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Clinical and Sociodemographic Features of *Trichomonas vaginalis* and Comparison of Methods Used in The Diagnosis

Trichomonas vaginalis'in Klinik ve Sosyodemografik Özellikleri ve Tanıda Kullanılan Yöntemlerin Karşılaştırılması

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ABSTRACT

Objective: *Trichomonas vaginalis* is one of the most common non-viral sexually transmitted infection in the world. The aim of this study is to determine the prevalence of *T. vaginalis*, the social, demographic and clinical features that affect it, and to compare the sensitivity of diagnostic methods.

Material and Method: The study was conducted on 150 female patients aged 18–65. Samples were taken from the posterior vaginal fornix during the pelvic examination, and the parasite was searched by direct microscopic examination, Giemsa staining, cysteine-peptone-liver-maltose, and Trichomonas Broth culture methods.

Results: *T. vaginalis* was detected in 25 (16.6%) patients. While the best sensitivity was found in the TB culture, the lowest sensitivity was found in DM. The highest positivities were found in the age group of 36–50 (26.1%) and in uneducated women (26.5%). The risk of *T. vaginalis* increased 3.21 times in people who wore white cotton underwear. The majority of positive cases had aqueous or purulent discharge, and 22.2% of the patients had complaints of discharge and itching.

Conclusion: Trichomoniasis is still an important public health problem. By using different diagnostic methods together, it will be possible to prevent cases that cannot be detected with a single method from being missed.

Keywords: *Trichomonas vaginalis, Diagnosis, Culture, Clinic, Türkiye*

ÖZET

Giriş: *Trichomonas vaginalis* dünyada en sık görülen viral olmayan cinsel yolla bulaşan enfeksiyonlardan biridir. Bu çalışmanın amacı *T. vaginalis* prevalansını, bunu etkileyen sosyal, demografik ve klinik özellikleri belirlemek ve tanı yöntemlerinin duyarlılığını karşılaştırmaktır.

Materyal ve Metot: Çalışma 18-65 yaş arası 150 kadın hasta üzerinde gerçekleştirildi. Pelvik muayene sırasında posterior vajinal forniksten örnekler alındı ve direkt mikroskobik inceleme, Giemsa boyama, sistein-pepton-karaciğer-maltoz ve Trichomonas Broth kültür yöntemleri ile parazit arandı.

Bulgular: Yirmi-beş (%16,6) hastada *T. vaginalis* saptandı. En iyi duyarlılık TB kültür yönteminde bulunurken, en düşük duyarlılık DM'de tespit edildi. En yüksek pozitiflikler 36-50 yaş grubunda (%26,1) ve eğitim seviyesi düşük kadınlarda (%26,5) görüldü. Beyaz pamuklu iç çamaşırı giyen kişilerde *T. vaginalis* riskinin 3,21 kat daha fazla olduğu tespit edildi. Pozitif vakaların çoğunda sulu veya purulent akıntı vardı ve hastaların %22,2'sinde akıntı ve kaşıntı şikayeti mevcuttu.

Sonuç: Trichomoniasis halen önemli bir halk sağlığı sorunudur. Farklı teşhis yöntemlerinin bir arada kullanılması ile tek bir yöntemle tespit edilemeyen vakaların gözden kaçmasının önüne geçilebilecektir.

Anahtar kelimeler: *Trichomonas vaginalis, Tanı, Kültür, Klinik, Türkiye*

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INTRODUCTION

Trichomonas vaginalis (*T. vaginalis*) is a protozoan parasite that lives in the human urogenital system. It is probably the most common non-viral sexually transmitted infection (STI) and ranks third among the most common causes of vaginitis in the world. TV has no cystic form, and transmission occurs by trophozoites and often through sexual intercourse (Vos et al., 2012; Edwards at al., 2016). Additionally, it can be transmitted by flush toilets, pools, non-sterile gynecological instruments, commonly used underwear, swimsuits, toilet paper, and the transition from mother to baby (Kissinger, 2015).

