

## *Trichomoniasis* in pregnant women in South-East Iran: Diagnosis, frequency and factors affecting

### Güneydoğu İran'daki hamile kadınlarda *Trichomoniasis*: Tanı, sıklık ve etkileyen faktörler

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#### ABSTRACT

**Objective:** *Trichomonas vaginalis* is considered to be the most prevalent cause of non-viral sexually transmitted infections globally and is a major health issue in developing countries. Since this flagellate protozoan parasite is associated with vaginitis, urethritis, cervicitis, and also pregnancy is one of the factors affecting trichomoniasis in women. Our aim is to identify the prevalence of trichomoniasis and the associated risk factors in pregnant women in the age group of 17-40 in the city of Zahedan (Southeast of Iran).

**Methods:** A cross-sectional study was conducted on 514 pregnant women who had referred to a midwifery clinic in Zahedan. Direct microscopic examination and Dorset egg medium culture were used to detect *T. vaginalis* in pregnant women. The demographic and personal records, along with the associated risk factors were collected using a questionnaire and then analyzed using the Frequency to calculate the age average, chi-square test, independent t-test with SPSS software version 20.

**Results:** The results showed that in 514 pregnant women, by the direct method, both the direct and

#### ÖZET

**Amaç:** *Trichomonas vaginalis*, küresel olarak viral olmayan cinsel yolla bulaşan enfeksiyonların en yaygın nedeni olarak kabul edilir ve gelişmekte olan ülkelerde önemli bir sağlık sorunudur. Bu kamçılı protozoan parazit, vajinit, üretrit, servisit ile ilişkili olduğundan ve ayrıca gebelik, kadınlarda trikomoniyazı etkileyen faktörlerden biridir. Amacımız, Zahedan (İran'ın Güneydoğu) şehrinde 17-40 yaş grubundaki hamile kadınlarda trikomoniyaz prevalansını ve ilişkili risk faktörlerini belirlemektir.

**Yöntem:** Zahedan'da bir ebelik kliniğine başvuran 514 gebe kadın üzerinde kesitsel bir çalışma yapılmıştır. Direkt mikroskopik inceleme ve Dorset yumurta besiyeri kültürü, gebe kadınlarda *T. vaginalis*'i saptamak için kullanıldı. Demografik ve kişisel kayıtlar, ilişkili risk faktörleriyle birlikte bir anket kullanılarak toplandı ve daha sonra yaş ortalamasını hesaplamak için Frekans, ki-kare testi, SPSS yazılımı sürüm 20 ile bağımsız t-testi kullanılarak analiz edildi.

**Bulgular:** Sonuçlar 514 gebede, *T. vaginalis* için direkt yöntemle, hem doğrudan hem de kültür

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culture methods and only culture method, 29(5.64%), 24(4.67%), and 24(4.67%) were reported positive sample for *T. vaginalis*, respectively. According to this result, there were significant differences between trichomoniasis and history of STI (OR=12,6; 95%CI=3,9-40,6) and previous abortion (OR = 6.840, CI:2.906-16.100) and vaginal discharge (OR= 2.9; 95%CI= 1.2-7.1) and antenatal care (OR=0.2; 95%CI= 0.1-0.7) in the studied infected pregnant women ( $p<0.05$ ).

**Conclusion:** This study has raised several important points that could contribute to the research on infectious diseases of pregnancy were also discussed. This data proves that trichomoniasis during pregnancy should be considered an important health issue. Furthermore, by using parasitological diagnostic tests in pregnant women, the complications of trichomoniasis can be prevented for both the mother and the fetus.

**Key Words:** *Trichomonas vaginalis*, diagnosis, frequency, pregnant women, Zahedan, Iran

yöntemleriyle ve sadece kültür yöntemiyle sırasıyla 29 (%5,64), 24 (%4,67) ve 24 (%4,67) pozitif örnek rapor edildiğini gösterdi. Bu sonuçlara göre, çalışma kapsamındaki enfekte hamile kadınlarda trikomoniyaz ile CYBE öyküsü (OR = 12,6;% 95 CI = 3,9-40,6), önceki abortus (OR = 6.840, CI: 2.906-16.100), vajinal akıntı (OR = 2.9;% 95 CL = 1.2-7.1) ve doğum öncesi bakım (OR = 0.2;% 95 CL = 0.1-0.7) arasında anlamlı farklılıklar vardı ( $p<0.05$ ).

