



Syndromic Management Approach and Wet Mount Microscopy of *Trichomonas vaginalis* in Pregnant Women and Sexually Transmitted Infection Clinic Attendants in Merawi Health Center, Merawi, Amhara Regional State, Ethiopia

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Authors' contributions

This work was carried out in collaboration between all authors. Author MA designed the study, performed the statistical analysis, wrote the protocol, and wrote the manuscript. Authors TK and NF managed the analyses of the study and they were advisors of the study. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Background: *Trichomonas vaginalis* is a flagellated single cell protozoan parasite, which carries the distinction of being the only truly sexually transmitted parasitic infection in humans.

Aim: To determine the prevalence of *Trichomonas vaginalis* among pregnant women and sexually transmitted infection clinic attendants in Merawi Health center. Syndromic management approach was also evaluated for its effective diagnosis of trichomoniasis.

Methodology: In this study, a total of 222 women were enrolled. Majority of them were in the age group of 15-25 years (48.6%, 108). Identification of the *Trichomonas vaginalis* trophozoites was performed by wet mount microscopy of vaginal samples (vaginal

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discharge and vaginal swab) and clinical diagnosis by the syndromic management approach was performed by health workers in the health center.

Result: Syndromic management approach has identified 4 women (1.8%) who had self-reported symptoms related to trichomoniasis. However, the laboratory wet mount diagnosis has identified 14 (6.3%) women who were positive for the trophozoites of *Trichomonas vaginalis*. The sensitivity of syndromic management approach with respect to the wet mount microscopy was 21.4%.

Conclusion: Syndromic management approach is highly insensitive in screening out trichomoniasis, even, as compared with the less sensitive laboratory test (wet mount microscopy). Hence, we support the need for improved diagnostic parameters to reduce adverse trichomoniasis associated reproductive health outcomes.

Keywords: *Trichomonas vaginalis*; *Syndromic Management approach*; *wet mount microscopy*; *vaginal swab*; *sexually transmitted infections*; *Ethiopia*.

1. INTRODUCTION

Trichomoniasis is a common sexually transmitted infection (STI) caused by the flagellated protozoan parasite *T. vaginalis* [1]. *T. vaginalis* is capable of invading and colonizing the heavily defended host uro-genital mucosa from both sexes, breaking through the primary innate defenses and withstanding induced innate and adaptive responses [2]. Mostly, trichomonal infections remain asymptomatic [3]. However, symptomatic trichomoniasis presents with an abnormal vaginal discharge, vulvar itching, ecto-cervical, prostatic and urethral inflammations [2,4]. *T. vaginalis* infection has been shown to be associated with sterility, pelvic inflammatory disease, cervical cancers, postnatal complications and adverse pregnancy outcomes. In particular, trichomoniasis results in premature rupture of membranes, pre-term delivery and low birth weight in pregnant women [4,5].

Trichomonas vaginalis is the most prevalent non viral sexually transmitted infection worldwide [6]. World health organization (WHO) estimates that *T. vaginalis* accounts for approximately half of all curable sexually transmitted infections worldwide [6]. The estimated global incidence of *T. vaginalis* infections is over 170 million cases per year [7].

No reliable reports are presently available on the actual extent of trichomoniasis in Ethiopia. However, Ethiopia has one of the highest human immunodeficiency virus (HIV) prevalence's in sub-Saharan Africa [8]. The HIV prevalence is between 14% and 20% among urban pregnant women, 12% in patients treated for STIs, and 74% in commercial sex workers [8]. The Amhara Administrative region, one of the largest regions in Ethiopia, has a very high burden of diseases [9]. A report from Dereje showed that the adult HIV prevalence for Amhara region is 2%, which is close to 2.2% of the national estimate [10].

