido como nevo epidérmico verrucoso inflamatório linear (NEVIL). **Métodos:** Realizada documentação de caso clínico de criança com diagnóstico de NEVIL em região vulvar. **Relato de caso:** Uma paciente do sexo feminino, 11 anos de idade, procurou o serviço de ginecologia da Universidade Federal de Alagoas queixando-se de lesões pruriginosas vegetantes em grande lábio vulvar esquerdo, regiões perianal e anal e introito vaginal. As lesões eram hipocrômicas, erosadas, recobertas por crostas ao longo da linha de Blaschko com lesões verrucosas no abdome e nos membros superiores e inferiores. Essas características se enquadram nos critérios clínicos de Altman e Mehregan e aos critérios histológicos de Dupre e Christol para diagnóstico de NEVIL. O tratamento foi realizado com Vitanol A® e complexo hidratante (Epidrat Ultra®) com melhora parcial das lesões. Optou-se pela exérese das lesões com controle do quadro. **Conclusão:** Essas lesões se caracterizam por fenômenos inflamatórios recorrentes, incluindo aspecto psoriasiforme ou eczematoso, normalmente em extremidades, sendo raro o acometimento genital. Outras variedades de dermatoses mais comuns são frequentemente confundidas com NEVIL, tornando a análise anatomopatológica de extrema importância para o diagnóstico. Apesar dos detalhes sobre diversos tipos de tratamento para NEVIL, não há estudos sobre vantagens relativas entre eles, uma vez que essa lesão é muito refratária ao tratamento.

Palavras-chave: nevo pigmentado; neoplasias; vulva.

INTRODUCTION

Inflammatory and linear verrucous epidermal nevus (ILVEN) is a rare hamartomatous lesion that histologically consists of hyperplasia of components of the epidermis[1]. The clinical presentation includes erythematous as well as hyperkeratotic and verrucous lesions in a linear arrangement following the Blaschko lines. They are typically pruritic and unilateral lesions, and commonly affect the limbs in a curvilinear pattern. It mostly affects female children[2].

ILVEN has been described since 1896 and corresponds to a linear inflammatory verrucous lesion variant of the verrucous epidermal nevus. It is characterized clinically by recurrent inflammatory phenomena, and may have psoriasiform or eczematous aspects; this necessitates differential diagnosis. This is normally present in one of the extremities; curiously, the left leg is more affected. Its location in the genital region is less common[3]. It is four times more common in women and usually appears within the first four years of life although it may also appear in adulthood[4].

The diagnosis of ILVEN is based on clinical and histological presentation. In 1971 Altman and Mehregan proposed classical clinical criteria for diagnosis. This was updated by Morag and Metzker

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in 1985 to include female sex, young age, common involvement of the left lower limb, itching, psoriasiform histology, and refractory to treatment[1].

Histological changes were described by Dupre and Christol and include alternation of orthokeratosis and parakeratosis as well as the presence or absence of the granular layer although these are not pathognomonic. Other microscopy findings have shown papillomatosis, acanthosis, lymphocytic dermal infiltration, or even Munro microabscesses, but these are non-specific markers[5].

The diagnosis of ILVEN is clinical; however, it can be confused with more common conditions, such as candidiasis or even psoriasis, necessitating anatomopathological studies for differentiation.

There are several treatment options documented. However, there are no established relationships as to the superiority of the treatment mainly because the lesions are extremely refractory to the treatment options.

CASE REPORT

An 11-year-old female patient was taken by her mother to the Dermatology and Gynecology Department at the University Hospital Professor Alberto Antunes (HUPAA) in Maceió, Alagoas. Since 2 years old, the patient had had pruritic lesions with a vegetative appearance on the large left lip, perianal and anal regions, and vaginal introitus. Initially, they were treated with antifungals and antibiotics but without success. The lesions were linearly distributed along the Blaschko lines to the left and characterized by hypochromic eroded areas-some were covered by crusts and verrucous lesions in the left abdomen, axilla, upper limb, and lower limbs (**Figures 1 and 2**). There was no family history of a similar pathology or cancer. There was no history of sexual abuse or HPV vaccine.

