Sexually Transmitted Viral Diseases in Women: Clinical and Epidemiological Aspects and Advances in Laboratory Diagnosis

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Sexually transmitted diseases (STDs) have long been known, but they have only recently been recognized as causes of significant long-term morbidity, mainly as a result of increased knowledge concerning viral STDs. The relationship of these diseases with conditions such as anogenital cancer and acquired immunodeficiency syndrome (AIDS) has made viral STDs an important issue in the healthcare of women and infants, and in reproductive health. The evolution of the AIDS pandemic is now characterized by growing differences between rich and poor nations. New diagnostic tools include rapid tests of blood, urine and saliva samples. New techniques, such as computerized cytology, have been developed for the diagnosis of human papillomavirus (HPV). Women infected with HIV are at a greater risk of being co-infected with HPV, and they are also more prone to the progression and persistence of HPV lesions. The herpes simplex virus presents high rates of co-infection with HIV, and it plays a particularly important role in increasing transmission rates of this virus.

Key Words: Sexually-transmitted diseases, viral; HIV; human papillomavirus; herpes simplex virus.

Sexually transmitted diseases (STDs) have long been known to cause acute pathological syndromes, such as genital secretion and ulceration. However, they only recently have come to be considered significant causes of long-term morbidity. This is principally due to the large amount of information that has been collected over the past 20 years on a group of agents that cause these diseases: the viruses. After the association between virus and anogenital cancer was established, and following the emergence of the first cases of Acquired Immunodeficiency Syndrome (AIDS), viral STDs began to be recognized as important diseases that influence the health of women and breastfeeding infants, as well as reproductive health. The medical diagnosis of STDs, AIDS, and genital cancer is currently a fundamental element of women’s healthcare [1].

Our capacity to diagnose STDs increased following the introduction of tests based on the amplification of nucleic acids. These tests were initially developed for the diagnosis of viral STDs and are particularly useful for this purpose. They present high sensitivity, and they permit the use of non-invasive specimens, such as urine and vaginal tissue, self-collected by the patients. Although the number of false results obtained with this technology has been low, there has been some reluctance to substitute traditional methods for this new methodology. One negative aspect is the high cost [2].

Our objective was to identify and describe the principal viral STDs, and to examine epidemiological factors and the relationship that exists between the different viruses. Finally, we examined the diagnostic methods traditionally used for their detection, as well as more recently developed methods.

Among the many sexually transmitted diseases caused by viruses, the principal STDs are AIDS (human
immunodeficiency virus – HIV), genital warts, intraepithelial lesions and genital squamous cell carcinomas (human papillomavirus – HPV), and herpes (herpes simplex virus – HSV-2 and HSV-1) (Table 1).

Acquired Immunodeficiency Syndrome (AIDS)

Almost 50 million people worldwide have been infected by HIV, and 12-13 million children have been made orphans by AIDS. Twenty years after its discovery, the HIV pandemic continues to evolve in magnitude and diversity, and it is currently a global public health problem [3,4]. The distribution of AIDS cases is characterized by a widening gap between the rich nations of North America and Europe and the poor nations of Africa, Asia and Latin America [3]. An important reduction in the number of new cases, morbidity and mortality from AIDS has been seen in the US and Western Europe, as a result of the use of high-cost, intensive therapies, principally HAART (highly active antiretroviral therapy). However, new HIV infections continue to occur every year at a constant rate in these same countries. Moreover, there is evidence of an increase in high-risk behavior in certain populations, indicating failure in primary prevention. However, the vast majority of new infections still occur in developing countries. While the two giants, India and China, are beginning to provide Asia’s ultimate contribution to the pandemic, the situation in Africa is the most desperate, with epidemics rapidly growing in various countries in the south and eastern parts of the continent, affecting all levels of African society. In these countries, expansion of the epidemic is not decreasing, and there are devastating effects on communities, families and individuals [4]. The development of a vaccine, seen as an essential step towards control of the epidemic, is complicated by the genetic diversity of the virus and the inability of the immune system to eliminate this agent.

