Prevalence of Trichomoniasis, Bacterial Vaginosis and Candidiasis in Women Attending a Sexual Transmitted Infections and Gynaecologic Clinic using an Immunologic Latex Agglutination Test

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Abstract

Objectives: Validation of a simple and rapid test for diagnosis of vaginitis diagnosis and to evaluate its performance in a population sample of women attending gynecologic and STIs clinic in the diagnosis of Candida albicans, Trichomonas vaginalis and Gardnerella vaginalis.

Methods: Clinical sensitivity and specificity were determined for Gardnerella vaginalis with 459 vaginal secretions samples obtained from a Gynecological Clinic of Havana, using Gram staining as reference method. Prevalence study was carried out in a total of 113 non-pregnant women attending a gynecology and STIs clinic that were screened for STIs (VIH/AIDS, Gonorrhea, Hepatitis B, Syphilis, Condyloma, and Genital Herpes) and for vaginitis by of Trichomonas vaginalis, Gardnerella vaginalis and Candida albicans by an immunological method. Risk factors were surveyed among attended women. Prevalence, confidence intervals (with 5% error) and oOdds ratios were calculated.

Results: In the case of Gardnerella vaginalis 24 (5.2%) of the clinical samples reacted with the negative control and were classified as inconclusive. Clinical sensitivity and specificity determined in the 435 remaining samples were 91% and 97% respectively. Diagnosed STIs were 3 women with Gonorrhea and 1 woman with Condyloma. Prevalence of trichomoniasis, bacterial vaginosis (gardnerellosis) and candidiasis were 43.36%, 31.86% and 13.27% respectively. From the declared risk factors and diagnosis was possible to observed a significant odds ratio >6 between trichomoniasis and promiscuous behavior confirming Trichomonas vaginalis STI condition. This was not the same for Gardnerella vaginalis where no relation was observed with STI risk factors., Ccandidiasis was not possible to correlate because of small number of cases diagnosed. The frequency of vaginitis symptoms and signs is shown in figure 2. Vaginal discharge was the most common symptom in women with any kind of vaginitis; the other more frequent symptoms or signs were colpitis, vulvar pruritus, abdominal pain, bleeding cervix and dyspareunia with no relation with the diagnosis.

Conclusion: The kit validation showed results as other more sophisticated techniques as DNA hybridization test and its performance was as expected detecting Ttrichomoniasis wasas the most frequent cause of vaginitis in this sample and hadwith significant relation with promiscuous behavior confirming the STI condition of this infection.

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INTRODUCTION

Infectious vaginitis is a frequent reason that women visit an obstetrician or gynecologist. Vulvovaginal candidiasis is the second most common cause of vaginitis in the United States and the most common cause in Europe $(_1)$. Its prevalence increases during pregnancy $(_2)$ and it facilitates infection with HIV and other Sex Transmitted Infections (STIs) $(_3)$. Trichomonas vaginalis is a flagellated protozoan that is considered to be sexually transmitted. The World Health Organization (WHO) estimates the worldwide prevalence of trichomoniasis to be 174 million and to account for 10% to 25% of vaginal infections (,). It is an important complication in pregnancy, as it has been related with prematurity and low birth weight (5). It has been supported the hypothesis that T. vaginalis may be an important cofactor in promoting the spread of HIV and, in some circumstances, may have a major impact on the epidemic dynamics of HIV in African-American communities (6). Bacterial vaginosis is produced when the local vaginal ecosystem by the effect of antibiotics, hormonal disbalance, anticonceptives, stress (7). The etiology is not well defined, however there are decreased quantity of lactobacillus spp and increased Gardnerella vaginalis associated with other bacteria such as Peptoestreptococcus spp, Bacteroides spp, Mycoplasma hominis, Peptococcus spp, Mobiluncus curtisti and Mobiluncus mulieris (8). Its is a bothersome disease because the abundant vaginal secretions with a characteristic smell. Occasionally is associated with fever, endometritis, salpingitis, pelvic inflammation, infertility and other gynecobstetrics complications (9, 10).

Diagnosis of non-viral vaginal infections has been largely contingent on the clinician's ability to do a sophisticated wet mount/potassium hydroxide (KOH) preparation examination (11). Clinical signs and symptoms make diagnosis unreliable being necessary laboratory confirmation (12). However, diagnosis relies on observing the presence of hyphae or pseudohyphae for candidiasis, vaginal epithelial cells coated with the coccobacilli (clue cells) for bacterial vaginosis and the presence of the protozoan for trichomoniasis. Microscopic examination of the saline fresh mounts is somewhat subjective with high specificity (>90%) but low sensitivity $(\pm 60\%)$ for the three microorganisms, which makes correct diagnosis elusive, complicating treatment and making it difficult to determine accurate prevalence rates $(_{13},$ 14, 15, 16). Indeed, several studies using this method to establish the prevalence of the most common infectious agents for vaginitis have shown widely varying results, which may in part actually be due to inaccurate diagnoses (2, 17, 18, 19, 20, 21, 22, 23, 24,25). The use of culture technique for these three agents has higher sensitivity but requires other equipment and relatively expensive culture media, further it can take up to 7 days for the results. In recent times the diagnosis sensitivity has been improved by the use of nucleic acid amplification technology however, is also outside the reach of many diagnostic centers in resource poor settings $(_{26}).$

We used before a less technologically demanding method,

that give results in three minutes, without the need of other equipment as microscopes and that can be used by not high qualified technician with good test performance characteristics for Candida albicans and Trichomonas vaginalis detection at pathological levels in pregnant women based on immunologic latex agglutination test (LAT) (₂₇).

