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Comparative Evaluation of Urine Based OSOM Assay and Traditional Tests in Detection of *Trichomonas vaginalis* among Asymptomatic Pregnant Women

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Abstract: Trichomoniasis is a sexual disease of worldwide distribution. Approximately 50-80% of infected women are asymptomatic. The disease was linked to pregnancy complications as pre-term birth and premature rupture of membranes. Detection of *T. vaginalis* by traditional tools as wet mount microscopy and permanent staining need trained technicians. The standard method of diagnosis is cultivation on modified Diamond's medium but this method is time-consuming. Recently, OSOM *Trichomonas* test has been introduced to detect *trichomonal* protein antigens in 10 minutes. Hence, the present study was done to evaluate the efficacy of the OSOM *Trichomonas* test in comparison with conventional methods like Wet mount examination, Giemsa stain and culture. Urine samples were collected from 260 asymptomatic pregnant females visiting rural antenatal clinics for routine follow up. Wet mount examination, Giemsa smear, culture in modified Diamond's medium and OSOM *Trichomonas* test were performed. Out of the 260 samples,10 (3.84%) and 14 (5.38%) were positive by urine mount and Giemsa stain. Forty-six (17.69%) cases were detected by cultivation on modified Diamond's medium. OSOM *Trichomonas* test detected 44 (16.92%) cases in urine samples. Using culture as a reference standard, sensitivity, specificity and PPV of the OSOM Trichomonas test was found to be 100%, 99.07% and 95.65% respectively. The OSOM Trichomonas test showed NPV 100% and accuracy 99.23%. It has a better performance than the classical urine microscopy and Giemsa smear. The test is rapid compared to culture and can be performed on-site especially in rural areas with limited facilities. Its high sensitivity and excellent specificity make it an ideal urine-based assay for trichomoniasis screening among asymptomatic pregnant women.

Key words: Modified Diamond's medium · OSOM *Trichomonas* test · *T. vaginalis* · Giemsa stain · Urine mount microscopy

INTRODUCTION

Trichomoniasis is a non-reportable sexually transmitted disease (STD). It is caused by a protozoan parasite called *T. vaginalis* [1]. Infections range from an asymptomatic carrier state to an acute inflammatory disease [2]. Unlike other STDs, it has a variant prevalence in different countries and approximately up to 50-80% of women infected with *T. vaginalis* are asymptomatic with high rates of re-infection [3]. The frequency of *trichomonal* infection is not monitored in most countries nor do control programs exist. Untreated infections can persist up to 5 years [4].

The Infected pregnant women may be at risk of adverse birth outcomes such as premature rupture of membranes, premature labor, low birth weight, post-abortion or post-hysterectomy infection and neoplastic transformation of cervical tissues [5-7]. Female infants can get the infection during birth; which may remain asymptomatic until puberty [8]. Furthermore, trichomoniasis increases the transmission of HIV and acquisition of other STDs [4, 9].

Diagnosis should not be based on the clinical symptoms of trichomoniasis only as it may be similar to those of other STDs. So, different laboratory methods have been developed to improve the detection of

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T. vaginalis with varied sensitivity and specificity in a different setting [10]. The traditional diagnosis rests on direct microscopic examination of wet mount preparations, staining and on culture of urine sediment, vaginal and urethral secretions. These methods have many limitations [2, 11]. The wet mount microscopy is a rapid cheap technique but needs trained microscopists and viable parasites [12, 13].

Since the performance of direct microscopy is not always possible due to the heavy patient load, permanent stains as Giemsa stain can be used [14]. The adequately fixed Giemsa stain, *T. vaginalis* features as nucleus, shape and the cytoplasmic inclusions can be easily recognized [15]. For the in-vitro cultivation of *Trichomonas*, Modified Diamond's medium is commonly used as a method of choice, but this media is not widely available [2]. This medium required samples incubation and daily microscopic examination for up to 7 days [16]. The OSOM *Trichomonas* test is a new rapid diagnostic tool that can be used at the point of care without special instrumentation. It is an immunochromatographic assay that is used to detect trichomoniasis in symptomatic, asymptomatic women and men within 10 minutes [17]. Its reported sensitivity (85 to 90%) and specificity (100%) are similar to those of culture [16].