In Brazil, AIDS, and its associated diseases, have spread more slowly in recent years [5]. This is probably due to a combination of factors: a) saturation of the higher risk segments, i.e. relative lack of a susceptible population [6]; b) a spontaneous change in behavior in certain segments of the population [6]; c) the impact of different prevention initiatives by governmental and nongovernmental organizations, and d) the use of HAART, which is readily available in our country [7]. Nevertheless, this relative deceleration of the spread of the epidemic is not homogenous, both with respect to the different geographical regions of the country and to the affected segments of the population [8]. The speed of expansion has decreased among men in large cities and in the southeast part of the country [9]. More recently, an increase has been seen in south Brazil, not only in the rate of AIDS cases registered but also in the number of cases resulting from heterosexual transmission. There has been dissemination of the epidemic from the large urban centers to the rural areas [10]. The AIDS epidemic in Brazil began among individuals of highly educated social groups and continuously progressed to lower socioeconomic level groups [11]. Irrespective of the focus, one constant finding of such studies is that certain segments of the population, such as injectable-drug users and women, continue to be disproportionately affected by the epidemic [8,10,11]. Women also tend to die younger than men; this hold true in all regions of the country [12].

Women are the segment of the AIDS population with the fastest growth rate. Maternal-fetal transmission and the high mortality and morbidity of the disease in children are additional reasons for strengthening efforts to combat AIDS in women. When the virus is diagnosed in pregnant patients, treatment (zidovudine) reduces the risk of maternal-fetal transmission by two-thirds [13]. Diagnosis is generally made late, since the first signs and symptoms of the disease are subtle. They include vaginal infections, abnormal cervical/vaginal cytology examinations, and other STDs with unusual clinical courses that are severe, recurrent or resistant to treatment [14]. The traditional diagnosis, which includes serological screening by enzymatic immunoassay (ELISA), is followed by a second confirmatory ELISA. A more specific, definitive diagnosis is reached using the Western Blotting technique. Alternatively, the cheaper...
immunofluorescence assay (IFA) has also been found to be efficient as a definitive test [15]. While ELISA measures antibodies against one or more proteins of the viral envelope, and can give false-positive results, Western Blot measures the presence of antibodies against each of the viral antigens, including the core protein (p24). Together, the serological tests have a >99.3% sensitivity and ≥ 99.7% specificity [16].

When rapid AIDS tests first began to be used in the 1980s, and at the beginning of the 90s [17], they provoked much controversy [18-21]. In more recent times, the debate has been renewed [22]. Rapid testing and self-testing have become widespread in some countries such as the US [23] and have been banned in others, such as Germany [24]. These tests include the detection of antibodies in drops of blood, urine [23] and saliva [25-28].

Rapid testing has a high sensitivity and is sufficient to confirm the absence of infection outside the immunological window period. Its specificity is ≥98.9% and requires supplementary serological testing to confirm diagnosis [29-31]. These tests are extremely useful in situations requiring fast detection of infection, such as following occupational exposure.

Saliva testing uses the same antibody detection methods as serological testing (ELISA, Western Blot). The agreement of results is quite high, permitting this method to be used as an alternative to serology [25].

Viral load tests and genotyping are used to select and evaluate therapeutic options. The use of these tests and other viral detection tests for diagnosis should be limited to situations in which serology is not definitive, such as in cases of neonatal infection or acute HIV infection, since these tests are less accurate than serology [32].

**HPV**

Genital warts, or condylomata acuminate (clinical form of the disease), are small papillary projections that generally occur on the vulva or in the vagina. They are caused by the human papillomavirus (HPV), a highly infectious agent. Many individuals exposed to HPV develop flat lesions (subclinical form of the disease) instead of visible warts. These lesions most often affect the cervix, are called intraepithelial lesions and are considered precursors of squamous cell carcinoma. More commonly, the genetic viral material installs itself in the mucosa, and it remains there for years without causing any lesion (latent form of the disease).

HPV is a circular, double-stranded DNA virus of the Papovaviridae family, with approximately 8,000 base pairs. More than 70 types of HPV have been identified. Among these, approximately 30 affect the genital mucosa, are transmitted sexually and are related to the above-mentioned lesions. Types 6, 11, 42, 43

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**Table 1. Virology of the principal agents of viral STDS**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Family</th>
<th>Genetic material</th>
<th>Size of virion</th>
<th>Target cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td>Retroviridae</td>
<td>Single stranded RNA</td>
<td>80-100 nm</td>
<td>CD4+ cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CD4+ T lymphocytes</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Monocytes / macrophages</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Microglia / MGC of SNC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Langerhan’s cells</td>
</tr>
<tr>
<td>HPV</td>
<td>Papovaviridae</td>
<td>Single stranded DNA</td>
<td>40-55 nm</td>
<td>Keratinocytes</td>
</tr>
<tr>
<td>HSV</td>
<td>Herpesviridae</td>
<td>Single stranded DNA</td>
<td>120-200 nm</td>
<td>Epithelial cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neurons</td>
</tr>
</tbody>
</table>