A biopsy was performed in 2008 that revealed hyper and parakeratosis, hypergranulosis, presence of neutrophils, acanthosis, and papillomatosis-features all consistent with ILVEN. The patient underwent cryotherapy sessions and then used tretinoin 0.025% cream (vitanol A®) and moisturizing complex (Epidrat Ultra®), with a slight improvement in the appearance of the lesions.

At the beginning of 2015, the proliferative lesions were removed from the perianal region, and the tretinoin dose was increased to 0.05% (**Figure 3**).

In 2016, there was a vegetative lesion on the large left lip and scars on the large right lip and perianal region. A biopsy of the lesion was consistent with ILVEN. The lesion was excised following its path that extended from the pubis to the smaller left lip. The pathology showed a squamous papilloma with important associated inflammatory changes and the absence of dysplastic alterations. The procedure and the postoperative period were uneventful. The patient is currently stable.

DISCUSSION

The case fulfills the clinical criteria (age of early onset, female, pruritus, and refractoriness to the treatment) and histological criteria (squamous papilloma with important associated inflammatory changes and absence of dysplastic alterations) for the diagnosis of ILVEN. The differential diagnosis of ILVEN should be made with

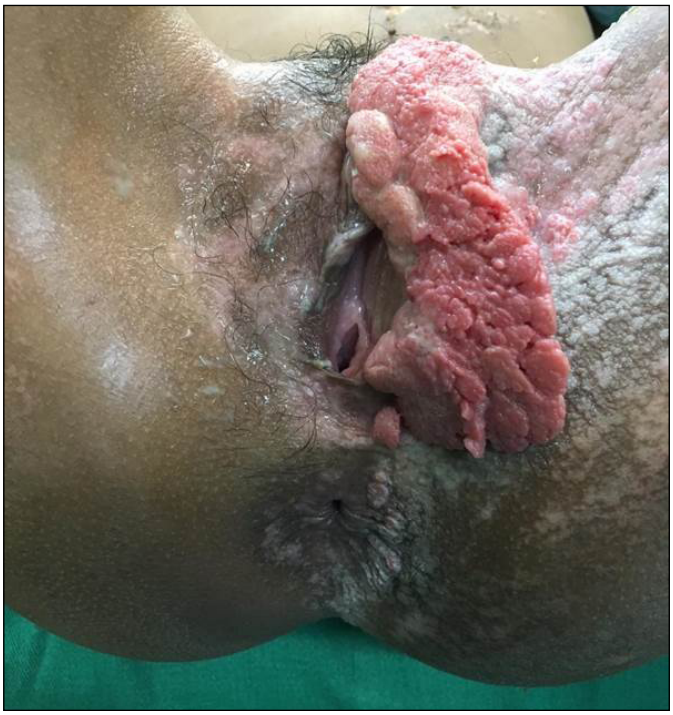

Figure 1 – Vegetative lesion on the large vulvar left lip, perianal and anal regions, and vaginal introitus in case of 11-year-old girl with inflammatory and linear verrucous nevus in the vulva.

