Evaluation of syndromic approach of vaginal discharge flowchart to diagnosis of trichomoniasis

Avaliação da abordagem sindrômica do corrimento vaginal para o diagnóstico de tricomoníase

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ABSTRACT

Introduction: *Trichomonas vaginalis* infection is the most prevalent non-viral sexually transmitted disease in the world. Among the different methods for diagnosis, the World Health Organization and Ministry of Health of Brazil proposes the use of flowcharts in the syndromic approach. **Objective:** To evaluate the syndromic approach of vaginal discharge to diagnosis of *T. vaginalis* infection. **Methods:** Transversal study with sample of outpatient population consisting of women in reproductive age. After exclusion of pregnant women and minor girls, the final sample consisted of women between 18 and 49 years old. The participants answered a questionnaire where the complaints were registered. They were examined, had the vaginal pH assessed and sample tested with 10% KOH solution to verify the exhalation of amine odor (whiff test). After this proceeding, a vaginal secretion sample was inoculated in a specific *T. vaginalis* culture medium. The culture results were used as the gold standard to evaluate the syndromic approach flowchart. The algorithm was evaluated according sensitivity, specificity, accuracy, and predictive values. **Results:** Among women with *T. vaginalis* infection, 10% were asymptomatic; among them, dyspareunia was significantly higher, if compared to women with no infection. Flowchart proposed by the syndromic approach had low specificity and accuracy, leading to unnecessary treatment in two-third of women. **Conclusion:** The diagnosis of trichomoniasis based only on the discharge complaint had low accuracy; the whiff test result improves the specificity of diagnosis of *T. vaginalis* infection, regardless of the vaginal pH value.

Keywords: sexually transmitted diseases; Trichomonas vaginalis: vaginitis; vaginal discharge.

RESUMO

Introdução: A infecção por *Trichomonas vaginalis* é a doença sexualmente transmissível não viral mais prevalente no mundo. Entre os diferentes métodos para seu diagnóstico, estão os fluxogramas previstos pela abordagem sindrômica. **Objetivo:** Avaliar o fluxograma de corrimento vaginal para o diagnóstico de tricomoníase em mulheres atendidas em equipe de saúde da família. **Métodos:** Estudo transversal feito com amostra consecutiva de população ambulatorial, composta por mulheres em idade fértil, exceto gestantes e menores de idade. As participantes responderam a um questionário onde foram registradas as queixas ocorridas nas últimas quatro semanas. Também foram examinadas e submetidas à medição do pH vaginal e teste das aminas. A cultura em meio específico foi considerada como padrão-ouro. **Resultados:** Dez por cento das mulheres infectadas pela *T. vaginalis* estavam assintomáticas; entre as infectadas, a dispareunia foi significativamente maior do que entre as mulheres negativas. O esquema proposto pela abordagem sindrômica tem baixa especificidade e acurácia. **Conclusão:** O diagnóstico de tricomoníase embasado apenas na queixa de corrimento tem baixa acurácia; o resultado do teste das aminas melhora a especificidade do diagnóstico da infecção por *Trichomonas vaginalis*, independentemente do valor do pH vaginal.

Palavras-chave: doenças sexualmente transmissíveis; *Trichomonas vaginalis*; vaginite; descarga vaginal.

INTRODUCTION

Among non-viral sexually transmitted infections (STIs), the *Trichomonas vaginalis* infection is estimated to be the most prevalent throughout the world[1], with different occurrences in various contexts. Associations between *T. vaginalis* infection and damage to sexual and reproductive health have been cited in several studies[2-8], including the increased risk of contracting and transmitting HIV[9-15].

The clinical condition of this infection is wide and varies from asymptomatic to intense vaginitis[16,17]. Among symptomatic women, it is usual the complaint of abnormal vaginal discharge, that is a common symptom of other infections or vaginal microenvironmental disorders. Any abnormalities of the vaginal exudate, either in quantity, appearance, or odor[18], which may be accompanied by other symptoms such as pruritus, burning, or dyspareunia are referred to as vaginal discharge[19].

