Detection of sexually transmitted pathogens Trichomonas vaginalis and Herpes simplex virus in women

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Abstract:

There are more than 340 million new cases of sexually transmitted infections (STIs) occurred annually throughout the world, the highest incidence is in people of developing countries. Pelvic inflammation, sterility, ectopic pregnancy, morbidity and mortality of newborns, and genital carcinoma have been assumed to be related to STIs.
Sexually transmitted diseases have various clinical symptoms while 70% gonococcal or non-gonococcal urethritis in males and genital tract infections in females are asymptomatic, both symptomatic and asymptomatic infections may cause severe complications, previous studies revealed that wide range of pathogens recognized as a causative agents of urethritis in males and genital tract infections in females, such as Trichomonas vaginalis and herpes simplex virus. More epidemiological studies are needed to evaluate the significant role of organisms other than the recognized genital pathogens in vaginal syndromes.
In summary we conclude that sexually transmitted diseases may increase reproductive morbidity rate causing difficulties in conception especially the infection with T. vaginalis and herpes simplex viruses, so concentrating on different methods in diagnosis is required. In addition, the cost and time of the test should be taken in consideration.

Key words: Sexually transmitted diseases, Trichomonas vaginalis, Herpes simplex virus

الكشف عن داء المشعرات وفايروس الحلأ البسيط المنتقلة جنسيا

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الخلاصة:

هناك أكثر من ٣٤٠ مليون حالة جديدة من حالات العدوى المنقولة عن طريق الاتصال الجنسي التي تحدث سنويًا في جميع أنحاء العالم ، وهي أعلى نسبة إصابة في البلدان النامية. التهاب الحوض ، والعقم ، والحمل خارج الرحم

، والاعتلال والوفيات بين المواليد الجدد ، وسرطان الأعضاء التناسلية وقد افترض أن تكون مرتبطة بالأمراض المنقولة بالاتصال الجنسي.
الأمراض المنقولة جنسياً لها أعراض سريرية مختلفة ، في حين أن ٧٠٪ من حالات التهاب الإحليل السيلاني أو غير المكورات التناسلية عند الذكور والتهابات الجهاز التناسلي عند الإناث غير متناظرة ، كلاهما قد تسبب الإلتهابات العرضية وغير المصحوبة بمضاعفات شديدة ، كشفت دراسات سابقة أن مجموعة واسعة من مسببات الأمراض التهاب الإحليل عند الذكور والتهابات الجهاز التناسلي عند الإناث ، مثل داء المشعرات وفيروس الحلأ البسيط..
هناك حاجة إلى مزيد من الدراسات الوبائية لتقييم الدور الهام للكائنات الحية بخلاف مسببات الأمراض التناسلية المعترف بها في المتلازمات المهبلية..
في الخلاصة ، نستنتج أن الأمراض التي تنتقل عن طريق الاتصال الجنسي قد تزيد من معدل الإصابة بالأمراض التناسلية مما يسبب صعوبات في الحمل وخاصة الإصابة ب داء المشعرات وفيروس الهربس البسيط لذلك التركيز مطلوب على طرق مختلفة في التشخيص. بالإضافة إلى ذلك ، ينبغي أن تؤخذ تكلفة ووقت الاختبار في الاعتبار.

لكلمات المفتاحية: الامراض المنتقلة جنسيا، داء المشعرات، فايروس الحلأ البسيط..

Trichomonas vaginalis

Trichomonas vaginalis (*T. vaginalis)* an anaerobic, flagellated parasite is the causative agent of trichomoniasis [1]. Infection rates are similar in men and women, the infections are symptomatic in the women, while in men are asymptomatic. The disease transmitted usually by direct skin contact with an infected patient mostly through sexual intercourse. The WHO has been estimated that each year 160 million individuals have acquired the infection through the world with a large number in North America which is between 5 and 8 million new infection annually [2,3].

