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REVIEW: A Review on Diagnostic Methods for Trichomonas Vaginalis

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ABSTRACT

Introduction: Trichomoniasis is the most common non-viral sexually transmitted infection in the world, caused by the protozoan parasite Trichomonas vaginalis, which infects the urogenital tract of men and women. Approximately, 250 million new cases of Trichomonas vaginalis Infection are reported worldwide each year. Trichomoniasis is also considered an important HIV co-infection. The infection is often asymptomatic but can be accompanied by symptoms such as severe inflammation, itching and irritation, foamy discharge, and malodorous smell mucus, but the signs and symptoms of the disease are not sufficient for specific diagnosis.

Material and Methods: In this study, the websites of PubMed, Google Scholar, SID, and Margiran were searched and related articles were reviewed.

Results: Only screening and the use of highly sensitive and specific diagnostic methods can identify asymptomatic individuals. Today, the most common way to diagnose the infection is to use wet slide, Pap smear and culture methods that do not have high sensitivity and specificity. Also, due to the increase in infection and its complications, finding an efficient, rapid, and easy test to detect the parasite and differentiate Trichomoniasis vaginitis from other sexually transmitted diseases is considered important and necessary.

Conclusion: Nowadays, there are several diagnostic methods that differentiate Trichomoniasis infection from other sexually transmitted infections with high accuracy and sensitivity. Of course, existing diagnostic methods mostly use women's urine and vaginal samples for diagnosis, and methods that specifically diagnose the infection in men are more limited.

Introduction

Vaginitis is an inflammatory disease that may be diagnosed as bacterial vaginosis, vulvovaginal candidiasis, trichomoniasis, or concomitant infection (1). Trichomoniasis vaginitis is the most common non-viral sexually transmitted infection in the world (2, 3). It is more common in women of childbearing age (4) because the hormonal changes produced during pregnancy cause more infections of the lower genital tract and as a result lead to maternal complications and perinatology (5).

Trichomonas vaginalis (T.V) is a protozoan with 5 to 20 microns in size and has

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maximum metabolic function and growth in oxygen-free and low pH environments (6). Each year, approximately 250 million new cases of T.V Infection are reported world-wide (7, 8), and women aged 16-53 are at the highest risk of infection (9). Risk factors for women with the infection include older age, African American descent, low economic status, and having multiple sexual partners in life (10, 11). The prevalence of this disease in Iran has been reported between 2.1% - 15.7%. Also, the prevalence of infection in com-munities with low levels of health and literacy is higher than other communities and studies have shown that illiteracy is one of the reasons for the increase in the incidence of this infection (12).

Trichomoniasis is an important disease associated with HIV, in terms of exposure to sexually transmitted infections, which increases the number of high-risk organs (13, 14). Trichomoniasis are often asymptomatic, but can be associated with symptoms such as vaginal discharge and urinary incontinence (in women), as well as urethral discharge and urinary incontinence (in men). Clinical mani-festations in women include vaginitis, itching and irritation, as well as increased discharge, and in addition can cause other side effects such as premature birth, miscarriage, low birth weight, premature rupture of the blad-der, ectopic pregnancy, and so forth. Clinical manifestations in men include urethral itching, clear or mucous discharge, irritation, and severe itching after urination (15, 16). This parasite has the ability to cause vaginitis, ulcers and acute inflammatory disease of the vaginal mucosa (17, 18). In women, trichom-oniasis can last for years, and the symptoms can be severely debilitating (19). The infection in men, is often asymptomatic; But in its symptomatic form, it is known to cause non-gonococcal urethritis (20).

Over the past decade, the discovery that T.V infection is associated with a range of more serious conditions, such as prostate cancer, cervical cancer, adverse pregnancy outcomes, and an increased risk of HIV infection, has increased the attempts to diagnose and treat patients who host the parasite (21, 22). Only screening can identify asymptomatic individuals who will remain infected until normal recovery (23). Like other sexually transmitted diseases, the signs and symptoms of trichomoniasis are insufficient for a specific diagnosis (24) and for several reasons cannot be diagnosed solely on the basis of clinical signs (1). The clinical signs may be similar to other sexually transmitted diseases (2).

Classical "strawberry" cervix is seen in approximately 2% of patients, and (3) foamy discharge is seen in only 12% of women with T.V infection. These classic features are used alone in the diagnosis of trichomoniasis, 88% of patients go undiagnosed, and 29% of patients without infection are falsely reported positive (16). Among the diagnostic methods for diagnosing T.V infection are wet slide, PCR and culture (25). Wet slide is one of the most common diagnostic methods due to its low cost, short duration and high accuracy. However, the primary diagnostic method is the examination of a wet slide prepared from vaginal discharge under a microscope (26). The use of culture in diagnosis is also practical, but this method is time consuming and it takes at least 2-5 days to determine the test result. On the other hand, PCR is a high-precision method that allows amplification of DNA fragments and reduces the possibility of misdiagnosis. In cases where the number of trophozoites in the male reproductive system is low, it is proved that this method is practical and can detect the presence of trophozoites despite its limited number (27).