Although the incidence of infection varies according to the lifestyle and sociocultural structure of society, it is more common in the 20–45 age group with high sexual activity (Walker, 2004). The prevalence of infection is estimated to be 8.1% in women and around 1% in men. According to the World Health Organization (WHO) there were more than 270 million cases in a single year', and approximately 90% of the infections occurred among people living in limited resource conditions (WHO, 2008). In trichomoniasis, 95% of male patients are asymptomatic, play a carrier role, and are a constant source of infection (Vos et al., 2012). The clinic ranges from asymptomatic carriage to rash, burning sensation in the vulva and vagina, itching, and vaginal discharge (Vos et al., 2012; Kissinger, 2015). 'Additionally, it has been claimed that T. vaginalis raises the risk of HIV. 'Even though trichomoniasis is thought of as a straightforward infection, if left untreated, it can cause low birth weight, an early rupture of the membranes, pelvic inflammation, and a higher chance of tubal infertility. Even so, it has even been reported to have a predisposing effect on prostate and cervical cancer formation (Kissinger, 2015; Edwards at al., 2016).

Clinical signs are helpful for preliminary diagnosis; however, the definitive diagnosis is based on observing the trophozoite form or detecting the DNA of the parasite. Vaginal and urethral secretions, prostate fluids, and sometimes urine are used as examination materials. Direct microscopic examination (DM) and PAP smear are frequently used in diagnosis, followed by various stainings, cultures, serological, and molecular methods (Edwards at al., 2016; Van Der Pol B, 2016). It is important that the method yields results in a short time, has a low cost, and has high precision.

In this study, we aimed to investigate the prevalence of *T. vaginalis* in female patients by DM, Giemsa staining, and culture methods, and to evaluate the infection in terms of clinical and social variables obtained with the questionnaire form.

MATERIAL and METHOD

This study was conducted on female patients aged between 18–65 who applied to the Gynecology and Obstetrics Service/Polyclinic in the State Hospital of Siirt between June–October 2019 with the complaint of vaginal discharge. Within the scope of the study, the sample collection period was determined to be five months, and a total of 150 patients were reached during this period. 'Only patients with written informed consent were used in the trial; those without consent were not enrolled.

The participants were asked to complete a questionnaire form, and patient consent was obtained after the procedure was fully disclosed. Vaginal discharge samples were examined by DM, Giemsa staining, cysteine-pepton-liver-maltose (CPLM), and Trichomonas Broth (TB) culture methods. Approval for the study was obtained from the Van Yüzüncü Yıl University Non-Invasive Clinical Research Ethics Committee (09.11.2018/08).

Questionnaire form

After receiving ethical approval and patient agreement, these questions were created by a parasitologist and given by the midwife employed by the agency

Samples collection

After the speculum was placed in the lithotomy position, three swab samples were taken from the vaginal posterior fornix with a sterile swab. The first swab sample was used for DM and Giemsa staining, and the second and third ones were used for culture methods.

Direct microscopic examination and Giemsa staining

The discharge sample was touched onto the slide and spread out in a thin layer. A drop of saline was dropped on it with a pipette and covered with a coverslip. For staining, a sample was spread on another slide and allowed to air dry. After fixation with methyl alcohol, it was stained with freshly diluted Giemsa dye for 15-20 minutes. The preparations were examined under a microscope at 100 magnification for the presence of *T. vaginalis* trophozoites (Figure 1).