Laboratory Diagnosis of *Trichomonas vaginalis*

Microscopy

Microscopic diagnosis of trichomoniasis has depended on the observation of motile protozoa in samples from vagina or cervix and urethral or prostatic secretions. This detection by microscope was discovered in 1836 by Donné. The differentiation of *T. vaginalis* can be based on its distinctive jerky movements. The flagellar movement can also be noticed. The sensitivity of this technique varies from 38% - 82% with a limitation because fewer than 10^4 organisms/mL of inoculum size will not be seen. As well as, the specimen must remain moist. The trichomonad is resemble lymphocyte in size which is approximately ranging from 10 µm to 20 µm or as the smallest neutrophil; but when a trichomonad is not motile, the differentiation from the nucleus of a vaginal epithelial cell will be difficult. It is necessary to keep the temperature of the specimen at room temperature and preserve in phosphate-buffered saline, in these conditions the organism will remain alive for more than 6 h, and the motility of the organisms becomes attenuated. Although the wet mount technique considered as the lowest costly diagnostic test, but the lack of sensitivity may lead to the under diagnosis of the disease. Also, the delay in transportation or the evaporation of moisture from the sample will affect the motility and the sensitivity of the test [4,5].

Staining techniques have been implicated to increase the sensitivity of microscopic examination. Acridine orange and periodic acid-Schiff are the most common staining used for this purpose. Although staining technique will increase the sensitivity of microscopic examination of *T. vaginalis*, there is a difficulty in elimination of motility due to the effect of the fixative and the fact that *T. vaginalis* does not always have its characteristic pear-shaped form. To overcome this complication, the staining method used in combination with direct wet mount motility test [6].

Culture

Since the past 40 years, the golden standard method in the diagnosis of *T. vaginalis* is the broth culture technique. It is required inoculum size of only about 10^2 organisms/mL. Diamond's TYI medium is the standard broth that used in glass tubes

[7]. Incubation periods ranging from 2-7 days are required for the identification of *T. vaginalis* in broth culture test. Adding antibiotics will eliminate vaginal flora. However, bacterial contamination is still a common problem. To reduce the bacterial contamination the culture must be passage after 2-3 days. The organism can enter lag growth and, even in well-established axenic culture, can sometimes have attenuated growth for 24 h to 48 h before re-establishing its characteristic log/day growth. The culture technique is the most effective method for identification of *T. vaginalis*. However, it is still expensive. To solve this problem, the InPouch system (BioMed Diagnostics, USA) has been developed when the sample is put into a two-chambered bag, and the sample is ready for wet amount microscopy technique and this bag provides the required incubation period for culture [8].
Because *T. vaginalis* is an anaerobic organism, the incubation with CO_2 is recommended for optimal growing. Cultivation on cell cultures is a good way for observation of *T. vaginalis* from an inoculum containing only 3 organisms/ml [6].

Nucleic acid detection
Molecular detection by recombinant DNA technology has been approved as a good diagnostic method for identification of STDs such as Trichomoniasis. More than one set of primers have been tested and reported by Riley *et al.* [9]. A different study in the same technology done by Crucitti *et al* showed that some primers are more specific than others in Africans when compared with culture-based techniques. In general, in the women urine sample is less effective than vaginal swabs, and self-administered vaginal swabs have had variable results depending on the experience of the population tested [10].

Dot blot hybridization is another technique which was implicated to detect the infection with trichomoniasis by employing a 2.3 kb *T. vaginalis* DNA fragment as a probe. This probe was unstable as a radioactive probe but can be improved with a fluorescent-labelled technique [11].

Antibody based technique
T. vaginalis has eight serotypes and a variety of antigenic markers. Several techniques, including complement fixation, hemagglutination, gel diffusion, fluorescent antibody and Enzyme-linked immunosorbent assay (ELISA), have been showed high sensitivity in the detection of trichomonal antibodies. However, these techniques are not specific in the differentiation between acute and chronic infection. While in some people with low incidence, a positive antibody may reflect that there is an interaction with nonpathogenic trichomonads. Specific 62 kDa and 65 kDa proteins were used as monoclonal antibodies for identification of *T. vaginalis* from clinical specimens; however, these techniques have been abandoned for PCR-based technology [5,12].

Herpes simplex virus
Herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2) are causing herpes genitalis in women and this infection appears as primary or secondary infection. Herpes genitalis is one of the most common sexually transmitted infections [13]. Both HSV-1 and HSV-2 are enveloped DNA viruses, biological similarities and antigenic cross reaction are present between these herpes simplex viruses [14].

Laboratory Diagnosis of Herpes simplex virus
Light microscopy
The cutaneous specimens or mucocutaneous scrapings for herpes infection diagnosis should be taken by unroofing vesicles and gently swabbing the vesicle base and send to the laboratory in cooled box. These specimens are stained with Giemsa, Methylene blue, or Wright stain and are examined for the presence of

HSV cytopathic effects (CPE) by light microscope [15].