As a result of strong demand for an appropriate point-of-care test with reasonable sensitivity and specificity, to detect the common STI pathogens, WHO introduced various guidelines for the syndromic treatment of symptomatic patients [6]. The syndromic approach uses clinical algorithms so designed that primary health care providers in resource poor settings may arrive at an appropriate clinical diagnosis based on a patient's symptoms and clinical signs [6,11]. The clinical diagnosis is then linked to a predefined antimicrobial prescription in which drugs that have shown efficacy against the different STI pathogens in clinical trials are advised [12].

Even though syndromic management is more economical than laboratory diagnosis; it has been fraught with problems [11]. For example, a spontaneous complaint of abnormal vaginal discharge (in terms of quantity, color or odor) may be physiological or pathological. Although abnormal vaginal discharge often prompts women to seek screening for STIs, vaginal discharge is poorly predictive of the presence of an STI [13]. In addition, Syndromic approach cannot be used to detect infections among asymptomatic individuals. Moreover, Syndromic approach leads to over-diagnosis and over-treatment that may result in increased drug costs, possible side-effects of multiple drugs, alterations in vaginal flora and potential for increased drug resistance [14].

A large burden of gynecological disease exists in developing countries [15]. The complications related to STIs (including trichomoniasis) are major causes of mother and child mortality and morbidity during pregnancy [16]. However, as a result of the absence of effective screening test in the routine diagnosis or the relatively mild nature of the disease, trichomoniasis has been largely neglected [17].

2. METHODOLOGIES

The study was carried out in Merawi Health center which is found in Amhara regional state, West Gojjam zone, 530 km northwest of Addis Ababa. The town has local popularity in exhaustively producing “*araki*” (Strong locally brewed alcoholic beverage in Ethiopia). The town is a market place for the surrounding rural districts, and a large number of farmers come to it on the weekly market days for various activities. The town hosts large numbers of female sex workers who operate in hotels, local *brew* and *araki* selling houses. They work as waitresses in these places, and meet clients.

2.1 Data Collection Techniques

Willing Pregnant women visiting the antenatal clinic and women attending STI clinic of Merawi Health center during the study period were participated for the study. Data collection took place from November 05-2010 to January 29-2011 using questionnaire, results from the syndromic approach and wet mount microscopy of vaginal samples.

2.1.1 Collection of biological sample

For women who had vaginal secretion, the secretion was carefully taken. However, for women with no secretion, the vaginal vault and walls were swabbed using sterile cotton wool swab. Two samples (swabs) were taken from each participant by trained professionals in order to get satisfactory amount of sample and to increase the chance of detecting trophozoites of *T. vaginalis*. The swabs were placed in a tube containing 0.5 ml physiologic saline and kept at room temperature for not longer than 30 minutes before examination.

2.1.2 Clinical diagnosis

As per the WHO guideline, Syndromic Management is identification of consistent groups of symptoms and easily recognized signs (syndromes), and the provision of treatment that will deal with the majority of or the most serious, organisms responsible for producing a syndrome. Hence, staffs in Merawi health center diagnosed the study participants based on the clinical sign and symptoms and treatment was provided using syndromic management approach. The data were used for comparison with the results of wet mount microscopy.

2.1.3 Wet mount microscopy

The swab (sample) was thoroughly mixed with physiologic saline to get well homogenized sample and the cotton swab was discarded. A drop of homogenized sample was taken using a pipette and put on dry microscope slide. The sample on the microscopic slide was cover-slipped. The above two procedures were repeated for extra two slides, tripling the number of wet smears to be examined, which could enhance the chance of detecting the parasite (trophozoite). The wet preparations were examined under microscope (Olympus-CX21, Model: CX21FS1, S.N: 5H06916, Olympus Corporation; Tokyo, Japan). The microscopic examination was begun using the 10x objective noting cellular distribution and the 40x objective was used to identify the presence of motile *T. vaginalis* trophozoites which are oval (pyriform), larger than White blood cells, and flagellated that produce remarkable motility called “cork screw motility”.