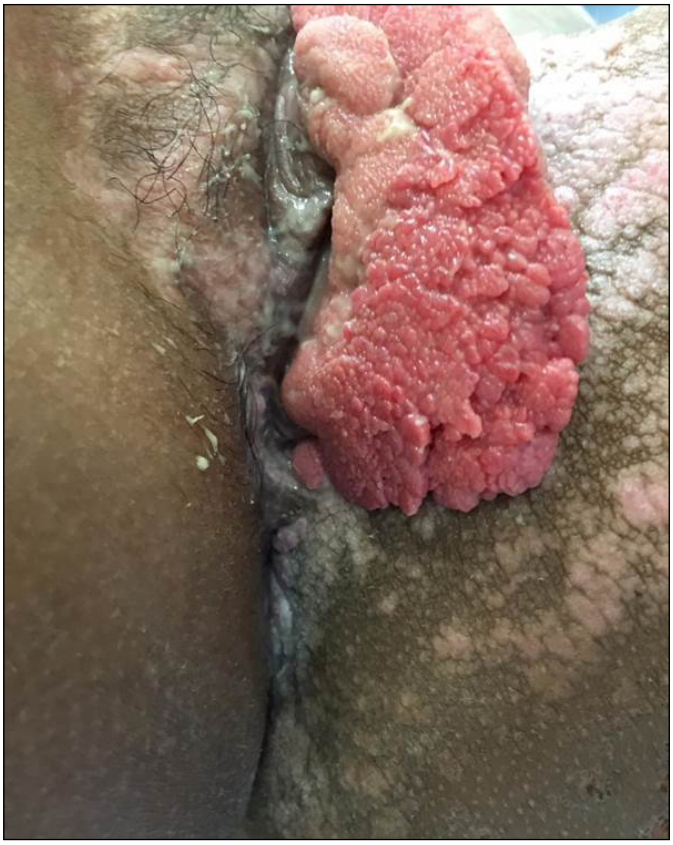

Figure 2 – Details of lesion in the large vulvar left lip of the inflammatory linear verrucous epidermal nevus' patient (11-year-old girl).

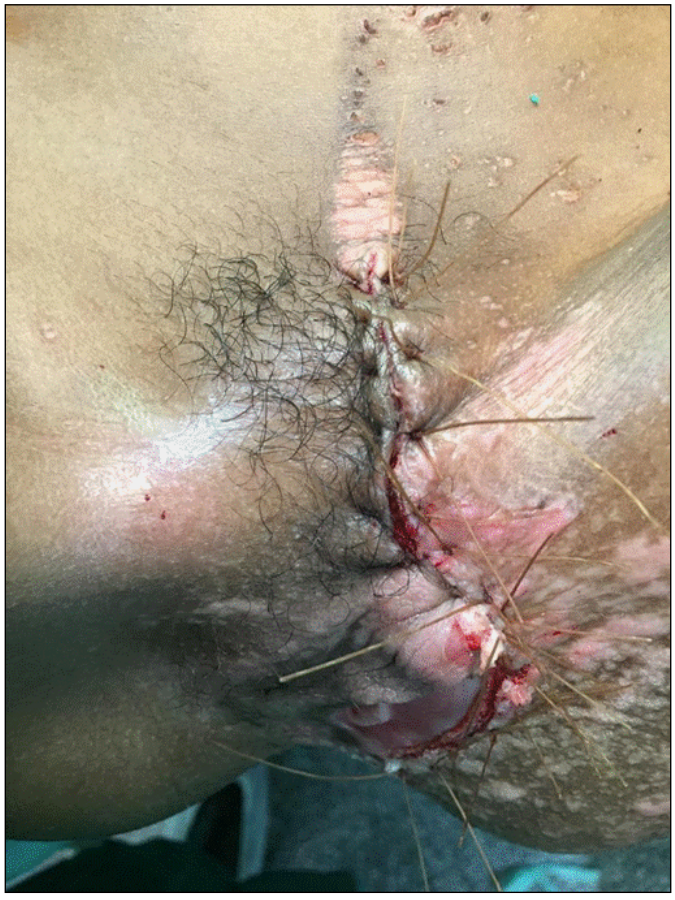

Figure 3 – Immediate postoperative aspect of the patient with inflammatory linear verrucous epidermal nevus (11-year-old girl).

a variety of dermatoses such as other epidermal nevi, linear psoriasis, and striated lichen. It is very important to do differential diagnosis with condyloma acuminatum and, in more severe cases, with Buschke-Lowenstein tumor, both HPV-induced lesions. Other diagnoses are often confused (vulvar candidiasis, for example), and the macerated appearance leads to intense itching[6] that is more severe than psoriasis or even eczema. In children, it is important to exclude the possibility of sexual abuse. When the diagnosis is in doubt, an anatomopathological study should be considered[7,8].

The antifungal and antibiotic treatment was based on a presumptive diagnosis of infection, but there was no response. Thus, a biopsy confirmed the suspicion of ILVEN and the patient was given tretinoin 0.025% cream (Vitanol A®) and a hydrating complex (Epidrat Ultra®). The proliferative lesions were removed from the perianal region followed by an increase in the dose of Vitanol A® to 0.05%.