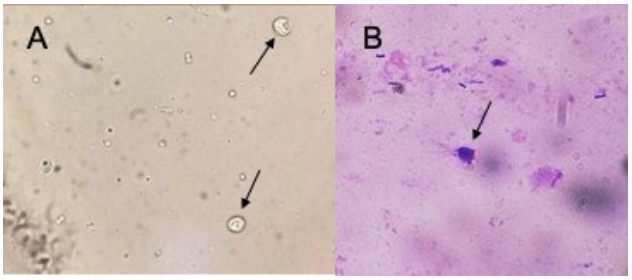

Figure 1. *T. vaginalis* trophozoites **A**. Direct microscopy, **B**. Giemsa staining

Culture methods

CPLM (Liofilchem Diagnostic, Italy) and TB media (Liofilchem Diagnostic, Italy) were used for the culture methods. Media were purchased commercially in powder form, adjusted to pH 6 after reconstitution. Sterilized in the autoclave at 121 °C for 15 minutes, 20% inactive horse serum, Penicilin G, Streptomycin, and Fluconazole were added to the media. It was aliquoted into each of the tubes as 5 ml, and the cultures were stored at +4°C. When the culture was to be done, the tubes were kept at room temperature, and the vaginal swab samples taken from the patients were transferred to this medium. Then, the tubes were placed in the incubator at 37°C, and after 48 and 72 hours, the slide-coverslip preparations were made, and the presence or absence of proliferation was examined under a microscope. The trophozoite detected in a sample by any method was evaluated as positive in total.

Statistical analysis

The chi-square and/or Fisher's exact test were used to examine the relationship between categorical variables or differences between groups, and the results were presented with the frequency of occurrence and percentages. SPSS 15.0 for Windows was used for the statistical analysis of the study, and $p \leq 0.05$ was accepted as the statistical significance limit.

RESULTS

The patients were divided into five age groups: 18–25, 26–35, 36–50, 51–64, 65 and over (Figure 2).

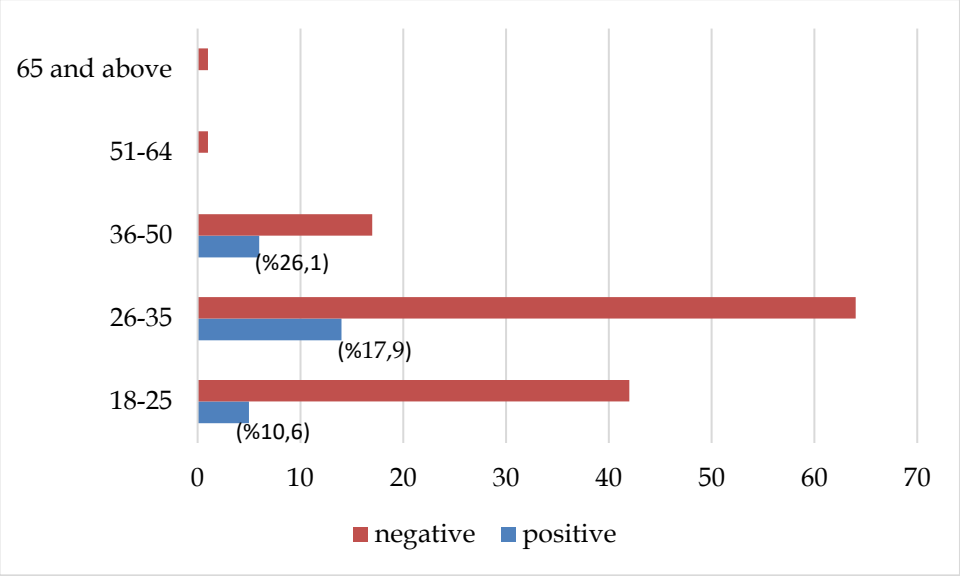

Figure 2. *T. vaginalis* infection by age groups (p:0.474)

According to their educational status, most of the patients (67/150) were primary school graduates (Figure 3).