2.2 Data Processing and Analysis

The data obtained from each study subject was carefully documented and analyzed using SPSS 16 software application and the final outcomes were presented using tables.

3. RESULTS

A total of 222 women participated in this study. Among these, 198 were pregnant women attending the antenatal clinic unit for their follow up and 24 were STI clinic attendant women. The age of women under study ranged from 15 to 45 years, and majority of them 48.6% (N=222) were within the age group of 15-25 years. Most of the participants were illiterate 73.9% (n=164), workless 58.6% (n=130), married 94.1% (n=209) and 86.9% (n=193) women didn't have awareness to any of STIs (Table 1).

From the total study participants, 4.5% (9/198) of pregnant women who visited the health center for their antenatal care and 20.8% (5/24) of STI clinic attendants who were complaints for the discomfort in the urogenital tract had laboratory confirmed trichomoniasis. Clinical Diagnosis (syndromic management approach) has identified 4 (1.8%) women who had self-reported symptoms related to trichomoniasis. However, the laboratory wet mount diagnosis has identified 14 (6.3%) women who were positive for the trophozoites of *T. vaginalis*.

Table 1. Frequency table of different variables for study participants

Demographic Characteristics	Variables	Total (%)
Age	15-25	108 (48.6%)
	26-35	101 (45.5%)
	36-45	13 (5.9%)
Educational status	illiterate	164 (73.9%)
	elementary	26 (11.7%)
	High school	16 (7.2%)
	college	16 (7.2%)
Occupation	governmental	16 (7.2%)
	private	76 (34.2%)
	none	130 (58.6%)
Marital status	married	209 (94.1%)
	not married	8 (3.6%)
	divorced	5 (2.3%)
STI awareness	yes	29 (13.1%)
	no	193 (86.9%)
Number of sexual partner ship	one sexual partner	209 (94.1%)
	have no sexual partner	5 (2.3%)
	have more than one sexual partner	8 (3.6%)

As shown from Table 2, a higher prevalence of *T. vaginalis* infection was found among young women with in the age group of 26 to 35 years (8.9%, 9/101), elementary school complete women (7.7%, 2/26), women who were single (50%, 4/8), women with more than one sexual partners(50%, 4/8), women who didn't have awareness to any of STIs (6.7%, 13/193) and women who work in restaurant, cafe, local 'beer' and 'caticala' selling houses (10.5%, 8/76).

The current diagnostic approach for STIs (syndromic management approach) was evaluated for its ability in correctly screening trichomoniasis cases. To do this, wet mount results of vaginal samples were taken as reference test to calculate the sensitivity and specificity of syndromic management approach (Table 3). The sensitivity and specificity of syndromic management approach were 21.4% and 99.5%, respectively. Similarly the positive predictive value and negative predictive values were 75% and 94.9%, respectively.

Table 2. Distribution of *T. vaginalis* among different age groups, marital status, education status, occupation, sexual partnership, STI awareness and symptoms with respect to *T. vaginalis*

		Laboratory (wet mount) result			Prevalence %
		<i>T. vaginalis</i>		Total	
		seen	not seen		
Age	15-25	4	104	108	3.7
	26-35	9	92	101	8.9
	36-45	1	12	13	7.7
Educational status	Illiterate	11	153	164	6.7
	Elementary	2	24	26	7.6
	High school	1	15	16	6.2
	College	0	16	16	0
Occupation	Governmental	0	16	16	0
	Private	8	68	76	10.5
	None	6	124	130	4.6
Number of sexual partnership	One sexual partner	9	200	209	4.3
	Have no sexual partner	1	4	5	20
	Have more than one sexual partner	4	4	8	50
Marital status	Married	9	200	209	4.3
	Not married	4	4	8	50
	Divorced	1	4	5	20
STI awareness	Yes	1	28	29	3.4
	No	13	180	193	6.7
Symptom with respect to trichomoniasis	Symptomatic	3	1	4	75
	Asymptomatic	11	207	218	5