Due to the increase in infection and its complications, finding an efficient, rapid, and easy test to detect the parasite and differe-ntiate T.V from other sexually transmitted diseases is considered important and necessary (24). However, recent advances in diagnostic tests for T.V raised hope for improved diagnosis and treatment, which may lead to better control of sexual transmitted infections (28).

Methods

In this study, diagnostic methods of T.V were

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evaluated. To collect this information, we searched the PubMed, Google Scholar, SID and Iran Medex databases using the keywords Diagnosis, Trichomoniasis, Trichomonas vaginalis and a combination of them.

Results

Wet Mount Examination

The primary diagnostic method for T.V is traditionally microscopic examination of the vaginal fluid and observation of motile trichomonas. In the wet mount method, a drop of vaginal discharge is placed on the slide and after placing the slide on it, the presence of the parasite in the sample is examined by a light microscope. The presence of parasites in the sample can be detected by regular and rapid movements performed by flagella (17). Although wet mount is a cheap and fast method, its sensitivity is low and shows only about 44-68% accuracy. However, if 0.85% physiological saline is used in slide preparation and sample examination with light microscope with 10X and 40X magnification, the sensitivity of wet slide method reaches 67.6% (15).

Culture

Culture is the gold standard for the diagnosis of T.V and the sensitivity of this method is 81%-94%. To culture the samples in TYI-S-33 medium, the tube containing the culture medium must reach room temperature first, then some vaginal secretions are added to the culture medium and incubated in 37 ºC. Finally, after 24 hours with an inverted microscope for 5 days, the environment can be examined for the presence of motile parasites (17). Diamond's medium is also a traditional culture method used to isolate *T. vaginalis*. However, contamination of the environment with common vaginal bacteria makes this procedure difficult. The InPouch culture system has similarities to the Diamond medium, except that by placing the sample in a two-compartment bag, it prevents the culture medium from being contaminated by bacteria. It also allows simultaneous sampling for wet mount and culture (15).

Affirm VPIII test

The first molecular assay for the diagnosis of T.V is Affirm VPIII test, a nucleic acid hybridization test that detects *Gardnerella vaginalis* and *Candida albicans* in addition to T.V. This method uses synthetic nucleic acid probes and color recognition probes complementing the unique genetic sequences of target organisms, which have at least 10 complex test steps and take 45 minutes to achieve the result, with a sensitivity of only 46% (13).

OSOM Trichomonas test

OSOM is an immunochromatographic method for measuring the immunity of capillary flow enzymes based on Trichomonas membrane proteins that can detect Trichomonas in 10 minutes. This assessment is a clinical laboratory improvement modification (CLIA) and has five stages. Compared to Wet Mount and Wet Culture methods, Trichomonas OSOM test is more sensitive and specific (29). The sensitivity and specificity of this method have been reported to be 86.1% and 100%, respectively (30). In addition, a home T.V test kit is available to perform an OSOM test at home. Studies have shown that this home test is very acceptable and can also be used in the emergency department to prevent over-treatment of vaginal infections (29).

ELISA (enzyme-linked immunosorbent assay)

Recently, the sensitivity of *T. vaginalis* diagnostic methods using nucleic acid amplification techniques has been greatly improved (4). However, these methods are expensive and not easily available in resource-limited settings. As a result, serological methods using monoclonal antibodies for the diagnosis of T.V have been supported and include latex agglutination, immune-fluorescence, ELISA, and lateral flow techniques (31, 32). However, these serological techniques can be technically difficult. Likewise, each technique has its own advantages and disadvantages.

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Sandwich ELISA is a method that detects T.V antigen in urine and vaginal fluid samples of women with a sensitivity and specificity of 95% and 97%, respectively (4).

GeneXpert T.V assay (Cepheid)

In this method, urine samples, intrauterine swabs and vaginal swabs of asymptomatic and asymptomatic women are tested by Probe APTIMA. The sensitivity and specificity of this method are 96.4% and 99.6% in the vaginal sample and 98.9% and 98.9% in the urine sample, respectively. This test is not specific to women. Recently it has been used to examine male urine samples for the presence of T.V infection. In comparison between this method and InPouch Culture method, it was found that InPouch method is more sensitive in diagnosis (33).