**Sonuç:** Bu çalışma, gebeliğin bulaşıcı hastalıkları ile ilgili araştırmaya katkı sağlayabilecek birkaç önemli noktayı gündeme getirmiştir. Bu veriler, hamilelik sırasında trikomoniyaz'ın önemli bir sağlık sorunu olarak kabul edilmesi gerektiğini kanıtlamaktadır. Ayrıca gebe kadınlarda parazitolojik tanı testleri kullanılarak hem anne hem de fetus için trikomoniyazın komplikasyonları önlenabilir.

**Anahtar Kelimeler:** *Trichomonas vaginalis*, tanı, sıklık, hamile kadınlar, Zahedan, İran

## INTRODUCTION

*Trichomoniasis* is a sexually transmitted disease caused by a flagellate protozoan parasite called *T. vaginalis* (1). Men are carriers of the disease and women are its reservoirs (1). This parasite is constantly transferred between sexual partners and thus is known as the Ping-Pong-Disease (2). This parasitic infection can lead to several conditions in women including vaginitis, cervix disorders, pelvic infections, premature rupture of membranes (PROM), low birth weight, respiratory problems and conjunctivitis in neonates, infertility, and preterm birth (3). In men, this parasite can cause inflammation in the urinary tract, cystitis, and prostatitis (3). *T. vaginalis* shows  $\beta$ -hemolytic characteristics, which can cause iron to be released from erythrocytes of the

menstrual blood upon their destruction (3). In men, the presence of zinc (Zn) in prostate secretions has a very effective protecting role against *T. vaginalis* (4). Using condoms is an effective method of prevention for trichomoniasis, and the sperm killer chemical nonoxynol-9 has destructive effects on the parasite (4). The most common complaint among patients infected with *T. vaginalis* is the presence of white or green vaginal discharge called leukorrhea (4). 70% of the infected patients are asymptomatic, while 10% display classic symptoms, and 20% non-classic (4).

Trichomoniasis is the most common non-viral sexually transmitted infection in the world (5). Age, sex, and poor economic and health conditions are among the most important factors contributing to the

prevalence of the infection (5). *T. vaginalis* survives on a wet sponge for a few hours, and in urine for up to 24 hours. The infection can be transferred non-sexually by using other people's bathing equipment and swimming in contaminated pools. It has been reported that *T. vaginalis* can spread from an infected mother to her infant daughter (6).

This parasite is visible in urinary precipitations and vaginal discharge of women, and in the urine and prostate discharges of men. It is noteworthy to mention that searching for the parasite in the urine sample should take place within 30 minutes (7). The most widely accepted method of detection is preparing a wet mount slide (58% sensitivity). In this fast and inexpensive method, a swab is taken from the vaginal epithelial discharge and gently coated with a warm drop of physiological serum or KOH on the lamellae, and then thoroughly analyzed. More recent molecular methods are also recommended in the case of negative results from direct testing and culture (5,8). Herein, this study aims to analyze the prevalence of trichomoniasis in pregnant women by using both direct microscopic examination and culture.

## MATERIAL and METHOD

### Area study and sample collection

This study covered the period of 2017-2019 in Zahedan city (Southeast of Iran). It was conducted on 514 pregnant women in the age range of 17-40 (with 24-36 week and more than 36 weeks pregnancy period) who had referred to a midwifery specialist and gynecologist in Zahedan. Demographic data and data associated with possible risk factors were collected. The sample size was estimated based on previous studies (9). With a prevalence of 0.3% was considered 696 pregnant women Because the study method was randomly multi-stage and available and it was not possible to obtain this volume of sample for this reason, the study was performed on 514 pregnant women. Demographic and personal records

of the patients along with the associated risk factors were collected by a questionnaire. Data collection was performed by trained midwifery experts in appropriate conditions to protect the privacy and confidential information of mothers. Furthermore, the informed consent of the participants was also obtained. The inclusion criteria for the study included a pregnancy of 24 to 36 weeks and above, and the exclusion criteria consisted of pregnancy with bloody vaginal discharge.