Here we wanted to validate the method for Gardnerella vaginalis diagnosis and to evaluate the performance of the LAT in a population sample of women attending gynecological and STIs clinic in the diagnosis of Candida albicans, Trichomonas vaginalis and Gardnerella vaginalis.

MATERIALS AND METHODS LAT FOR DIAGNOSIS VALIDATION

The kit was validated in our laboratory using culture as reference method for Candida albicans and Trichomonas vaginalis as described before (₂₇). In the case of Gardnerella vaginalis detection limit was 10⁶ CFU/mL cross reaction was carried out with the other two agents at concentrations of 10⁸ CFU/mL for Candida albicans and cells/mL for Trichomonas vaginalis. Clinical sensitivity and specificity were determined with 459 vaginal secretions samples obtained from a Gynecological Clinic of Havana, using Gram staining as reference method following Nugent's criteria, where Bacterial vaginosis showed mixed flora (gram-positive, gram-negative or gram-variable bacteria) and absent or decreased L. acidophilus.

PERFORMANCE OF LAT IN STIS CLINIC

Another group of 113 non-pregnant women attending a gynaecology and STIs clinic during August 1 to October 31 of 2003 were screened for STIs (VIHHIV/AIDS, Gonorrhea, Hepatitis B, Syphilis, Condyloma, and Genital Herpes) and for vaginitis by Trichomonas vaginalis, Gardnerella vaginalis and Candida albicans by an immunological method validated before. Only women assisting for first time to the clinic and that voluntarily accepted, were included. Clinical examination was made and vaginal secretions samples were taken of the vaginal walls with a sterile swab using a speculum. The presence of Candida albicans in concentrations $>10^6$ CFU/mL (considering normal flora misinterpretation), Gardnerella vaginalis in concentrations > 10⁶ CFU/mL and Trichomonas vaginalis in concentrations 10^{6} cells/mL were determined in 3 minutes by a simultaneous simple method using latex particle agglutination, following manufacturer instructions (Newvagin C-Kure, La Habana, Cuba).

All women were surveyed looking for the following risk factors, among others: a) a new sexual partner since six months before, b) three or more sexual partners since six months before, c) not married, d) sexual partner with urethral secretions.

Data were introduced in an MS ACCESS database. Prevalence, confidence intervals (with 5% error) and Odds ratios were calculated using the WINEPI - RATIOS (2.0) software, Chi^2 analysis with SAS (version 8.02).

RESULTS LAT FOR DIAGNOSIS VALIDATION

The results of the validation of the method of Candida albicans and Trichomonas vaginalis were shown before ($_{27}$). In the case of Gardnerella vaginalis 24 (5.2%) of the clinical samples reacted with the negative control, which is nonspecific rabbit gammaglobulin bound to latex particles, and were classified as inconclusive; usually this is due to the presence of blood or sperm in the sample and can not be determined by this method, that is why they were excluded. Clinical sensitivity and specificity determined in the 435 remaining samples were 91% and 97% respectively calculated from data shown in table1.

Figure 1

Table 1: True positives, false positives, true negatives and false negatives samples by the Latex anti-Gardnerella agglutination test compared with Gram staining as reference method.

| | | Gram staining | |
|-----|-----------|---------------|-----------|
| | | Positives | Negatives |
| LAT | Positives | 163 | 8 |
| | Negatives | 16 | 272 |

PERFORMANCE OF LAT IN STIS CLINIC

In the epidemiological study age distribution varied from 16 to 58 years, with a mean and standard deviation of 32.6 ± 8.2 , 2.7% with less then 20 years, 33.6% with 20 to 30 years, 46.9% with 30 to 40 years and 16.8% with more than 40 years.

Frequency of declared risk factors by women is shown in

table 2 were 19.5%, a new sexual partner since six months before, 16.8% more than one sexual partner since six months before, 16.8% single and 1.8% sexual partner with urethral secretions.

Figure 2

Table 2: Frequency of detected risk factors among 113 nonpregnant women attending an STD clinic.

| Risk factor | N | % |
|---|----|-------|
| New sexual partner | 22 | 19.47 |
| Sexual relations with≥3 sexual partners | 19 | 19.47 |
| Single | 19 | 16.81 |
| Sexual partner with urethral secretion | 2 | 1.77 |

Diagnosed STIs were 3 women with Gonorrhea and 1 woman with Condyloma.

Prevalence of diagnosed vaginitis and mixed infections cases are shown in Ffigure 1.