Table 3. Sensitivity and specificity of syndromic management approach with respect to wet mount microscopy

Clinical diagnosis (Syndromic management approach)	Wet mount result (<i>T. vaginalis</i>)		Total
	Positive	Negative	
suspected	3	1	4
Trichomoniasis not suspected	11	207	218
Total	14	208	222

Sensitivity = 21.4 %, Specificity = 99.5%

4. DISCUSSION

Trichomoniasis prevalence of 6.3% in this study was higher than the reports from 1.2% of Addis Ababa [18] and 1.8% of Argentina [19]. However, the prevalence in this study was lower than the reports from 17.3% of Uganda [12], 11% of Nigeria [20], 9.1% of Peru [17] and 14% of rural South Africa [21]. Despite the use of different laboratory diagnostic tests

(some reports used culture while others used saline wet mount), the difference in the prevalence's was probably because asymptomatic pregnant women and women who were STI clinic attendants were included in this study. However, either symptomatic women or pregnant women in the family planning clinic were included in the other reports.

As shown by significant association in this study ($P = 0.01$, $CI = 0.018$ to 0.65), women who had more than one sexual partner were more affected by trichomoniasis (50%, 4/8) than women who had no sexual partner (20%, 1/5) or with one sexual partner (4.3%, 9/209). This finding agrees with the report from Peru [17] which stated that *T. vaginalis* infection occurred more often among women who reported unprotected sex with a non steady partner and women who reported multiple sexual partners.

Syndromic approach was found to be less sensitive in screening trichomoniasis cases (sensitivity taking wet mount results as reference = 24.1%). This finding agrees to the report from China [22] which revealed that the syndromic approach algorithm had poor sensitivity of 10%. However, the report from Uganda [12] showed that the sensitivity of syndromic management in screening *T. vaginalis* was 66.7%. The differences in the sensitivity figures might be attributed to the differences in the study participant because the inclusion of symptomatic women increases the prevalence which in turn increases the sensitivity. However, the inter personnel difference between health workers either in strictly following the syndromic management algorithm (flow chart) or difference in taking adequate trainings related to the syndromic management approach might increase or decrease the sensitivity.

According to the laboratory wet mount results of this study, 25% (1/4) of trichomoniasis cases diagnosed by the syndromic approach did not have *T. vaginalis* infection ($PVP = 0.75$). This finding supports the result from Addis Ababa [18] and China [22], which revealed that 50% of the cases diagnosed by syndromic approach were false positives. These misdiagnosed women by the syndromic approach would be unnecessarily treated that could bring potentially harmful side effects and drug resistance too.

5. CONCLUSION

As a result of high rates of asymptomatic infections and the tendency of health workers to deviate from the syndromic management guidelines and rather follow their personal clinical judgment, many trichomonal infections remain untreated. These, untreated women, will be predisposed to further complications because they didn't get appropriate treatment. Untreated women with the infection are, also, the potential sources to unknowingly transmit the infection and increase the prevalence.

Syndromic management approach is highly insensitive in screening out trichomoniasis, even, as compared with the less sensitive laboratory test (wet mount microscopy). Hence, syndromic management approach should not be the only choice to screen out trichomoniasis. We support the need for improved diagnostic parameters to reduce adverse trichomoniasis associated reproductive health outcomes. In addition, the screening and treatment of sexual partners of infected women must be also prioritized as a public health measure to prevent re-infection and reduce infection prevalence.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

The research proposal was ethically cleared by the department ethical review board of the Department of Microbiology, Immunology and Parasitology of Addis Ababa University. Prior to data collection, the medical director of the Merawi Health center was informed on the general overview of the study and permission was asked. Then, eligible study subjects were informed on the aim of the study and written consent was taken. Finally, the wet microscopy result with respect to trichomoniasis was disclosed to all study participants through health professionals in the health center. The accurate treatment has proceeded according to the laboratory result. In addition, Health information on STIs was delivered to all participants.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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