Hence, the present study has been undertaken to evaluate the performance of classical methods (wet mount microscopy, culture, Giemsa stain) and the OSOM test in *T. vaginalis* detection using urine sediment collected from asymptomatic pregnant women living in rural areas. This study group is seldom tested for trichomoniasis during routine follow up in antenatal care health units.

MATERIAL AND METHOD

Study Design: A cross-sectional study was conducted from November 2016 to June 2017 in the Fayoum Governorate, Egypt. The study groups consisted of 260 asymptomatic pregnant women attending antenatal care clinics for routine follow up in the rural health care unit in Abgeg village. The enrolled pregnant women were between 15 - 40 years old with normal pregnancy. Pregnant females who had urinary antiseptic or topical antiparasitic agents within 72 hours prior to testing were excluded from the study. Oral informed consent was taken from all participants, followed by an explanation of the study aims and methods for them. The enrolled participants asked to provide about 20-40 ml of first catch urine sample in order to perform wet mount examination, Giemsa stain, culture on modified Diamond's medium and OSOM *Trichomonas* test.

Laboratory Methods:

Urine Specimen Processing and Wet Mount Examination: The urine samples were collected into clean, grease-free, wide-mouth urine containers. Each sample was coded using a serial number and was quickly handled.

The urine was mixed thoroughly and centrifuged at 1000 rpm for 5 min. The supernatant was decanted and the pellet was re-suspended in 250µL of distilled water. A drop of the resuspended sediment was placed on a clean, grease-free microscope slide, covered with a coverslip and examined at10x and 40x magnification. The *trichomonads* were identified by their size (10-20 µm), ovoid shape and characteristic jerking motility. *T. vaginalis* was recorded as positive if motile *trichomonads* were seen.

Giemsa Stain: A drop from urine sediment was used to prepare Giemsa smears and the procedure was done according to Ichhpujani and Bhatia [18]. The prepared smears were fixed by methanol for one minute and allowed to dry. Smears then stained in 1:10 Giemsa stain (Egyptian Dignost. Co., Egypt) in a phosphate buffer (pH 7.2) for 10 min. The preparations were scanned at ×100 magnification in order to detect violet, pear-shaped *trichomonad* trophozoites with characteristic morphologic features.

Modified Diamond's Culture Medium: Following the preparation of modified Diamond's medium [19], culture tubes were warmed to 37°C for 15 minutes to inoculate the samples. 100 µl of the re-suspended urine sediment was pipetted into culture tubes, followed by incubation with 5% CO_2 at 35-37°C. The tubes were examined daily for 5 days or until a positive result was obtained. A positive culture was defined as a visualization of the motile of *T. vaginalis*. No motile parasites were observed in negative cultures.

The OSOM Trichomonas Test: The OSOM ®Trichomonas Test (Genzyme Diagnostics, Cambridge, USA) was performed according to the manufacturer's instructions except that the included sterile swab was placed into the remaining part of the urine sample for several minutes. The urine swab was then placed into a tube containing 0.5ml of sample buffer with 0.01% sodium

azide and mixed vigorously. The swab was squeezed and removed. The test stick was then placed into the tube and the test was read after10 minutes. The appearance of a visible blue test line along with the red control line indicates a positive result. Only a red control line was visible in the negative result. The test result was considered invalid if no red control line was visible. All the above laboratory methods were done under standard laboratory conditions.

Data Analysis: The Data were coded and analyzed using the statistical package SPSS version 26. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated.

Ethical Considerations: Oral consent was taken from each pregnant female after a brief description of the research idea.

RESULTS

A total of 260 asymptomatic pregnant women of age group (15-40years) were included in this study. The mean age of them was 27.5± 0. 43 years. All collected urine samples were subjected to wet mount, Giemsa stain, modified Diamond's culture and OSOM *Trichomonas* test.