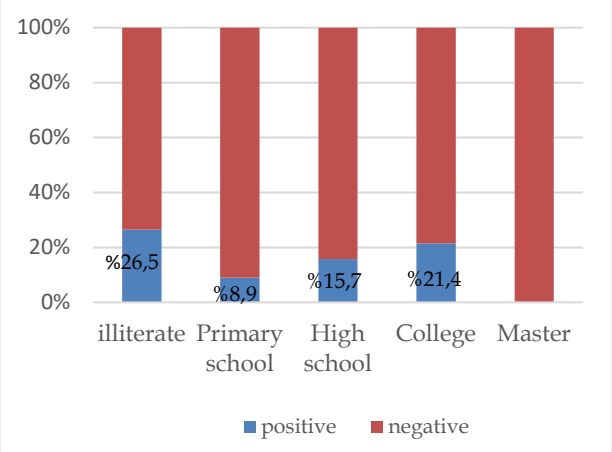

Figure 3. *T. vaginalis* infection by education level

Eighty-one of the patients were settled in the city center, 31 in the district center, and 38 in the village. One hundred thirty-three patients were using Turkish-style toilet; 10 were using both Turkish and flush toilet; and seven were using only flush-toilet. For self-cleaning after the toilet, 74 people used only water and 23 people used toilet paper. 116 people were taking a shower while sitting, and 34 people were standing. The underwear types of patients mentioned in figure 4.

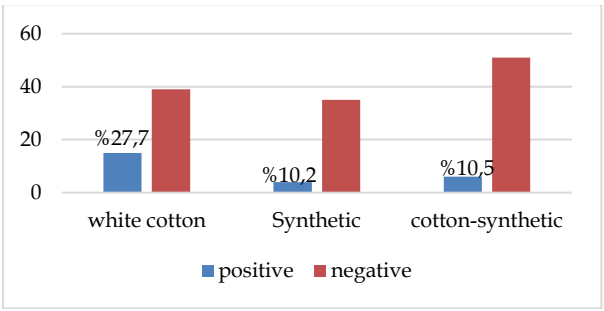

Figure 4. *T. vaginalis* infection by underwear type

Only 93 people change their underwear every day. The patients used five different methods for sexual protection (Figure 5); eight people stated that they had sexual intercourse every day, and 66 people every other day (Figure 6). While 70 of the patients felt pain during sex once in a while, 31 in every intercourse, and 9 in frequent sex, 40 of them did not feel pain. Sixty-seven of the patients had purulent, 54 had

aqueous, 15 had an egg-white discharge, and 14 left the question unanswered. The color of the discharge was white in 89 patients, yellow in 52, green in seven, and brown in two. While 98 of the patients were asymptomatic, 18 of them had itching, 17 of them had burning in the urine, and 17 of them had both itching and frequent urination. The discharge occurred every day in 109 patients, five times a month in 27 patients, up to treatment in five patients, and nine patients did not answer this question. The odor of vaginal discharge was like musty in 46 cases, spoiled cheese in 27, dead fish in three, and 74 people stated that it did not smell. Sixty-three of the patients were uncomfortable when there was excessive discharge, 22 when it smelled, and five when there was a color change; however, 60 patients were not disturbed.