AmpliVue assay

This new technology uses the isothermal helicase-dependent amplification (HDA) technique, and in 3 steps, examines vaginal samples taken from asymptomatic and asymptomatic women for 45 minutes and reports the result. AmpliVue Assay, similar to the method Solana, uses HDA technology, but this test can be performed in a small manual cartridge that does not require additional equipment (15).

The test targets a protected DNA replication sequence of T.V and uses a helicase to isolate DNA before amplification. That patients are still in the clinic, be used. This method has been approved by the FDA for vaginal specimens of asymptomatic and asymptomatic women and its sensitivity and specificity are 100% and 97.9%, respectively (34).

Vaginal pH Test

T.V grows best in low acid environments and the increase in vaginal pH may be due to trichomoniasis infection. A health care provider performs an experiment based on the contact of the pH paper with the vaginal wall or a sample of the vaginal swap, and finally, by comparing the color of the sheet with the specified color scale, determines the pH of the vagina, resulting in an initial diagnosis of trichomoniasis (6).

Papanicolaou test (Pap smear)

The Papanicolaou test is a microscopic examination of a stained specimen that is primarily used as a diagnostic test to screen for a variety of cervical abnormalities and genital infections and may also detect T. vaginalis. It should be noted that this method has a high diagnostic error rate and is often not suitable for screening unless being used in conjunction with more sensitive testing (6). However, Papanicolaou staining is the only technique that proves the distinct pyriform shape of T.V (14).

Potassium Hydroxide (KOH) "Whiff Test"

The whiff test is a basic procedure that may be used as part of a clinical diagnosis. The test is performed by mixing a vaginal swab with a 10% solution of potassium hydroxide and sniffing it after mixing. Strong smell of amines (fish); It can be a sign of trichomoniasis infection (6).

Acridine Orange Staining

In this staining method, the prepared smear is fixed with methanol for 2-3 minutes, then the slide is covered with acridine orange and placed at room temperature for 2 minutes. After rinsing with distilled water, the slide is examined under a fluorescence microscope at 40x magnification. If *T. vaginalis* is present in the sample, its trophozoites are red brick with a yellowish green core (35).

Gram staining

In gram staining, the vaginal sample is fixed on a slide first, then the slide is immersed in crystal violet for 45 seconds, and after rinsing with water, the crystal violet is fixed by adding Gram's iodine to the slide, and after 45 seconds, the slide is rinsed again with distilled water and wait for it to dry. The next step is the decolorization step, in which the slide is exposed to 95% ethanol for 30 seconds. Finally, the slide surface is covered with 0.25% safranin for 30 seconds and after rinsing with water, the slide is examined under a microscope. In the presence of

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trichomoniasis infection, T.V trophozoites appear under a microscope in the form of pears with round or oval nuclei with flagella and wavy membranes (19).

Giemsa staining

A drop of saline is impregnated with vaginal discharge onto a glass slide and allowed to air dry and then fixed by immersion in methanol for one minute. The slides were then stained with Giemsa diluted with phosphate buffer solution at a dilution of 1: 9 for 10 minutes, washed with water and air-dried again. Finally, the slides are examined under a microscope at a magnification of 10 × 100 and, in case of the presence of T.V, a round or oval trophozoite nucleus, flagella, and corrugated membrane appear (19, 32).

IFA

In the IFA method, the washed parasite is used as an antigen to cover the antigen on the slides. The patient's serum is then added to the antigens, and after incubation and washing, human FITC-labeled antibodies are added to the slides. Each slide is also used for a single sample with a negative control and a positive control to control the test method. If T.V antibody is present in the individual's serum, it binds to the antigen coated on the slide. This complex of antigen and antibody will be detected by the secondary antibody conjugated to the fluorescent material and will be visible under a fluorescent microscope (17).

Solana Trichomonas assay

The Solana Trichomonas assay is a new rapid test for qualitative diagnosis of T.V DNA that can report results within 40 minutes of sample collection. The FDA method was used in 2017 to diagnose T.V from asymptomatic and symptomatic vaginal and urinary specimens of women. The sensitivity of this method is estimated to be 98% and 92% for vaginal and urine samples, respectively. The Solana method requires a special test tool to process the samples, so the initial cost of using it is high. In this method, after collecting the sample, it is heated to make it slippery and diluted then it is added to a reaction tube containing Helicase-based Amplifying Reagents (HDA) (15, 36).

Polymerase Chain Reaction

PCR is a method in which samples are treated with enzymes that can detect and amplify specific regions of T.V DNA. After amplification, the number of DNA fragments increases, making it easier to detect. Studies have shown that PCR is the most accurate diagnostic method in the diagnosis of T.V It should be noted that this method is mostly used in research laboratories (6, 37).

