

Study on *Trichomonas vaginalis* infection in women with type-2 diabetes mellitus and vaginal discharge in Thi-Qar Government

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Abstract

There is a wide variation in the prevalence of Trichomonas vaginalis in Iraqi women. There are no data on prevalence in Iraq women with diabetes, a known risk factor for increased genital infection. We wished to ascertain the prevalence of Trichomonas vaginalis infection in diabetic women with vaginal discharge in Thi-Qar, Iraq. A cross-sectional study was performed at one specialized polyclinic between November 2019 and December 2020. One hundred and ten pregnant and non-pregnant diabetic women aged 17-52 years who complain of vaginal discharge were interviewed and high vaginal swabs were taken and tested with wet mount and culture to detect Trichomonas vaginalis, candida and bacteria. Random blood glucose levels and VDRL tests were also done for all patients. Twenty seven out of 110 vaginal swabs (24.5%) were positive for Trichomonas vaginalis. The age of the patient, color and smell of the vaginal discharge significantly concurred with the Trichomonas vaginalis infection status. Highest rate of infection (50%) observed in diabetic women over 40 years of age. Non-pregnant diabetic women had a slightly higher prevalence (27.7%) than pregnant diabetic women (22.2 %). Eighteen of all vaginal discharge (16.4%) were positive for Candida albicans. Bacterial vaginosis was mostly due to Staphylococcus spp (100%). Additionally, 4 patients (3.6%) had a positive VDRL test.

Conclusion: Nearly quarter of women with type 2 diabetes mellitus presenting with vaginal discharge in Thi-Qar had Trichomonas vaginalis infection. This is markedly higher than previously reported rates in the general female population of the city.

Keywords: *Trichomonas vaginalis*, *Wet mount*, *Culture*, *Diabetes*.

INTRODUCTION

Trichomonas vaginalis is a sexually transmitted flagellated protozoan. The World Health Organization (WHO) estimates the worldwide prevalence of Trichomoniasis to be 174 million (1). Prevalence of both candidiasis and Trichomonasises increases during pregnancy. This may be attributable to the increased levels of estrogens and corticoids reducing the vaginal defense mechanisms (2). Possible side effects of cervico-vaginal infection on the gestation have been suggested over the years. It has a direct effect on the fetus and indirect fetal damage secondary to premature labor and/or premature rupture of membranes (3). Prior studies have shown that certain high-risk behaviors such as poor sexual activity hygiene and multiple sexual partners, reproductive age, pregnancy, diabetes, contraception, antibiotic use are risk factors for vaginitis especially candidiasis (4). Patients with diabetes mellitus have a high prevalence rate (46%) of vulvovaginal candidiasis which has been linked to the degree of hyperglycemia (5).

MATERIALS AND METHODS

Patients and Methods

A cross-sectional observational study was conducted in a specialized polyclinic in the city of Thi-Qar, Iraq between November 2019 and December 2020. A total number of 110 diabetic women (both pregnant and non-pregnant) who presented with vaginal discharge were invited to be included in the study. Patients were interviewed and high vaginal swabs were taken and tested with the wet mount and culture to detect candida and bacteria. Descriptive statistics were used to characterize groups and Chi-square test was used to test differences between groups. P value less than 0.05 was considered statistically significant.

Molecular identification of *T. rubrum* by conventional PCR

The genomic DNA were extricated by utilizing a processor in nearness of fluid nitrogen. The measure of 6 µl of DNA arrangement was utilized as a layout in the accompanying PCR, examples were checked and evaluated on 2% agarose gel and by utilizing Nanodrop spectrophotometer. The PCR-intensified inward interpreted spacer (ITS) district of ribosomal DNA (rDNA) was performed with groundworks ITS-1 forward (5'- CGGGCGAAAATACA GATGAT-3') and ITS-1 turn around (5'-TGGTGCCATTTGCTATCGTA-3') under the accompanying PCR conditions.

RESULTS

Out of the total 110 patients, 27 vaginal swabs (24.5%) were positive for *Trichomonas vaginalis* with the wet mount method. The age of the patient ($X = 7.67$, p value 0.02), Random blood sugar (Pearson $X^2 = 69.1$, p value = 0.02) and color ($X^2 = 97.9$, $df = 4$, $P = 0.000$) and smell of vaginal discharge ($X^2 = 21.6$, $df = 1$, $P = 0.000$) was significantly correlated to *Trichomonas vaginalis* infection. While pregnancy ($X^2 = 0.063$, $df = 1$, $P = 0.802$) and nationality ($X^2 = 0.95$, $df = 1$, $P = 0.32$) were not. Highest prevalence was for diabetic women above age 40 (50%) (table 1). Non-pregnant diabetic patients and women with Iraqi nationality have slightly higher prevalence (27.7 %, 25.7% respectively). The most common color of the vaginal discharge for all diabetic patients in the sample was white (65.5%). While patients with positive vaginal swabs for *Trichomonas vaginalis* infection had green and yellow color being most common (40.7% each) (Table 2). Mostly the discharge had fishy smell (65%) (Table 3). Moderate to high pus cells were present in around (90%). *Candida albican* was positive in 18 (16.4%) and *Bacterial vaginosis* was evident in 100% of all vaginal discharge (Table 4). *Staphylococcus* species were the most common bacteria isolated (60.9%) and the second were *pseudomonas* (11.8%) while *Neisseria gonorrhoea* isolated in 10% of patients (Table 4). VDRL test of syphilis was positive in 4 (3.6%) of the patients.