HIV: human immunodeficiency virus; HPV: human papilloma virus; HSV: herpes simplex virus; DNA: desoxyribonucleic acid; RNA: ribonucleic acid; MGC: multinuclear giant cells; SNC: central nervous system.
and 44 cause low risk lesions for malignancy, while types 16, 18, 45 and 56 are associated with high-risk lesions and are also referred to as oncogenic. Nevertheless, the mere presence of a high-risk form of HPV is not in itself sufficient to trigger the carcinogenic process. Co-factors, such as immunosuppression, tabagism, micro-traumas, nutritional and hormonal factors, number of sexual partners and a history of infections (vaginosis, herpes simplex, etc.) have to be present [33]. The progression and natural course of cervical disease are still not fully understood [34].

The Papanicolaou test is a morphological screening method for viral infections and its consequent intraepithelial lesions [35]. Colposcopy permits visualization of the lesions and histopathology allows a definitive diagnosis to be made. Techniques for the amplification and hybridization of nucleic acids have been used since 1980 for the detection, typing and quantification of viral load [36]. Indications for their use include low-grade squamous intraepithelial lesions (LGSIL), which in 30% of cases are associated with oncogenic types of HPV, and lesions that are difficult to characterize by cytology (ASCUS – atypical squamous cells of undetermined significance) [37]. Although molecular biology techniques, such as polymerase chain reaction (PCR) [38-40] and hybrid capture (HC) [41-43], present high sensitivity and specificity for HPV detection and are frequently used as quality controls for the other techniques, the use of these methods for screening purposes is still not accepted. The high cost and the small increase in sensitivity gained when this method is added to cytology are the principal justifications for not using molecular methods as a screening tool [44-47]. Other adjuvant methods of cytology are being developed and improved in parallel with these methods; however their development has been less emphasized. Such methods include macroscopic inspection of the cervix, cervicography and colposcopy [48]. Liquid phase cytology has led to an improvement in the quality of the material collected and has offered the possibility of associating cytological and molecular methods in the same sample. Meanwhile, computerized cytology has improved the quality of cytological scrutiny by substituting cytotecnickians for computers [49]. Finally, following a trend currently observed with other STDs, a study has reported adequately sensitive HPV molecular detection in self-collected vaginal, vulvar and urine samples [50]. We found a sensitivity of from 45 to 86% and a specificity of 54% to 70% in self-collected samples, compared to 98% sensitivity and 52% specificity with physician-collected cervical samples from outpatients.

HIV-HPV Interaction

Systemic and local cellular immunity are factors of extreme importance in HPV infection and its manifestations [51]. In fact, immunosuppressed women have a high risk of developing intraepithelial and invasive squamous cell neoplasia of the lower genital tract [33]. This group includes patients who have been submitted to organ transplant, patients with Hodgkin’s disease, those being treated with immunosuppressive therapy in general, and HIV-positive women [33].

The HIV-HPV interaction is particularly important, since the two viruses are sexually transmitted, placing high-risk populations in contact, making co-infection common [33]. A study carried out in São Paulo showed a high prevalence of high-risk HPV infection (34.8%) and of high-grade intraepithelial lesions. Two or more subtypes of HPV were found in 45% of the patients [52]. Case-control studies have shown HIV infection to be an independent risk factor for HPV, both in its latent and clinical forms [33]. These results reinforce the need for regular gynecological follow-up of HIV-positive patients to ensure early diagnosis of preinvasive lesions and for the prevention of cervical cancer.

Invasive cervical cancer and its precursors are the most important gynecological manifestation of HIV infection [53]. Infection by this virus is related to an increase in prevalence (2-3 times greater) and persistence (7 times greater) of HPV infection. Persistence of HPV infection is known to be important in the development and progression of cervical intraepithelial lesions, and this is one of the factors that may explain the higher occurrence rate of these lesions in HIV-positive patients [54-59]. HIV-positive women
have a 3-5 times greater risk of developing intraepithelial lesions [53,57,60] and a 3-4.5 times greater risk of developing invasive neoplasia [53,60]. In a study carried out by Ellerbrock [53], 328 HIV-positive women and 325 HIV-negative women, followed up for a mean of 30 months, 20% of the HIV-positive women and 5% of the HIV-negative women developed intraepithelial lesions (p<0.01, RR = 3.2).