### Direct microscopy examination and culture for medical diagnosis

514 vaginal specimens were obtained from vaginal discharges of the fornix by 2 sterilized wet swabs during clinical examinations by midwifery specialists (2 swab samples were taken from each pregnant woman). Thereafter, each specimen was separately inserted in a 0.5 mL normal saline buffer and sent to a Diagnostic Parasitology Laboratory for detection. From each sample, a smear was prepared and analyzed under a light microscope by a medical parasitologist. Also, samples were cultured on Dorset egg medium. In this section, the steps for preparing the culture medium are explained.

Egg and distilled water with a ratio of 1/10 (the added water was equivalent to 0.1 of the egg's mass), streptomycin (2 drops), starch powder as the solid phase (0.5 grams), and Ringer's lactate or normal saline buffer as the liquid phase of the culture medium. The second swab of each sample was added to the liquid medium and incubated at 37 °C for 3 days. The liquid culture was evaluated to detect the growth of the parasite daily, and wet smears were used to search for trophozoite of *T. vaginalis* (10). In case the parasite was detected in direct examinations but the culture results were negative, two new samples were taken for direct and culture re-examination from the patient in their follow-up visit, and if the culture resulted negative for the presence of *T. vaginalis*, the sample would be considered negative. The most acceptable method of diagnosing *T. vaginalis* is to

prepare a wet smear with direct microscopic and search for a parasite in the culture medium, which is up to 90% reliable (5,8).

### Statistics analysis

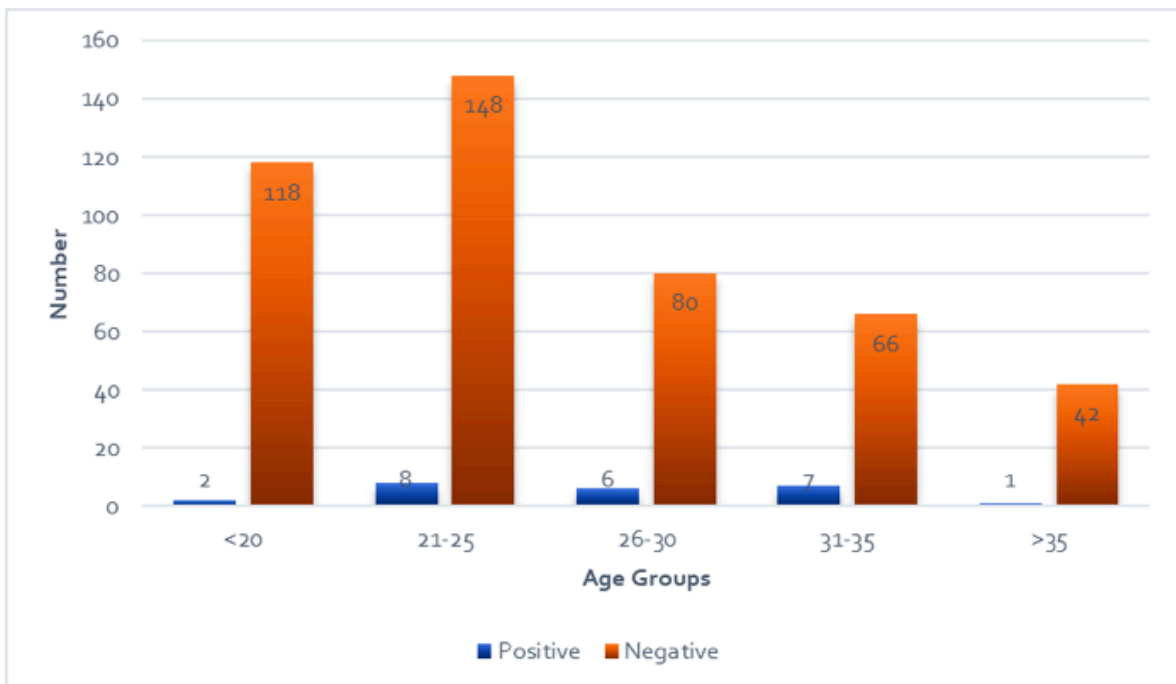
After conducting experiments and obtaining verbal questionnaires from each participant, the data were analyzed using Chi-square tests (linear-by-linear association) and for result test direct microscopy examination and culture *T. vaginalis* based different Age groups was calculated using Independent t-test and for quantitative data (mean age range) were used to describe the frequency (SPSS software version 20). In order to calculate (95% confidence interval) in age