ILVEN is quite refractory to treatment, making clinical work frustrating. Several modalities in the management of this condition have already been reported; however, no study has yet demonstrated superiority between any of the treatments. Topical treatments such as topical corticosteroids with or without occlusion and intralesional steroids are rarely beneficial. In contrast, vitamin D analogs have proved useful in some cases[9,10]. Other documented options are a combination of 0.1% tretinoin with 5% fluorouracil, anthralin, tar, vitamin D 3 analogs, surgical excision, cryotherapy with nitrogen liquid, and carbon dioxide laser therapy[11-14].

CONCLUSION

We describe a rare case of ILVEN including the differential diagnosis with lesions of infectious origin such as those induced by human papillomavirus (HPV) (condyloma acuminatum and Buschke-Lowenstein tumor), as well as other dermatoses such as epidermal nevi, linear psoriasis, lichen striatus, candidiasis, psoriasis, and eczema. Both children and adults can have ILVEN. It presents in unusual regions such as the vulva and perineum. The investigation must proceed with an anatomic-pathological study to differentiate it from other conditions. The literature describes several treatments although there is no documented superiority among them. Surgical excision is common in refractory cases.

Participation of each author

José Humberto Belmino Chaves followed the case and wrote the article. Julia Espíndola Guimarães followed the case and wrote the article. José Eleutério Jr. wrote the article, reviewed the English version, and formatted the text.

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Conflict of interests

The authors declare no conflicts of interest.

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AIDS e TB: finalmente boas notícias na frente de combate?

AIDS and TB: do we finally have good news on the front?

Ethel Leonor Noia Maciel[1]

The World Health Organization's (WHO) "End TB" strategy has highly ambitious targets to reduce TB deaths and new cases by 95 and 90%, respectively, by 2035 compared to 2015, and to ensure that no family is burdened with catastrophic expenses due to TB. The strategy is based on three pillars[1]:

1. integrated, patient-centered care and prevention;
2. bold policies and integrated information systems, including social protection for patients and recommendations for universal health coverage;
3. intensified and innovative research and inclusion of new technologies.

In BRICS countries, such as Brazil, the current strategies are not focused on achieving these goals, since the efforts have led to a modest reduction of 2% per year over the last decade[2]. To meet the targets proposed by WHO, the rate must decrease 10% per year over the next 20 years – an enormous challenge.

Brazil has a TB cure rate of 55% among people living with HIV (PLWH). It still has room for improvement in the pillar one (Integrated, patient-centered care and prevention) strategy[2]. Likewise, preventive therapy for latent infection is vastly underutilized in this high-risk population[3]. Currently, the primary focus of Brazilian TB control program is on diagnosis and treatment of active disease, with few actions in contact investigations and preventive therapy activities[4], although since 1995[5] WHO and Brazil have guidelines[3] calling for these strategies. WHO guidelines on preventive therapy[3] emphasize the need to increase rates of initiation and completion of Latent Tuberculosis Infection (LTBI) treatment, mainly in groups of higher risk of progression to TB, such as PLWH.

Based upon local[6] and global evidence[7], Brazilian guidelines[4] recommend Isoniazid Preventive Therapy (IPT) for at least six months using a dose of 300 mg/day. Recently, there seems to be, at last, a new guideline and a more effective and efficient strategy. The AIDS program assumed responsibility for IPT, as they have done related to the prevention of other diseases, and the treatment for LTBI is now recommended for all HIV patients with a CD4 count equal to or below 350 – regardless of whether the Tuberculin Test (TT) was performed or not, since active tuberculosis was excluded[8]. The good news is a new presentation of the drug Isoniazid, in a single tablet of 300mg. A study has been conducted by Universidade Federal do Espírito Santo, funded by Ministry of Health, to evaluate the adherence and adverse effects of this new presentation, but the latter is already available for PLWH.

Furthermore, there is a new surveillance system for notification and monitoring of cases of latent TB, but still not for the whole country, and an indicator to address specifically preventive therapy is not clear yet. So, these are indeed good news to celebrate.

If Brazil added a preventive therapy indicator, it would be the new target to be met. Hence, it would result in innovative strategies to reach this goal. Moreover, with an integrated surveillance system, both, AIDS and TB programs would be able to monitor LTBI treatment more effectively.

Finally, TB and AIDS programs should definitely work together to control TB in PLWH, since one target is not achievable without the other across the whole country.

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Conflict of interests

Nothing to declare.

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