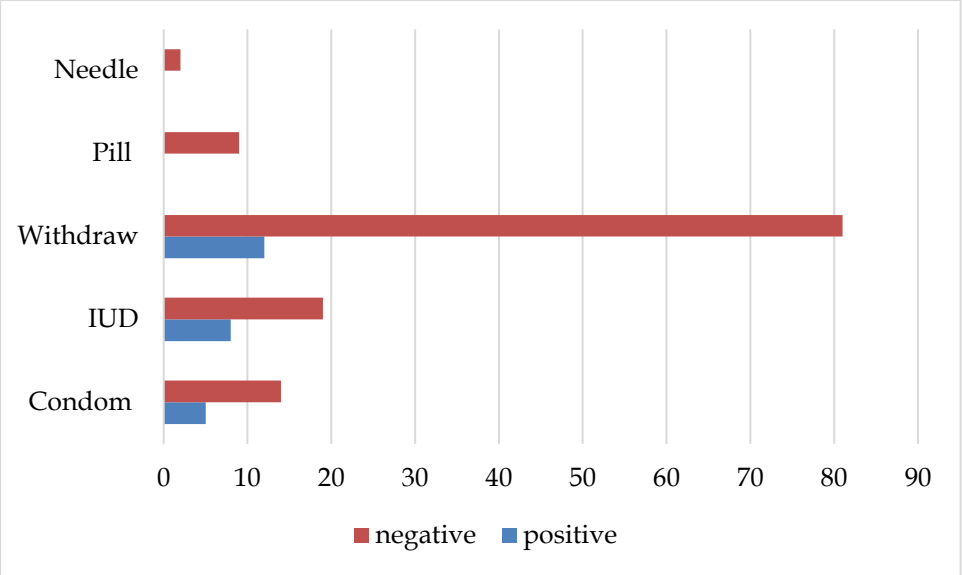

Figure 5. *T. vaginalis* frequency according to sexual protection method

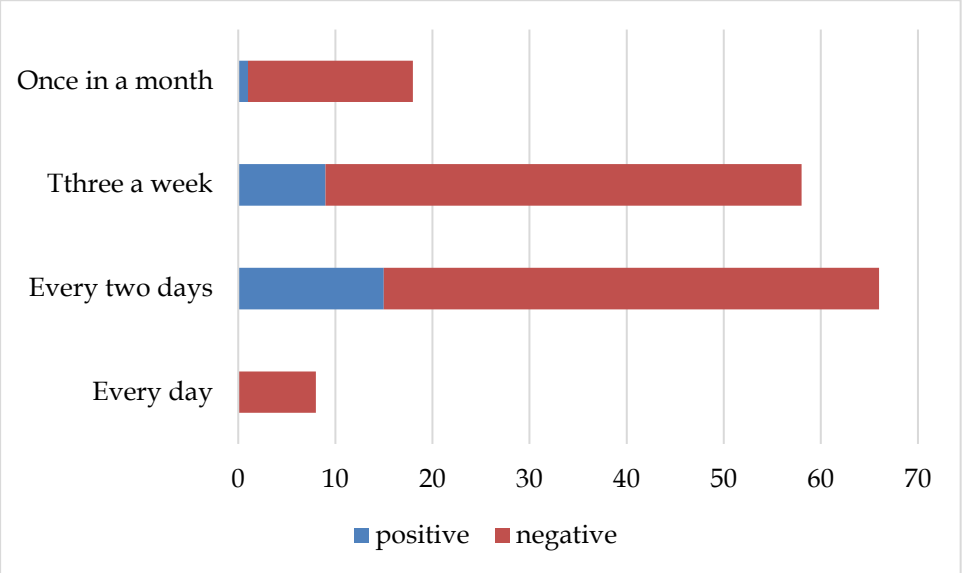

Figure 6. Distribution of *T. vaginalis* according to the frequency of sexual intercourse

T. vaginalis was detected in a total of 25 (16.6%) of 150 patients: 12 (8%) of them by DM, 16 (10.6%) of them by Giemsa staining, 10 (12%) of them by CPLM culture, and 18 (%) of them by TB culture (Table 1).

Table 1. Comparison of diagnostic methods

No	DM	Giemsa	CPLM	TB	Total
1	+	+	-	-	+
2	-	+	-	-	+
3	-	+	-	-	+
4	+	+	+	+	+
5	-	+	-	+	+
6	+	+	+	+	+
7	-	+	-	+	+
8	-	-	+	+	+
9	-	+	-	+	+
10	+	+	+	+	+
11	-	+	-	+	+
12	-	-	+	+	+
13	-	+	+	-	+
14	+	+	+	+	+
15	+	-	+	+	+
16	+	+	+	+	+
17	+	-	+	-	+
18	+	+	+	+	+
19	+	+	+	+	+
20	-	-	+	-	+
21	-	-	-	+	+
22	+	+	+	+	+
23	-	-	-	+	+
24	+	-	+	-	+
25	-	-	-	+	+
Total positivity	12 (8%)	16 (10.6%)	15 (10%)	18 (12%)	25 (16.6%)