Out of 260 samples, 10 (3.84%) were positive by wet mount and 14 (5.38%) were positive using Giemsa stain. The maximum number of positive trichomoniasis cases 46 (17.69%) were detected by culture on modified Diamond's medium. With the OSOM rapid test, *T. vaginalis* was detected in 44 (16.92%) of 260 enrolled pregnant females. Considering culture on modified Diamond's medium as the gold standard test, the diagnostic performances of the above-mentioned tests are shown in Table (1).

All 10 positive samples by wet mount were also positive by culture. urine mount examination showed high 100% specificity and 100% PPV, but sensitivity, NPV and accuracy were 21.74%, 85.60% and 86.15% respectively. The Giemsa stain results showed 14 (5.38%) positive samples with 99.53% specificity. The PPV was 92.86%. Only one positive Giemsa stained sample was negative by culture. Sensitivity, NPV and accuracy were 28.26%, 86.64%and 86.97%, respectively.

The OSOM *Trichomonas* test results showed 44 (16.92%) positive samples. Compared to the culture result, *T. vaginalis* antigens were detected by the OSOM *Trichomonas* test in 44 of 46 culture-positive samples, indicating 100% sensitivity. All positive OSOM samples were also positive by culture. The test showed specificity 99.07% and PPV 95.65%. *Trichomonas* antigens couldn't be detected in 2 culture-positive samples by the OSOM *Trichomonas* test and showed NPV 100% and accuracy 99.23%.

DISCUSSION

Detection of *T. vaginalis* among asymptomatic pregnant women by simple rapid tools especially in rural areas can play an effective role in reducing the adverse complications as premature rupture of membranes, premature labor, low birth-weight and other combined infections as bacterial vaginosis and candida albicans. Unfortunately, in rural antenatal care unites, asymptomatic pregnant women are seldom tested for trichomoniasis at all during routine follow up, perhaps due to caution in vaginal sampling during pregnancy. This leads to a limitation of studies and published data on rates of colonization in pregnant women. In the present study, the detection rate of *T. vaginalis* in urine sediment among the target group was low (3.84% and 5.38%) by the classical wet mount and Giemsa stain. This rate was elevated by the use of culture (17.69%) and the OSOM test (16.92%). Similar results were found by Lawing *et al* [20] Akbari and Matini [21]. Moreover, Lawing *et al*. [20] reported a lower detection rate of *T. vaginalis* in urine specimens when compared to vaginal samples.

Urine microscopy is a relatively cheap rapid test that has the ability to detect motile trichomonads. This test was used in the present study as a simple diagnostic technique that showed high specificity (100%) and PPV (100%), but NPV and accuracy were 85.60% and 86.15%

Table 1: Performance of diagnostic tests used for detection of *T. vaginalis* compared to culture on modified diamond media as a gold standard test.

	Wet mount	Giemsa stain	OSOM *Trichomonas* test
Number (%) of +ve cases / total	10/260 (3.84%)	14/260 (5.38%)	44/260 (16.92%)
Sensitivity%	21.74%	28.26%	100%
Specificity%	100.00%	99.53%	99.07%
Positive predictive value% (PPV)	100.00%	92.86%	95.65%
Negative predictive test% (NPV)	85.60%	86.64%	100%
Accuracy%	86.15%	86.97%	99.23%

respectively. Similarly, Smith *et al* [22] reported low sensitivity (22%) of wet mount preparation, high (100%) specificity, PPV and NPV was 79.8%.

The current data shows also, that Giemsa staining is more sensitive (28.26%) than urine mount (21.74%) but still less sensitive than culture and OSOM test. The Giemsa stain showed (99.53%) specificity, (92.86%) PPV, (86.64%) NPV and (86.97%) accuracy. The reported results for urine mount and Giemsa smear in the current work remain within range as confirmed by Ojuromi *et al* [23] and Hussein *et al* [24].

The performance of the diagnostic tests may be affected by the volume of collected urine. The expected concentration of parasite in urine is low in large urine specimens [25]. Perazzi *et al* [11] explained the reason for Low performance of Giemsa smear and urine mount mainly in asymptomatic patients by the low number of organisms in the sample. Also, Radonjic *et al* [26] noted that the trophozoites could be damaged during the process of Giemsa staining and overlooked during microscopic examination.