The causes are diverse and can originate from idiopathic changes of endogenous microbiota or from exogenous infections, including the sexually transmitted infections (STIs). The term STI has been used to replace the term STD (sexually transmitted disease), considered to be wider, and encompassing the asymptomatic infections[20]. In this study the two terms are taken as synonyms. Preference is given to the second, once the emphasis is on the symptomatic cases.

The diagnosis and treatment of trichomoniasis are contemplated in the syndromic approach of sexually transmitted diseases, more specifically in the vaginal discharge algorithms. The syndromic approach of STDs has been recommended by the World Health Organization (WHO) since 1991[20] and adopted by the Ministry of Health of Brazil since 1993[21]. Its use has the advantage of timely diagnosis and treatment in a single contact, even in the absence of laboratory facilities.

While recommending the adoption of the syndromic approach as a strategy for the control of sexually transmitted diseases[20], WHO recommends that flowcharts must be adapted to the local epidemiological reality. Two flow charts – with and without microscopy – are available for the management of vaginal discharge.

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OBJECTIVE

To evaluate the accuracy of vaginal discharge flowchart for the diagnosis of trichomoniasis.

METHODS

The sample examined in this research is part of a larger study of the prevalence of *T. vaginalis* conducted in a family health clinic of the Federal District, Midwest region of Brazil.

Type of study

A cross-sectional study was conducted between November 2014 and March 2015 in a Primary Health Unit, located in the Estrutural City, one of the administrative regions of the Federal District.

Ethical Considerations

The study was approved by the Research Ethics Committee of the State Health Department of the Federal District (CAAE 28186514.5.0000.5553).

Sample Population

The outpatient population sampled consisted of women of reproductive age, except pregnant women and minor girls (less than 18 years of age). The final sample consisted of women between 18 and 49 years.

The invitation to participate in the research was made to all women who were within these criteria, regardless of the reasons that led them to seek medical assistance at the clinic. The team has about 700 women registered in this age group. Considering this number of women as a finite population, assuming a 10% prevalence of infection and a 5% confidence interval, the sample should be composed of 116 participants. Anticipating loss of 20%, the calculation was extended to 139 women. At the end, 201 women agreed to participate, and 193 were examined.

Data collection

After obtaining the signature of participant in the consent form, a trained female interviewer applied a questionnaire, and the participants were asked about the presence of some gynaecological complaint. They subsequently underwent a genital examination for measuring the vaginal pH and realization of whiff test. The pH was measured using graduated strips at intervals of 0.3 units and range from 3.6 to 6.1 (pH-Fix®, Macherey-Nagel, Ref. 92130).

The whiff test was performed by adding a drop of 10% KOH to vaginal discharge, previously disposed on a glass slide. The test was considered positive if was perceived a characteristic fishy odor.

For the culture of *T. vaginalis*, a sterile swab was applied to the posterior side wall of the vagina and plated in TYM (Trypticase - Yeast extract - maltose) culture medium, as proposed by Diamond[22]. The samples were transported to the Laboratory of Parasitology and Vector Biology of the Faculty of Medicine at the University of Brasília where they were placed in an incubator at 37º C.

The plates with TYM culture medium were viewed after 24, 48, and 72 hours. After 96 hours, the culture was instilled with 5 mL of 0.9% saline solution, transferred to test tubes, and centrifuged at 2,500 g for five minutes. The supernatants were discarded and the remaining pellets were examined. For each sample, 100 µL of the pellets were placed on glass slides and examined using an optical microscope at 40x. A second slide was prepared and allowed to dry at room temperature. This second slide was fixed with methanol, stained with Giemsa, and examined under a microscope at 100x. The result was considered positive when the presence of the parasite was observed on either of the slides.