DM: Direct Microscopy, CPLM: Cysteine-Pepton-Liver-Maltose, TB: Trichomonas Broth

Although no significant relationship was found between age groups (p=0.474), the highest positivity was found in the 36–50 (26.1%) and 26–35 (17.9%) age groups in accordance with active sexual age (Figure 2). The parasite was more common in illiterate women (26.5%) (p=.110) (Figure 3); in the group with a monthly income of 3000–6000 TL with 27.5% (p=.366); in people living in the city center with 20.9% (p=.296). 9.4% of water users for cleaning and 21.7% of toilet paper users were found to be infected (p=.086). Parasites were detected in 18.9% of people who had a bath while sitting (p=.163); 27.7% in those using white cotton underwear (p=.024) (Figure 4); 20% in those who changed their underwear once a week (p=.278); and 22.7% of patients who had sex once every two days (p=.219) (Figure 6).

It was observed that approximately half of the cases with parasites experienced pain in every intercourse (p=.001) (Table 2).

Table 2. *T. vaginalis* infection according to the frequency of pain in sex

Frequency of pain in sexual intercourse	Positive	Negative	N	*p*
In every sex	12 (%38,7)	19	31	0,001
Occasionally	12 (%17,1)	58	70	
In frequent sex	0 (%0)	9	9	
None	1 (%2,5)	39	40	
Total	25	125	150	

While 68% (17/25) of the infected patients were found to be asymptomatic, only 32% of them had clinical symptoms. 22.2% of the patients had discharge plus pruritus (p=.661). The vast majority of positive cases had aqueous (11 people) or purulent discharge (10 people); however, the highest parasite positivity was detected in women with egg white consistency discharge with 26.6% (p=.167) (Table 3).

Table 3. Relationship between vaginal discharge consistency and infection

The consistency of the discharge	Positive	Negative	N	*p*
Aqueous	11 (%20,3)	43	54	0,167
Purulent	10 (%14,9)	57	67	
Egg white	4 (%26,6)	11	15	
None	0 (%0)	14	14	
Total	25	125	150	

Different rates of positivity were detected according to the color of vaginal discharge; 57.1% in green, 19.2% in yellow, and 12.3% in white ones (p=.034).

The best sensitivity was detected in the TB culture method and the lowest in DM. The same high selectivity was detected in all of them. While false positivity was 0.00 in all methods, false negativity was found to be 0.28 in TB and 0.52 in DM. Here, it was understood that DM gave one false-negative result for 0.52 true negative results. Again, the highest total correct prediction was found to be in the TB culture method.

The results of the methods in the diagnosis of *T. vaginalis* infection are evaluated separately, and eight patients (32%) were detected by all methods: four patients (16%) by Giemsa+TB, three patients (12%) by only TB, two patients (8%) by only Giemsa, two patients (8%) by DB+CPLM, one patient (4%) by only CPLM, one patient (4%) by DB+Giemsa, one by Giemsa+CPLM (4%), and one (4%) by DM+CPLM+TB methods (Table 1).

DISCUSSION

T. vaginalis is one of the leading sexually transmitted diseases that survives in the urogenital system. The prevalence of the infection may vary according to the lifestyle, socio-cultural structure and age of the society, and the presence of clinical symptoms (WHO, 2008; Vos et al., 2012). The prevalence rate was found to be 50–70% among women working in the brothel (Çulha et al., 2006). It is thought that around 170 million people are infected each year worldwide, and 25 million of them are pregnant (Salakos et al., 2018).