and education levels, groupings were changed and divided into two distinct groups (11).

This study was approved by the Zahedan University of Medical Sciences Ethics Committee (Date: 12.07.2016 and Number: IR.ZAUMS.REC.1395.285).

### RESULTS

The participants of this study consisted of pregnant women in the age range of 15-42, Most of the patients were between 17 to 25 years old and the mean age of pregnant women was  $25.49 \pm 6.04$  years (Figure 1).



**Figure 1.** The results of direct microscopy examination and culture tests for *T. vaginalis* based on different age groups in pregnant women (P value= 0.20).

Table 1 shows the prevalence of trichomoniasis in terms of demographics, behaviors and clinical characteristics. 74.5% of the studied women were urban residents and 25.5% rural. 16% of these women were illiterate, while the remaining 84% had

different education levels. In addition, most of them were housewives (88.7%), while only 11.3% were employed. The results showed that in 514 pregnant women, by the direct method, both the direct and culture methods and only culture method, 29(5.64%),

24(4.67%), and 24(4.67%) were reported positive sample for *T. vaginalis*, respectively. Only 5 positive samples by direct method, were negative by culture method. The most important reasons for the negative culture of positive samples were the destruction of the parasites in samples, lack of growth of all parasites in culture medium and insufficient number of it for culture. Therefore, there was Medium or balanced agreement ( 90.56%) between the results of direct microscopic examination and culture test (kappa index was 0.42).

In table 1 and figure 2 The relationship between trichomoniasis and demographics, behaviors and clinical characteristics in pregnant women were reported. According to this result, there were significant differences between trichomoniasis and history of STI (OR=12.6; 95%CI= 3.9-40.6) and previous abortion (OR=6.8; 95%CI=2.9-16.1) and Vaginal discharge (OR= 2.9; 95%CI= 1.2-7.1) and Antenatal care (OR=0.2; 95%CI= 0.1-0.7) in the studied infected pregnant women ( $p<0.05$ ).

**Table 1.** The relationship between trichomoniasis and demographics, behaviors and clinical characteristics in pregnant women of Zahedan (Southeast of Iran)

Characteristics	Total cases N (%)	Cases with trichomonas N (%)	OR (95% CI)	P value
Residential Place				
Urban	383 (74.6%)	17 (4.4%)	0.82 (0.33-2.03)	0.672
Rural	131 (25.4%)	7 (5.3%)		
Education				
Under Diploma	383(74.5%)	16(9.8%)	0.67 (0.28-1.60)	0.367
Upper Diploma	131(25.5%)	8(6.5%)		
Occupation				
Housewife	456(88.8%)	22(4.9%)	1.41 (0.32-6.19)	0.640
Employed	58(11.2%)	2(3.5%)		
Illicit drug abuse				
Yes	11(2.1%)	0(0.0%)	1.02 (1.00-1.03)	0.459
No	503(97.9%)	24(4.8%)		
Previous STIs				
Yes	15(2.9%)	5(33.3%)	12.63 (3.93-40.59)	<0.001
No	499(97.1%)	19(3.8%)		
Previous abortion				
Yes	111(21.6%)	15(13.5%)	6.84 (2.90-16.10)	<0.001
No	403(78.4%)	9(2.2%)		
Vaginal discharge				
Yes	79(15.3%)	8(10.1%)	2.95 (1.21-7.15)	0.013
No	435(84.7%)	16(3.7%)		
Antenatal care				
Yes	488(94.9%)	20(4.1%)	0.23 (0.1 -0.74)	0.008
No	26(5.1%)	4(15.4%)		
Gestational age				
24-36 week	82(15.9%)	6(7.3%)	1.81 (0.69-4.72)	0.216
More than 36 weeks	432(84.1%)	18(4.2%)		