Calculated Odds Ratios of Trichomonas vaginalis and Gardnerella vaginalis vaginitis with risk factors are shown in table 3 vulvovaginitis by C. albicans and sexual partner with urethral secretions risk factor were not possible to correlate because of very few cases.

Figure 3

Figure 1: Prevalence with Confidence intervals (95%) and mixed infections cases in 113 non-pregnant women attending an STD clinic.



Figure 4

Table 3: Odds ratios between risk factors and Trichomoniasis and Vaginosis. *** p

| Risk factor | Trichomonas Vaginalis | Gardnerella Vaginalis |
|--------------------------|--------------------------|--------------------------|
| \geq 3 sexual partners | 6.62 *** | 0.72 |
| New sexual partner | 0.42 | 1.29 |
| Single | 0.94 | 1.71 |

The frequency of vaginitis symptoms and signs is shown in Ffigure 2. Vaginal discharge was the most common symptom in women with any kind of vaginitis; the other more frequent symptoms or signs were colpitis, vulvar pruritus, abdominal pain, bleeding cervix and dyspaurenia.

Figure 5

Figure 2: Frequency of symptoms and signs in women with vaginitis. VD vaginal discharge, C colpitis, VP vulvar pruritus, AP abdominal pain, BC bleeding cervix, D dyspareunia.



DISCUSSION

Kit validation results have shown similar specificity but higher sensitivity to Candida albicans and to Trichomonas vaginalis compared with reported direct microscopic observations and very similar to a rapid nucleic acid hybridization test ($_{27}$). In the case of Gardnerella vaginalis sensibility of 91% and specificity of 97% is quite similar to results of 89% sensibility and 97% specificity obtained with an nucleic acid hybridization test ($_{28}$, $_{29}$).

New laboratory tests that that can diagnose trichomoniasis as well as other types of vaginitis with high sensibility and specificity, fast enough to give results immediately and acquirable for third world countries are a demand ($_{30}$). In this study with relatively high number of cases with STIs risk factors, the LAT behave as expected diagnosing higher number of trichomoniasis cases than in previous study in a sample of pregnant women that attended obstetrics clinics where candidiasis was the most prevalent cause of vaginitis ($_{27}$).

From the declared risk factors and diagnosis was possible to observed a significant odds ratio >6 between trichomoniasis and promiscuous behavior confirming Trichomonas vaginalis STI condition. This was not the same for Gardnerella vaginalis where no relation was observed with STI risk factors.

Prevalence of Trichomoniasis was also high in this women's sample followed by Bacterial Vaginosis (BV) and very few cases of candidiasis, which were also combined. Considering the presence of risk factors for STIs and no presence of pregnant women in this sample explains the predominance of trichomoniasis over BV and Vulvovaginitis.

Fast and reliable diagnoses of vaginitis lead to precise and fast treatment. A delay in diagnosing and treating STDs can lead to chronic complications and irreversible sequelae. Women and children suffer the main consequences. In women, the most serious consequences are acute and chronic pelvic inflammatory diseases, infertility, ectopic pregnancy, and cervical cancer. Infection during pregnancy may cause spontaneous abortion, stillbirth, prematurity, low birth weight, congenital syphilis, and ophthalmia neonatorum $(_{31})$. Non-ulcerative STDs were risk factors for sexual transmission of HIV-1 in women, after controlling for sexual exposure. Because of their high prevalence in some populations, non-ulcerative STD may represent a considerable population-attributable risk in the transmission of HIV-1 worldwide. The identification of treatable STD as risk factors for HIV-1 transmission offers an important additional strategy for the prevention of HIV/AIDS $(_{32})$.

Also there is strong evidence that the flora associated with BV increases the acquisition of HIV ($_{33}$). It is independently associated with HIV seroprevalence. HIV infection may promote abnormal vaginal flora, or BV may increase susceptibility to sexual transmission of HIV. Alternatively, this association may result from intervening variables; in this case BV may be a marker or a cofactor of HIV transmission ($_{34}$).

An study demonstrates that high-risk heterosexual HIVnegative and HIV-positive women should receive regular gynaecological evaluation regardless of self-report for symptoms of vaginal Candida infections and that high-risk heterosexual HIV-negative women may benefit from gynaecological management and care regarding the prevention and treatment of vaginal Candida infections. These same authors hypothesize that treating Candida infection could be another cost-effective strategy for slowing the spread of heterosexual-related HIV infections among high-risk, sexually experienced women, especially in developing countries, until a vaccine or other inexpensive HIV therapies are made available (35).

The most frequent symptom was vaginal discharge in women who tested positive for bacterial vaginosis, candidiasis and trichomoniasis; however, it was a symptom present in fewer than 50% of the women with positive samples. The other commonly but less detected symptoms in positive cases were colpitis, vulvar pruritus, abdominal pain, bleeding cervix and dyspaurenia, confirming that clinical criteria are not very useful for diagnosis ($_{36,37}$).

The kit validation showed results as other more sophisticated techniques as DNA hybridization test and its performance was as expected detecting trichomoniasis as the most frequent cause of vaginitis in this sample and with significant relation with promiscuous behaviour confirming the STI condition of this infection.

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