The main diagnosis of the illness is based on appropriate sample and close examination of the parasite's trophozoite form under a microscope, even though clinical signs are useful in making the diagnosis. In the DM, a large number of microorganisms are needed for detection, and it may not be possible to see the parasite in chronic cases or in cases that the parasite is rare (Ertabaklar et al., 2004; Khatoon et al., 2015). By culture, accepted as the gold standard method, even a small number of microorganisms (about 1–10) can be detected in the sample. However, the disadvantages of the method are that it requires 2–7 days, and patients continue to spread the infection in the meantime because they are not treated (Kissinger, 2015; Van der Pol B, 2016). Nabweyembo et al. (2017) reported that the sensitivity of DM is very low (25%), on the contrary, Sönmez Tamer et al. (2008) determined the sensitivity and specificity of the CPLM method as 58% and 100%, respectively. In Sri Lanka, *T. vaginalis* positivity was found to be 2.8% by DM and 4.21% by the culture method (Banneheke et al., 2013). Cevahir et al. (2002) detected the parasite in 20 of 16310 patients with DM and in exactly twice as many patients (40 patients) by the culture method. In a study in which DM, Giemsa staining, and CPLM culture methods were used, the researchers detected 2.23% of the patients only with the CPLM culture method (Yazar et al., 2002). However, there are also studies in which all *T. vaginalis* positive cases can be detected by both DM and culture methods (Akarsu, 2006).

In this study, 12 samples were detected as positive by CPLM and 15 samples by TB culture methods. Although this difference may vary according to the people who applied the method and the characteristics of the commercially sold chemicals, it was determined that the parasite had better growth potential in the TB medium.

In women, the clinical presentation varies from asymptomatic to severe vaginitis. Symptoms such as vaginal and vulvar itching, redness, burning sensations, a small or large amount of foamy and foul-smelling discharge may be observed (Vos et al., 2012; Edwards et al., 2016). In addition, embarrassment, restlessness, insomnia, irritability, and weakness also disturb women. Mainly, the clinic and the severity of the disease are influenced by the host immune response, and hormone levels, vaginal flora, parasite count, and strain types are also important in this regard (Çetin, 2006; Kissinger, 2015).

Approximately 60% of positive cases in Alabama were found to be symptomatic (Schwebke and Burgess, 2004). This situation was 40% in Sri Lanka (Banneheke et al., 2013) and 50% in Iran (Ahady et al., 2016). In the clinic of infection, basic complaints such as discharge, odor, and itching are mainly encountered (Schwebke and Burgess, 2004; Doğan and Gitmez, 2019). However, Östan et al. (2005) detected *T. vaginalis* in only 4.7% of patients with vaginal discharge and itching complaints, and Akdemir et al. (2010) found it in only 8% of 237 cases with vaginal discharge. Apart from these findings, complaints of pelvic pain, vulvovaginitis, irritation, urethritis, and dysuria were found in fewer cases (Abdul et al., 2011; Houso et al., 2011; Doğan and Gitmez, 2019). As seen in general studies, approximately half of the cases of trichomoniasis can be asymptomatic. In this current study, 32% of the positive cases were found to be symptomatic, and complaints of discharge, itching, frequent urination, and burning in the urine were observed. In this infection, discharge is generally defined as an abundant, fluid, greenish, foamy, malodorous, and mucopurulent discharge. In addition, it has been reported that the incidence of the parasite is higher in purulent discharge (Kissinger, 2015; Edwards et al., 2016). In a study, a smell of discharge was reported in 5.3% of the positive cases (Doğan and Gitmez, 2019), and 16.2% in another one (Erbil et al., 2019). Çetinkaya et al. (2011) observed green-foul-smelling discharge in 18.18% of positive patients, and Younis and Elamami (2016) observed green-yellow discharge in 40.7% of patients. We observed that 57.1% of the patients with green discharge were infected, and 44% of the infected patients had a musty smell. Erbil et al. (2019) found that 18.2% of the patients who frequently experienced pain during sex were positive, while Doğan and Gitmez (2019) reported that this complaint was seen in only five of the 35 positive cases. In this study, it was observed that 38.7% of positive patients felt pain during each intercourse, and only 2.5% did not feel any pain.