Light microscopy is recommended for herpes virus detection because several advantages, inexpensive, rapid, and simple to perform with the ability to observe other pathologies, such as bullous impetigo, candidiasis, or pemphigus [16].

Cell culture

Before the development of molecular methods, viral culture was the main technique for diagnosis of acute HSV infection. High-quality collected sample and a perfect transportation condition are required to maintain infectivity and virus culture. Because the virus is enveloped and highly labile, specimens collected using a swab must be transferred to suitable viral transport media (VTM), such as M4, M6, or universal transport medium, and others [15].

Both rabbit kidney and mink lung cell lines were used for culturing of HSV-1 and HSV-2, with (100% and 95%, respectively) and showed that the sensitivities with a low viral inoculum were higher than those with the MRC-5 and Vero cell lines (77% and 64%, respectively) [17].

Direct immunofluorescence assays (DFA) is another method developed for direct diagnosis of patient specimens which giving the result in one day. With 100% specificity and 50-100% sensitivity in a comparison with viral culture. Using of DFA in combination with cell culture can increase the sensitivity for HSV diagnosis from about 50% when DFA is used alone to 80% when it is used in combination with cell culture [18].

Molecular tests

HSV can be detected by viral genome targets. Quantification of conserved regions of the herpesvirus DNA polymerase by real-time PCR assays will amplify both HSV-1 and HSV-2 such as, glycoprotein B a viral envelope protein required for virion fusion and glycoprotein D. The Polymorphisms in these targets allow for type-specific probes which can be used for subtyping [19]. Molecular methods have been proved to be more sensitive than culture technique and differentiate HSV-1 and HSV-2 without additional steps. A study was done by Whiley *et al.*, described the use of PCR followed by melt analysis of the gene encoding glycoprotein D (gD) lead to accurate typing [20].

There were three molecular tests for HSV-1 and -2 detection available in 2010 and approved by Food and drug administration (FDA). The first test, PCR-based MultiCode-RTx kit (Luminex, Austin, TX) was developed to detect HSV-1 and -2 infection in the vagina. This test has been reported a sensitivity of 92.4% and a specificity of 98.3% for the detection of HSV-1, while the sensitivity of HSV-2 was 95.2% and specificity was 93.6% [21].

The second test, ProbeTec HSV Qx test (BD) designed to diagnose herpes simplex viruses from both men and women. The sensitivity and specificity for HSV-1 detection were 96.8% and 97.6%, respectively and the sensitivity and specificity for HSV-2 detection were 98.4% and 83.7%, respectively when compared with viral culture. A third test, the IsoAmp HSV assay (Biohelix, Beverly, MA), also used for testing HSV-1 and HSV-2 in both male and female. This test uses isothermal helicase-dependent amplification of the HSV gB gene and detection with a target-specific colourimetric probe [22].

An advantage of this assay is that it does not require thermocycling, allowing it to be performed using only a heat block, while the disadvantage is its inability to distinguish between HSV-1 and HSV-2, which may increase the rate of recurrence of HSV-2 than of HSV-1 in genital ulcers [23].

Serology

Whole-antigen-based (non-gG-specific) detection methods

Western blotting (WB) assays were described for detection of HSV-1 and -2, whole-antigen prepared from HSV-1 or HSV-2 infected cell lines and separated by

electrophoresis, adsorbed to a nitrocellulose gel, and exposed to patient serum. HSV detection is determined by the presence of banding patterns specific to HSV-1 or HSV-2. The 92,000-*M*r band correspond to HSV-2- specific glycoprotein G (gG) which is the key factor in differentiating between HSV-1 and HSV-2 infections. The drawbacks of WB assays are expensive and time-consuming. As well as, false-negative HSV-2 results can occur in individuals who are seropositive for HSV-1. This is due to a strong immune response to HSV-1 common antigens, resulting in obscuring antibodies on the blot [24].

gG-based detection methods

HSV glycoprotein G (gG)-based ELISA is another technique utilized HSV-2 glycoprotein G (gG), it was available in 1999. The sensitivity of this assay is (80 to 98%) and the specificity is ≥96% [25]. The development of HSV-2 antibodies occurred within 2-3 weeks, and if the patient has a new infection the absence of IgG specific for gG and appeared subsequently after 12 weeks revealed a new infection with HSV. The differentiation between newly HSV infection and reactivated may help in epidemiological studies to clarify the source of infection [26].

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