Various studies have shown that HIV-positive patients with low CD4+ lymphocyte counts have greater viral loads (copy count twice as high for patients with CD4+ < 200 cells/µL) [33,61] and a greater persistence of HPV infection [54], as well as a higher prevalence of low, intermediate, and high-grade cervical intraepithelial lesions [55,58,61]. The prevalence of persistent infection by subtypes of high grade HPV also appears to be significantly greater in HIV-positive patients [54].

Studies have shown that co-infection with HIV is also a risk factor for the other neoplasias for which HPV is a co-factor. According to a study carried out by Frisch [60], HIV-positive patients have a greater relative risk of developing intraepithelial lesions of the vulva and vagina (RR 3.9), anus (RR 7.8 for women and 60.1 for men) and penis (RR 6.9). Similar results were found for invasive neoplasias of the vulva and vagina (RR 5.8), anal canal (RR 6.8 in women and 37.9 in men), penis (RR 3.7), tonsils (RR 2.6) and conjunctiva (RR14.6).

It is still not clear whether the HIV-HPV interaction is related directly or indirectly to the immunosuppression caused by HIV. Co-infection does not occur in the cervix; however, molecular interactions do occur between the two viruses. They are therefore probably caused by extracellular factors [33]. The increase in HPV gene expression in HIV-positive women may be due to interactions involving the HIV-1-trans-activating (Tat-1) protein and the p97 HPV 16 promoter protein, leading to reversal of HPV E2 gene repression [33].

**Herpes**

The *herpes simplex*-2 virus (HSV-2) is a predominantly genital pathogen; while HSV-1 is detected in approximately 15% of herpetic genital infections. Since the seventies, the prevalence of HSV-2 infection has been steadily increasing; this problem has become a public health issue in recent years, even during the “HIV decade” [62]. One recent study on the prevalence of HSV-2 infection in middle-aged Brazilian women found 42% seropositivity [63]. Carvalho et al. [64] found varying prevalence rates according to the subpopulation studied: 6.9% of students evaluated, 22.6% of pregnant women and 53.1% of the individuals with sexually transmitted diseases presented antibodies to HSV-2. They also reported low occurrence of recognized symptoms in seropositive patients.

Primary infection with HSV-2 lasts around three weeks, and during this period the virus is released from the lesions [65]. This infection may be asymptomatic or mild; it presents systemic symptoms in around 70% of cases, pain and localized pruritus in 98%, dysuria in 63%, and painful lymphadenopathy in 80% [66]. Locally, the infection appears in the form of painful mucocutaneous, vesicular and ulcerative lesions, situated on the outer genitalia or cervix [65,66]. This clinical condition and the resulting complications tend to be more severe in women, in whom they are frequently associated with unbearable pain [65]. Complications arising from the primary infection include aseptic meningitis (in up to 25% of women), sacral radiculomyelitis and neuralgias [65]. According to a study carried out by Lafferty, the recurrence rate of genital infections caused by HSV-2 is 33% per month, in contrast with 0.1% recurrence in the case of orolabial infection by the same virus [67].

Recurring episodes are milder and of shorter duration (7-10 days) than the first infection and are characterized by the presence of vesicular and ulcerative lesions on an erythematous base, or by local irritation only. The virus is released from these lesions for 2 to 5 days [65].

The virus is most frequently transmitted by symptomatic patients but transmission may also occur in symptomless patients [68]. Two other factors aggravate the control of the disease: the facts that antiviral therapy does not completely eliminate the virus and that condoms
are not totally effective in prevention since the herpes virus frequently affects the outer genitalia. Cytopathology of exfoliated cells may permit diagnosis, but this depends on adequate sample collection and the evolutive phase of the lesion. General sensitivity of this method is around 60-70% [65]. Enzymatic serological tests for HSV antibodies (ELISA) have been available for many years, but they present low sensitivity and specificity and have traditionally rarely been used to define treatment of infected patients. The time required for serological diagnosis is longer than that available for the initiation of treatment [65]. The definitive epidemiological test has been the Western Blot; however, this is generally only available in research institutes and is very expensive [69]. Recently, new serological tests for specific antibodies for HSV-1 and HSV-2 have become commercially available [70-72]. These tests have made it possible to identify the infection in symptomatic as well as in asymptomatic patients [73].