T. vaginalis is seen more frequently at the ages of 20–45 when sexual life is more active, and its incidence decreases in young girls and in the periods after menopause (Walker; 2004; Akarsu, 2006). The highest positivity was observed in the 41–50 age group by Değerli et al. (2011) and in the 18–39 age group by Ambrozio et al. (2016) In a study conducted in Nigeria, the positivity rate was 17.28% in the 21–30 age group, 14.45% in the 31–40 age group, and 9.52% in the 41–50 age group, but parasites could not be detected in people aged 61 years or older (Abdul et al., 2011). In the studies carried out, the infection can be encountered in all age groups. In this study, the highest positivity was found in the 36–50 age group with 26.0%.

The educational status of the individuals has a significant effect on the infection. Çetinkaya et al. (2011) stated that 88.9% of positive cases were primary school graduates. Erbil et al. (2019) found 83 patients positive, and of these, 17.4% were illiterate, 13.2% were primary school graduates, 15 were high school graduates, and six were university graduates. Similarly, Ahady et al. (2016) found that 22% of infected people were illiterate and 46.6% were primary school graduates. On the other hand, Nourian et al. (2013) did not detect *T. vaginalis* infection among postgraduates. In this study, no relationship was found between education level and infection, and the parasite was found at a high rate in both the illiterate (26.5%) and the bachelor (21.4%).

The hygiene and toilet habits of the public affect the prevalence of infections. In Nigeria, 77.8% of positive cases were seen in those who made their toilet outdoors (Adeoye and Akande, 2007). Crucitti et al. (2011) detected 10% positivity in patients who used the common toilets. In this study, it was not possible to obtain a meaningful result for comparison since the majority of the patients used a Turkish-style toilet (133/150 patients). Also, in our study, a much higher level of infection was found in those who took a bath while sitting (18.9%). It is thought that the reason for this is that taking a bath while sitting increases the risk of contamination. In our study, the infection was detected more frequently in those who took a bath while sitting (18.9%). This may be due to the fact that sitting facilitates the transmission of the agent.

Since the primary route of transmission of the parasite is sexual intercourse, it is important to use protection methods to avoide infection. Even though it was stated that 48% of the cases with *T. vaginalis* did not use any protection method (Banneheke et al., 2013). Likewise, Çetin (2006) found that three out of nine positive cases did not use any contraceptive method. In another study, the highest positivity was detected with 16.2% in those who did not use any method, 12% in those using outside ejaculation, 11.6% in those using the IUDs, 9.2% in those using pills, and 8.6% in those using condoms (Erbil et al., 2019). However, it has been reported that no parasites are encountered in those who use oral contraceptives, needles, or tubal ligation methods (Çetin, 2006). In this study, the highest positivity was found in those using IUDs (29.6%) and condoms (26.3%). No agent was found in patients using oral contraceptives or needle methods. It is thought that the parasite is found more frequently because the IUD and condom damage the epithelial cells by causing local irritation, and thus *T. vaginalis* can settle more easily.

Conclusions

Trichomoniasis still appears to be an important public health problem. In this study it was determined that, approximately 2/3 of *T. vaginalis* infected patients were asymptomatic; it is more common in sexually active ages, illiterate people, IUD and condom users; the risk of positivity increased in those with green-colored discharge. The best sensitivity was found in the TB culture method; therefore, this culture method should be applied in addition to DM for a more accurate diagnosis.

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Conflict of interest: The authors have no conflicts of interest to report.

Ethical approval: Approval for the study was obtained from the Van Yüzüncü Yıl University Non-Invasive Clinical Research Ethics Committee (09.11.2018/08).

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