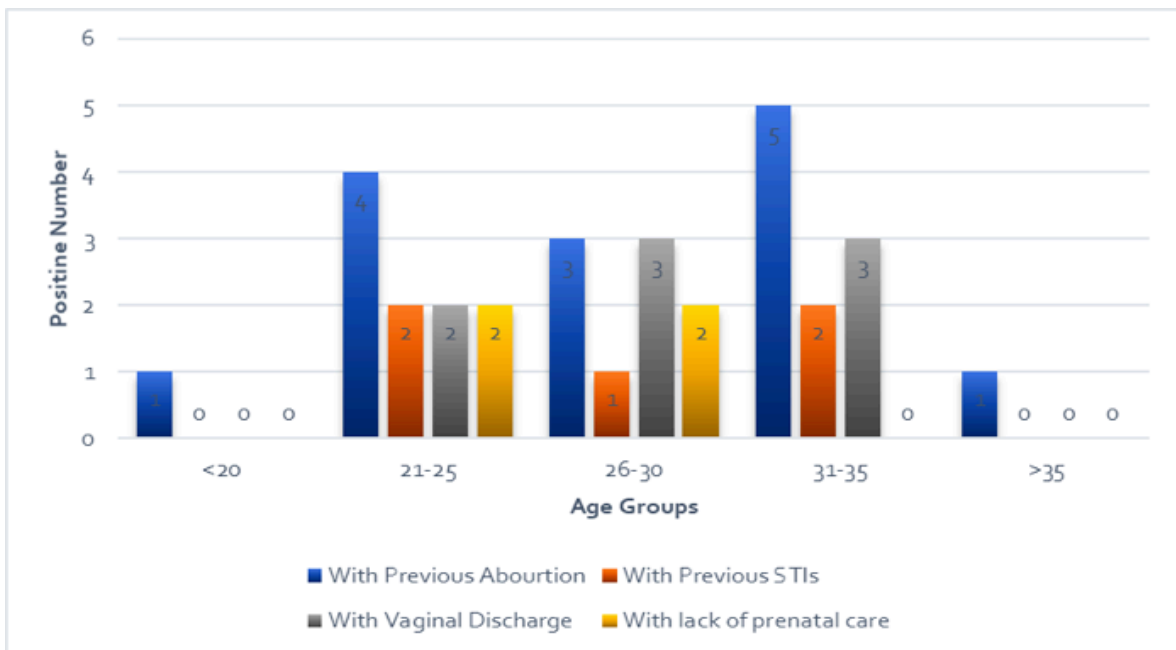


Figure 2. Number of positive individuals by various factors and Age groups

## DISCUSSION and CONCLUSION

This study set out to diagnosis, frequency and factors affecting on Trichomoniasis in pregnant women in South-East Iran. To investigate this, direct microscopic examination and Dorset egg medium culture were used to detect *T. vaginalis* in pregnant women. In the following, the demographic and personal records, along with the associated risk factors were collected using a questionnaire and then analyzed using the frequency to calculate the age average, Chi-square test, Independent t-test and SPSS software version 20. Analysis of data revealed that in pregnant women there were associated elements with trichomoniasis to be a history of STI and previous abortion. The current findings add substantially to our understanding of relationship and effect of trichomoniasis in pregnancy from epidemiological and clinical aspects on pregnant mothers.