Furthermore, urine sample processing time and centrifugation should be considered as disabling factors that may be affect the integrity of T. vaginalis. Such a delegate parasite also couldn't stand for the change of temperature or survive outside the body in temperature other than 37ºc and die within half-hour [27].

The most accurate method used in the diagnosis of trichomoniasis is culture as wet mount microscopy and Giemsa staining are subjective and the result largely depends on the experience of the examiner. In the current work, culture technique detected the maximum number of positive cases 46 (17.69%) and was considered a reference standard test with high sensitivity as in Literature [28, 29]. In a recent study done by Adjei *et al.* [30] low performance of culture methods was observed. The Adjei study revealed that wet mount examination of urine specimens failed to detect any positive case of *T. vaginalis* and urine culture showed very low (11.1%) sensitivity, (100%) specificity and accuracy of 55.6%. Also, the Adjei study concluded that the exclusive use of urine-based detection of *T. vaginalis* may not be appropriate.

The culture technique is expensive and time-consuming as it requires incubation of samples in microaerophilic conditions and daily microscopic examination for up to 5-7 days. This test wasn't also available in every health care setting [16]. This often results in delayed diagnosis and loss of patients in follow-up which can be overcome by the use of rapid tests as the OSOM *Trichomonas* Test. This OSOM *Trichomonas* test is an immunochromatographic capillary-flow assay that detects *Trichomonas* antigens directly from the samples. Results are obtained rapidly within 10?minutes. In the present study, OSOM *Trichomonas* Test results showed high performance in detection of infection among asymptomatic pregnant women with sensitivity 100%, specificity 99.07%, PPV 95.65%, NPV 100% and accuracy 99.23%. All positive samples (44/46) by OSOM *Trichomonas* Test were also positive by culture. Being this test is an antigen detection method, only 2 culture-positive samples were falsely negative by the OSOM *Trichomonas* test.

The present results are comparable to different studies findings that reported good performance of the OSOM test in comparison to wet mount examination or culture. El-Moamly and Rashad [31] reported 84% sensitivity, 98% specificity, 70% positive predictive value and 99% negative predictive value for the OSOM rapid test compared to 68%, 100%, 100% and 98%, respectively, for mount microscopy. Hegazy *et al* [32] study reported 97.98% sensitivity and 99.37% specificity for OSOM *Trichomonas* Rapid Test. Katz *et al* [33] reported that the OSOM *Trichomonas* test is a sensitive point of care tests than classical microscopy, but false positives may occur. Madhivanan *et al* [34] concluded that the OSOM test had superior sensitivity (86.1% v. 83.3%) to wet mount but performed less well compared with culture (94.4% v. 86.1%) excellent specificity 100% and excellent PPV 100%, NPV 97.1% and accuracy of 99.5% compared composite reference standard (wet mount or culture positive).

As compared to traditional techniques, The OSOM test is rapid. It can be performed on-site by medical practitioners with the limited skill required to process and interpret the test. Its results were available in 10 minutes [35].

This test doesn't depend on the viability of parasite as in other classical methods and eliminates the need for expert microscopists to detect *T. vaginalis* in the clinical setting.

Moreover, the OSOM test doesn't cost as much as culture and nucleic acid amplification methods. The expenses are generally proportionate to wet mount when the cost evaluations rely upon expert's time and the utilization of laboratory facilities [36]. Its high sensitivity and excellent specificity make it an ideal assay in limited-resource settings. Furthermore, the test is useful in screening multiple samples at a time especially in antenatal clinics in poor rural areas where there is a rapid turnaround of pregnant women.

CONCLUSION

The classical microscopy, staining and culture methods used in the diagnosis of *T. vaginalis* are time-consuming and need experience. The new OSOM test is a simple, rapid objective test. This test can be helpful as screening asymptomatic pregnant women, especially in poor rural areas. Its high sensitivity and excellent specificity make it an ideal urine-based screening assay to promote national public health program for trichomoniasis detection, treatment and control to prevent adverse pregnancy outcomes.

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