Nucleic acid amplification tests

As with other STIs, the advent of highly sensitive and specific NAATs has provided vital new tools for the diagnosis of T.V infections. Millions of copies of specific DNA or RNA target sequences are identified. Thus, NAAT analytical sensitivity is inherently greater than microscopic testing, culture, antigen detection, or nucleic acid probes that detect organisms or their constituents. The high analytical properties of NAAT are due to the use of nucleotide primers and probe sequences that are unique to the target organism.

Standard methods during internal and commercial development confirm that these tests do not detect pathogens and common microbial inhabitants of the genitourinary tract other than the intended target and do not interfere with the presence of other microorganisms in the diagnosis (28).

APTIMA T. vaginalis assay

APTIMA is performed using specific rRNA targeting, transcriptional amplification (TMA) and detection of amplified products by a special method that requires a high-complexity instrumentation system and trained laboratory personnel. As a result, APTIMA is not a point-of-care experiment and is more expensive than many non-NAAT methods. Also, the sensitivity of this method is 76% to 100% (28).

Latex agglutination test

Latex agglutination test has been used in

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various studies and has been shown to be highly sensitive and specific. It is now used as a normal diagnostic test in many countries. The vaginal swab is immersed in glycine buffer and stored at -20 ° C, then is used to test latex agglutination. To demonstrate the effectiveness of the latex agglutination kit in detecting T.V antigens, dissolve some parasites in the glycine buffer and place a drop of this antigen-containing buffer on the black latex agglutination slide and mix with a drop of polyester-Coated antibody. The slide is then placed in a shaker for 5 minutes and the slide is examined for agglutination.
Also, in studies, this experiment was repeated with different concentrations of antigen and antibody to determine the appropriate concentration that has the best agglutination. This experiment was repeated with different concentrations of antigen and antibody to determine the appropriate concentration that has the best agglutination. The sensitivity and specificity of this method are 100% and 81%, respectively (9). (***Table 1***).

Table 1. Demographic information of students surveyed in the study

Diagnostic Method	Sensitivity (%)	Specificity (%)
Affirm VPIII test (13)	46	-
OSOM Trichomonas test (29,30)	86.1	100
ELISA (4, 32)	95	97
GeneXpert TV assay (33)	96.4	99.6
AmpliVue assay (15, 34)	100	97.9
Solana Trichomonas assay (15, 36)	98	92
APTIMA T.V assay (28)	76-100	-
Culture (15, 17)	81-94	-
Latex agglutination test (9)	100	81
Gram staining (19)	54.5	93.1
Giemsa staining (19, 32)	63.3	100

Discussion

Trichomonas vaginitis is one of the most common sexually transmitted infections that affects women between the ages of 16 and 53 (especially pregnant women) and can affect a person's health. In this study, we examined the diagnostic methods of T.V Studies have shown that wet culture and propagation methods, which are common diagnostic methods in the laboratory today, do not have sufficient accuracy and sensitivity. Therefore, we should look for alternative methods that can give us the most accurate results with less cost and time. Affirm VPIII test is a method that can determine the test result by 10 steps in 45 minutes. OSOM Trichomonas test is a method with high accuracy and sensitivity that can detect T.V by 5 steps in 10 minutes. ELIZA is a method that uses the nucleic acid amplification technique in diagnosis, but despite its high sensitivity and accuracy, due to its high complexity and high cost, it is not commonly used in laboratories.

GeneXpert T.V assay is a method with high accuracy and sensitivity that detects the presence of T.V in the urine sample and vaginal swab of symptomatic and asymptomatic women. Amplivue assay this method uses the HDA technique and detects T.V in symptomatic and asymptomatic women by 3 steps in 45 minutes. PCR is a method with specific enzymes that can detect specific regions of T.V DNA after amplification and it's the most accurate diagnostic method in diagnosis. Solana Trichomonas assay is a new rapid test for diagnosis of DNA in 40 minutes. Among this diagnostic methods, OSOM Trichomonas test, GeneXpert TV assay, AmpliVue assay, Solana Trichomonas assay and APTIMA TV assay, are ideal assay due to their high accuracy and sensitivity.

Conclusion

Today, there are several diagnostic methods that differentiate trichomoniasis infection fro m other sexually transmitted infections with

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high accuracy and sensitivity. Of course, exi sting diagnostic methods mostly use women' s urine and vaginal samples for diagnosis, an d methods that specifically diagnose the infe ction in men are more limited.

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Authors' contributions

All authors have intellectually committed to the study design and process. The final manuscript was revised and accepted by all authors.

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