Table (1): Age-related prevalence of *Trichomonas vaginalis* infection among diabetic women with vaginal discharge

| Age group (years) | Status | | Statistical result |
|-------------------|----------|----------|------------------------------------|
| | Negative | Positive | |
| 17-29 (N=42) | 33 | 9(21.4%) | $x^2 = 7.67$, $df=2$, $P = 0.02$ |
| 29-40 (N=50) | 41(82%) | 9(18%) | |
| >40 (N=18) | 9(50%) | 9(50%) | |
| Total | 83 | 27 | |

Significant ($P < 0.05$)

Table (2): Characteristic of vaginal discharge for all samples of diabetic patients and their percentages

| Color | Frequency | Percent | Valid % | Cumulative % | <i>T. vaginalis</i> status | |
|--------|-----------|---------|---------|--------------|----------------------------|------------|
| | | | | | Negative | Positive |
| Bloody | 6 | 5.5 | 5.5 | 5.5 | 6 | 0 (0%) |
| Green | 11 | 10.0 | 10.0 | 15.5 | 0 | 11 (40.7%) |
| Clear | 9 | 8.2 | 8.2 | 23.6 | 9 | 0 (0%) |
| White | 72 | 65.5 | 65.5 | 89.1 | 67 | 5 (18.5%) |
| Yellow | 12 | 10.9 | 10.9 | 100.0 | 1 | 11 (40.7%) |
| Total | 110 | 100 | | 83 | 83 | 27 (100%) |

$$x^2 = 97.9, df = 4, P = 0.000$$

Significant (P<0.05)

Table (3): Characteristic smell of vaginal discharge for patients with positive vaginal swab for *T. vaginalis* infection and their percentage

| <i>T. vaginalis</i> status | Smell of vaginal discharge | | Total |
|----------------------------|----------------------------|-----------|-------|
| | Fishy | Not fishy | |
| Negative | 7(8.5%) | 76(91.5%) | 83 |
| Positive | 13(65.0%) | 14(15.5%) | 27 |
| All | 20(18.2%) | 90(81.8%) | 110 |

$$x^2 = 21.6, df = 1, p = 0.000$$

Significant (P<0.05)

Table (4): Type of bacteria isolated using culture from the high vaginal swab in diabetic patients and their percentage

| Organism | Frequency | Percent | Valid percent | Cumulative percent |
|----------------------------|-----------|---------|---------------|--------------------|
| <i>E. coli</i> | 11 | 10.0 | 10.0 | 10.0 |
| <i>Klebsiela spp.</i> | 8 | 7.3 | 7.3 | 17.3 |
| <i>Nieseria gonorrhoea</i> | 11 | 10.0 | 10.0 | 27.3 |
| <i>Pseudomonas</i> | 13 | 11.8 | 11.8 | 39.1 |
| <i>Staphylococcus spp.</i> | 67 | 60.9 | 60.9 | 100.0 |

Significant (P<0.05)

Confirmation of *T. vaginalis* isolates

The findings of our investigation, affirmation procedure to *T. vaginalis* detaches have likewise led through customary PCR system for recognizing of nearness to a particular 5.8SrRNA quality. Separated of gnomically DNA for those confines have utilized as format to intensification with groundworks for Internal Transcribed Spacer1 (ITS1). Separates had named *T. vaginalis* to size product equivalent 601bp post bands electrophoresis as well as UV of the item (Figure1).