Cultures positive for HSV from the contents of the vesicula or the edges of the ulcers is considered the traditional definitive diagnostic method. However, diagnosis by this method requires 7-28 days and has been shown to underestimate the number of patients infected. The sensitivity of this method is around 50% and is higher during the primary infection than during recurring episodes. During recurrences, samples should preferentially be collected in the vesicular phase, not when lesions are crusted [74]. Moreover, most patients who are seropositive according to Western Blot are unaware of their symptoms (unrecognized infection), or they present subclinical infection and do not undergo culture [69]. Diagnostic technology examining nucleic acids has also proved to be viable for the detection and typing of HSV and may be able to substitute culture as the definitive method [75,76]. The molecular method presents an increase in sensitivity of up to 30% compared to standard virological methods (detection of antigen by immunofluorescence followed by isolation and culture) [76], but it has still not been proven to be cost-effective, except for the detection of the virus in spinal fluid, for which it is the method of choice [65].

**HIV-HSV Interaction**

The prevalence of HSV infection is considerably higher in HIV-positive patients. Santos et al. found 73% positivity for HSV-2 in serological testing of HIV-positive patients in Brazil [77], a finding that is similar to data from studies carried out in other countries [78-80]. These data reflect the behavioral risk factors common to infection by the two viruses.

According to various studies, genital ulceration, of which HSV is the most common cause, is an important risk factor for the acquisition and transmission of HIV and contributes to the dissemination of this virus [77,81-85]. Telzak et al. reported that patients with genital ulceration have a three-fold greater risk of acquiring HIV than a similar population without ulceration [86]. A study carried out by Latif et al. in Zimbabwe in serodiscordant couples (only one member of the couple was HIV-positive) showed that the HIV-negative partners of HIV-positive individuals who had genital ulceration had a five-fold greater risk of acquiring HIV than did HIV-negative partners of individuals with no ulceration [87].

The explanation of how genital herpes serves as a co-factor in HIV contagion does not appear to be limited to the simple idea of continuity determined by the herpetic infection that would serve as an entry or exit route for HIV. There is evidence supporting an increase in HIV expression in the mucosa during reactivation of HSV [88]. The migration of CD4+ lymphocytes to the infection site may be one of the factors responsible [89]. Activation of these lymphocytes previously infected by HIV leads to a greater replication of the former virus in response to the HSV infection [90]. During reactivation, as well as stimulating the CD4+ lymphocytes, some HSV proteins seem to trans-activate the long terminal repeat of HIV, increasing replication [91-92]. In a study by Schacker et al., HIV RNA was detected in 25/26 episodes of HSV reactivation, in independent titers of the HIV plasmatic viral load [90]. According to Heng et al., the HSV infection enabled replication of HIV in the keratinocytes, which are cells normally not infected by HIV because of the absence of CD4 markers on their
surface [93]. In this same study, potentialization of the replication of HSV in the presence of HIV was seen.

These findings, taken together, may explain the greater risk of HIV transmission in patients co-infected with HSV. Although the use of preservatives is apparently not totally efficient in preventing HSV transmission, condom use makes HIV transmission rates similar among individuals with or without genital ulceration [83].

In addition to the higher rate of HSV infection in HIV-positive patients, these patients also present higher resistance to treatment with acyclovir or foscarnet [94,95]. Also, the course of the HSV infection in these patients is more prolonged and there is a greater risk of complications [65,96-97] – mucocutaneous infections lasting more than 30 days; bronchitis, pneumonitis or esophagitis are defining diseases for AIDS [98].

Final Remarks

In conclusion, great advances have been made in the diagnosis of STDS in recent years due to increased knowledge and awareness of the risks associated with these diseases. Data on the mechanisms of interaction between the different viruses that cause STDS, as well as clinical observations showing greater morbidity in co-infected patients, prove the importance and necessity of testing for the various common STDS as soon as diagnosis of a sexually transmitted disease is made.

The new methods of rapid diagnosis will certainly bring important changes in the management of acute situations such as accidental exposure. Diagnostic testing of self-collected samples promises to increase patient acceptability of testing, a very important aspect in the control of the disease dissemination and in the timely treatment of affected individuals.

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