Flowchart Evaluation

We evaluated the vaginal discharge flowchart without microscopy for the management of vaginal discharge. The algorithm was evaluated according its sensitivity (S), specificity (E), positive predictive value (PPV), negative predictive value (NPV), and accuracy (Ac) for the diagnosis of trichomoniasis. It was also calculated the positive and negative likelihood ratios (PLR) and (NLR). These measures are defined below:

- True positive (TP): individuals diagnosed as positive by the test and the gold standard;
- False positive (FP): individuals diagnosed as positive by the test, but negative by the gold standard;
- False negative (FN): individuals diagnosed as negative by the test, but positive by the gold standard;
- True negative (TN): individuals diagnosed as negative by the test and the gold standard;
- Sensitivity (S): proportion of individuals diagnosed by testing among the infected; the result can be found by the formula (TP/TP+FN) x 100;
- Specificity (Sp): proportion of individuals with negative results among uninfected; the result can be found by formula (TN/FP+TN) x 100;
- Accuracy (Ac): proportion of correct results (TP and TN) among all individuals in the sample, or (TP+TN/n) x 100;
- Positive predictive value: probability of an individual with positive test actually being infected - (TP/TP+FP) x 100;
- Negative predictive value: probability that an individual with a negative test is not infected – (TN/FN+TN) x 100.

RESULTS

The calculated values of S, Sp, PPV, NPV, and Ac are expressed as percentages in **Table 1**. RVP and RVN are expressed in whole numbers.

The prevalence of women infected with *T. vaginalis* was 16% (30 of 193). In general, gynaecological complaints were more frequent among those with positive cultures. However, the difference was significant only among those who complained of dyspareunia. Of the 30 women with positive culture, three (10%) were asymptomatic. Among those with negative cultures (n=163), the proportion of asymptomatic was the double (20%), with a significant difference (**Table 2**).

For the diagnosis of trichomoniasis, the isolated complaint of vaginal discharge confers sensitivity, specificity, and accuracy below 60% (diagnosis 1). Higher sensitivity (73%) occurred when the discharge complaints were associated with the pH value or test of amines (diagnosis 5). Higher specificity (85%) occurred in diagnosis 4, which included only women who presented vaginal pH values above 4.5 and positive results on the test of amines, simultaneously.

DISCUSSION

Infection with *T. vaginalis* is considered the most prevalent curable STI in the world[23]. Nevertheless, the proper diagnosis of this infection still faces barriers that start because there are many asymptomatic carriers[24]. Even among symptomatic women, complaints are invariably nonspecific. Such complaints are common for many different conditions that affect the female genital tract[7].

In this sample, if the complaints of vaginal discharge were taken solely into consideration for the diagnosis of trichomoniasis, 46% of women would receive a wrong diagnosis, with 75 negative women receiving a positive diagnosis (FP) and 13 positive women diagnosed as negative (FN). This demonstrates that the vaginal discharge complaint alone is not sufficient for the adequate diagnosis of trichomoniasis.

The pH values above 4.5 increased the diagnostic specificity (77%), which also reflects in accuracy (70.5%). However, there was a 24% lower sensitivity, with two-third of the women undiagnosed.

Table 1 – Evaluation of trichomonas diagnoses according to the results of cultures for *T. vaginalis*, 2015.

Trichomoniasis	Growing of *Trichomonas vaginalis* Diamond medium						
	S	Sp	PPV	NPV	Accuracy	PLR	NLR
Diagnostic 1	57	54	18,5	87,1	54	1,2	0,8
Diagnostic 2	33	77	21,3	86,3	70,5	1,5	0,9
Diagnostic 3	33	84	27,8	87,3	76,2	2,1	0,8
Diagnostic 4	27	85	24,2	86,3	75,6	1,7	0,9
Diagnostic 5	73	59	24,7	92,3	61	1,8	0,5

Diagnostic 1: vaginal discharge; Diagnostic 2: vaginal discharge AND pH above 4.5, regardless whiff test result; Diagnostic 3: vaginal discharge AND positive whiff test, regardless pH value; Diagnostic 4: vaginal discharge AND positive whiff test AND pH above de 4.5; Diagnostic 5: vaginal discharge AND positive whiff test AND/OR pH above de 4.5; S: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value; PLR: positive likelihood ratio; NLR: negative likelihood ratio.

Table 2 – Distribution of women according vaginal complaints and culture results for *T. vaginalis*, in 2015.