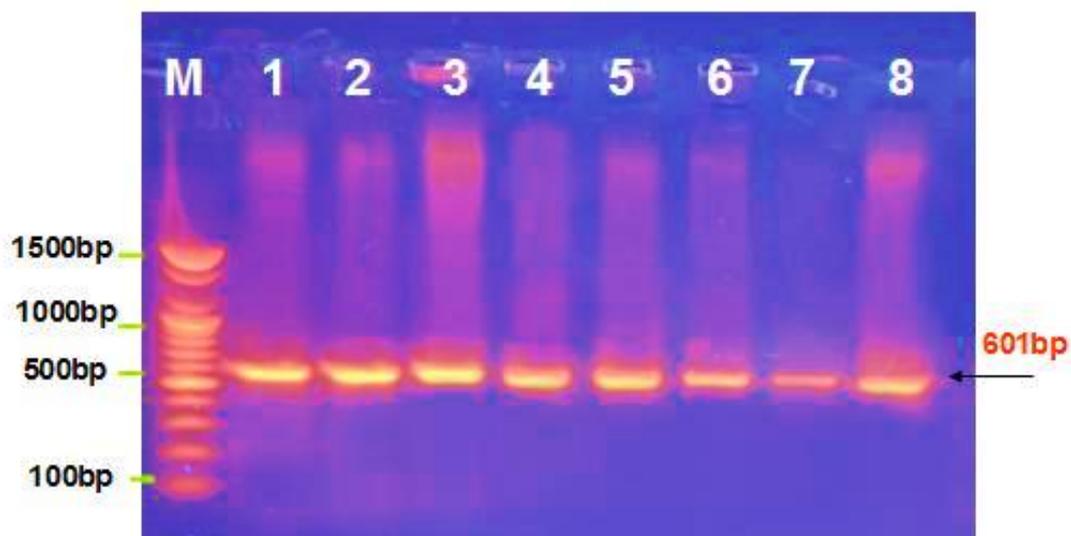


Figure (1): Agarose gel electrophoresis of PCR products stained with ethidium bromide for amplifying the primer of ITS1 gene to *T. vaginalis* at an amplicon size of 601bp

The lane M represent Ladder marker (100-1500bp) and lanes 1-8 represent positive samples to *T. rubrum* isolates

DISCUSSION

There is a wide variation in the prevalence of *Trichomonas vaginalis* in Iraq women likely these results of the present study. Whereas in the Shattrah district at the far southern of the country, it was reported at 26.8% (6), it was reported at much higher level of 36.7% in Rifaa district in the northern region (7). In contrast, its prevalence in Nassirya city was very low at 1.2% only (8). To our knowledge, there are no data on the prevalence of *Trichomonas vaginalis* in diabetic patients in Iraq. Therefore, this study aimed to estimate that prevalence of *Trichomonas vaginalis* in diabetic patients in a gynecology clinic setting in Nassirya. In our study, nearly quarter of the high vaginal swabs from diabetic women complaining of chronic vaginal discharge were positive for *Trichomonas vaginalis* with the wet mount method. This prevalence is significantly high when compared with the previously reported prevalence in general female population in Nassirya (8). This may be partially explained by defective immune system in diabetic patients and increase risk of infection especially in those uncontrolled. The prevalence is nearly similar to the general female population in Albatnan District in 2012, which was 26.8% and lower than its prevalence in general female in Zawia district, which was 36.7%. (6, 7). In our study the age of the patients was significantly correlated to *Trichomonas vaginalis* infection. Actually, this result is in agreement with many studies, but in contrast, highest prevalence was for diabetic women of 40 years (50%) Figure 2. While most of the previous studies show highest prevalence below age of 40 (10) where there is great sexual activity and high estrogen level making good environment for growth of *Trichomonas vaginalis*. Although our result can be explained by the fact that as the diabetes duration increases the immunity, become more defective especially in uncontrolled individual. The color and smell of vaginal discharge were significantly related to *Trichomonas vaginalis* infection, which is in agreement with most of the previous studies. Green and yellow color being most common (40.7% each) and fishy odor was reported in 65% of the *Trichomonas vaginalis* infected patients. Pregnancy was reported to be one risk factor for vaginal infection especially *Trichomonas vaginalis*. In contrast, our results showed pregnancy was not significantly correlated to *Trichomonas vaginalis* infection and non-pregnant diabetic women have slightly higher prevalence (27.7%) compared to pregnant diabetics women (22.2%). These findings are in agreement with some previous reports that showed slight difference of 5.7% versus 3.8% (11) but at variance with other studies which showed markedly greater prevalence in non-pregnant women than in pregnant women (83.8% versus 16.2% respectively) (6,12). Surprisingly, the prevalence of *Candida* in our diabetic women was low compared to *Trichomonas vaginalis* infection (16.4%, 24.5). The presence of

moderate to high pus cells in the vaginal discharge of diabetic women was 90% and invariable presence of bacterial vaginosis (100%) is not unusual. Lakshmi K, et al showed a high prevalence of bacterial vaginosis in diabetic women (98%) (12) with *Staphylococcus spp.* being the most common bacteria isolated (60.9%) and *Pseudomonas* being the second (11.8%). However, this is in contrast to other studies, which showed that *E. coli* as the most prevalent bacteria followed by *Staphylococcus spp.* (12, 13). Furthermore, our findings of *Neisseria gonorrhoea* in around 10% and positive VDRL test in 3.6% of patients are not strange as sexually transmitted diseases may coexist.

In conclusion, this study revealed high rates of *Trichomonas vaginalis* infection affecting nearly quarter of all women with type 2 diabetes mellitus presenting with vaginal discharge in Nassirya. This is markedly greater than the previously reported rates in the general female population of the city.

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