The studies at hand show that the prevalence of trichomoniasis in pregnant women in different continents of the world is as follows: ranged from 3.9-24.6% in Latin America and Southern Africa (12), less than 10% in Europe (13) and between 0.4 % to 45%, especially Middle East, in Asia (14-16). The results of the current paper demonstrated that 4.6% of pregnant women in Zahedan were infected with *T. vaginalis*. The infection rates reported in this study were lower in comparison with the several studies in Nigeria and Brazil (17,18). Furthermore, in this study, the history of vaginal discharge, STI and previous abortions were the associated variables with increasing trichomoniasis in the studied pregnant women, however, there was no significant correlation between *T. vaginalis* infection and other variables. In addition, there was no significant correlation between trichomoniasis in pregnant women and the independent variable of age groups. Based on the fact that the prevalence of trichomoniasis in women in

the age groups of 16-35, according to the conducted research, is due to increased sexual activities, only 4.02% of the studied pregnant women who were below 30 were infected with *T. vaginalis*. These results matched the study carried out on pregnant women by Uneke et al in 2010 (19), which reported the highest prevalence of trichomoniasis to be in the age group of 26-30, while the study of Alo et al in 2012 was reported 36% of the prevalence in the age group of 31-35 (19,20).

The infection rates of urban pregnant women were 2.4 times higher than those living in rural places. Considering the factors affecting the prevalence of trichomoniasis, including economic, cultural, and health conditions, further investigation is needed on the Residential Place variable with the aforementioned associated variables. The prevalence of trichomoniasis infection in this study was twice as high in pregnant women without a high school diploma than those with higher education levels, suggesting that education and awareness of women regarding personal and sexual health and STD prevention methods can reduce their prevalence and complications. No significant correlation was found between occupation and trichomoniasis infection. Uncertainty in the results of this study could be due to the low levels of employed participants.

In study conducted by Alo et al in 2012 showed that 10% of the pregnant women in their second trimester and 5.5% in their third trimester were infected with trichomoniasis, the infection was not observed in the first trimester of pregnancy (20). In the current study, women in their third trimester of pregnancy were available and tested. Since untreated trichomoniasis infections last for at least three months, and during this period can lead to reproductive health consequences, timely screening can prevent negative consequences of pregnancy (21-23). Therefore, screening tests for *T. vaginalis* are recommended for women in their second or early third trimester of pregnancy. In the present study,

33.3% of pregnant women had a history of previous STIs, and a higher prevalence of trichomoniasis in women with STIs was reported in some regions (24). Furthermore, 13.5% of the infected women had a history of a previous abortion (24). This significant correlation proves that these elements should be considered as risk factors to assess the prevalence more accurately and screen the patients with greater sensitivity in women with such histories. According to some comparative studies in pregnant women with and without trichomoniasis, infected women show a 30% risk of low weight birth (25). Also, the role of trichomoniasis in causing dangerous complications during pregnancy for mothers and fetus has been proven in various studies, for example: Abortion, Uterine rupture, etc (28-31). The current data show that discussing routine screening for trichomoniasis during pregnancy and before labor can prevent the associated complications.

As for the survey on examine the subject in more detail, there were two main limitations. First, although maximum attempt was made to use a questionnaire with items that were clear and relevant, further access to clinical information for pregnant women was not possible. Second, many pregnant mothers do not participate in the study due to lack of sufficient knowledge about the subject. There is clearly much room for further research in this respect. For example, simultaneous effect of sexually transmitted infections such as Chlamydia trachomatis and *T. vaginalis* on the severity of clinical symptoms in pregnant women and their fetuses.

In conclusion, this study has raised several important points that could contribute to the research on infectious diseases of pregnancy were also discussed. In this paper, we presented a study in which we investigated the prevalence of trichomoniasis in pregnant women (514 specimens of vaginal discharges). Pregnant women with trichomoniasis were studied based on terms of clinical symptoms (a history of vaginal discharge,

STI and previous abortion). Our finding suggests that 24/514 (4.6%) were infected with *T. vaginalis* by two methods and A statistically significant difference between a history of STI and a previous miscarriage and prevalence of diseases in pregnant women was found. So, this data proves that trichomoniasis

during pregnancy should be considered an important health issue. Furthermore, by using a parasitological screening test in pregnancy, the complications of trichomoniasis can be prevented for both the mother and the fetus.

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### ETHICS COMMITTEE APPROVAL

\* The study was approved by the Zahedan University of Medical Sciences Ethics Committee (Date: : 12.07.2016 and Number: IR.ZAUMS.REC.1395.285).

### CONFLICT OF INTEREST

The authors declare no conflict of interest.



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