Complaint	Positive (n=30)		Negative (n=163)		Variation
	n	%	n	%	
Discharge	17	57	75	46	11
Dyspareunia	15	50	53	33	17
Lower abdominal pain	15	50	86	53	-3
Malodorous discharge	12	40	54	33	7
Burning	11	37	43	26	10
Pruritus	10	33	46	28	5
Asymptomatic	3	10	33	20	-10

The test of the amines provided similar sensitivity (33%) to the measurement of pH, but had greater specificity and accuracy, and was a better discriminator among women with negative cultures, preventing them from being wrongly diagnosed as carriers of trichomoniasis. By this procedure, 24% (46 of 193) of the women would be incorrectly diagnosed, with 26 of them receiving positive diagnoses without effectively being carriers the parasite.

Similar findings have been reported in the literature[25]: the test of amines increased the predictive value for diagnosis, at the expense of increase in specificity, although the adequacy of testes for sensitivity was reduced. There are, therefore, some limitations in the comparison of results. In the study mentioned above, the population was composed of pregnant women and the gold standard was considered the wet mount.

The scheme proposed by the flowchart of syndromic approach (diagnostic 5) had the higher sensitivity, but moderate specificity and accuracy (around 60%), with an elevated number of negative women diagnosed with trichomoniasis (67 of 193). Among the women diagnosed with trichomoniasis by flowcharts and negative cultures, many may have had bacterial vaginosis (BV), as this is also associated with positive test of the amines and higher values of pH[19].

The drug and recommendations for its use in the treatment are the same: metronidazole in a single dose of 2 g or 14 doses of 400 mg divided into twice daily doses during 7 days. The difference is that in the case of trichomoniasis, women should be instructed to refer their partners to treatment, a situation that can be delicate considering the stigma surrounding sexually transmitted diseases. In this case, the greater sensitivity of the flow chart would have better predictive value and accuracy in situations of high prevalence.

As mentioned, clinical cases of trichomoniasis and BV are similar. It is also common the occurrence of BV accompanying the infection by *T. vaginalis*[26,27]. In both cases, positive results from the test of amines and higher pH values can be expected. That said, the value of microscopy cannot be denied for the distinction between these two nosological entities since microscopic examination permits a view of moving parasites[28]. Although presenting less sensitivity in comparison to culture[29], the relative practicity of its use and the possibility of immediate diagnosis can improve the resoluteness of care provided in family health teams.

The increased specificity made possible by the use of the microscope in the diagnosis of trichomoniasis has been demonstrated by Vishwanath et al. (2000)[30]. Although there may be no increase in sensitivity, the increased specificity improves the predictive value for a positive diagnosis. As explained above, the syndromic approach strategy has two flowcharts for the management of vaginal discharge – with and without microscopy. The latter is not used in the primary care units of the Federal District, because there are no available microscopes. To implement this method, an analysis of the cost of equipment, training, and the necessary changes in the work processes of the teams would need to be considered.

This study is not the first evaluation of flowcharts proposed by the syndromic approach in Brazil. However, most studies of the vaginal discharge flowchart have been more focused on infection by *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Among the studies that examined *T. vaginalis*, the gold standard used was the wet mount of discharge[25,31]. In research reported in Scielo, Pubmed,

and Google Scholar, no flowchart validation studies for the diagnosis of *T. vaginalis* using culture as the gold standard in primary health care were found.

CONCLUSION

The diagnosis of trichomoniasis based only on complaints regarding vaginal discharge has low accuracy. The test results of the amines improve the specificity of the diagnosis of *T. vaginalis* infection, independently of the value of the vaginal pH. The flowchart proposed by syndromic approach led, in this study, to unnecessary treatment in more than one third of symptomatic women.

AKNOWLEDGEMENTS

The authors would like to record their gratitude to professionals Lana, Cláudia Lopes, and Edilene Florêncio, who contributed directly to receiving and/or interviewing the participants, and Hian Silva for support in processing the samples in laboratory.

Conflict of interests

The authors declare no conflict of interests.

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Received on: 12.11.2016
Approved on: 02.12.2017