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Detection of Three Sexually Transmitted Infections and Associated Risk Factors among Symptomatic Women using Multiplex-Real Time PCR

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Abstract

Sexually transmitted infections (STIs) are posing a challenge to public health, globally. The aim of this study was to determine the frequency of three STIs in married women, clinical characteristics, and their associated risk factors. A cross-sectional study was performed among married women who attended the Gynaecological Hospital in Zakho City, Iraq from October 2021 to April 2022. A total of 150 high vaginal swabs were collected from each subject aged between 18 and 48 years (32.64 years ±8.01 SD). DNA was extracted from swabs to determine STIs using Multiplex Real-Time PCR. The frequency of STIs was 26 out of 150 (17.33%). *Chlamydia trachomatis* accounted for 17 (11.33%), *Trichomonas vaginalis* 7 (4.66%), and *Neisseria gonorrhoeae* 2 (1.33%). The highest rates of *C. trachomatis*, *T. vaginalis*, and *N. gonorrhoeae* were reported among young age groups. The level of education (p=0.043) and type of contraceptives (p=0.01) were identified as potential risk factors for *T. vaginalis*. The number of births (p=0.001) and using contraceptives (p=0.02) were reported as major risk factors for *N. gonorrhoeae*. Abnormal discharges (p=0.004), vaginal itching (p=0.03), painful intercourse (p=0.04), genital ulcers (p=0.03), and bleeding in the urine (p=0.001) were significantly associated with infections. The frequency of STIs was low in symptomatic patients but high among the reproductive age group. Education level, contraceptive usage, and number of births were identified as potential risk factors. Early detection of these factors is crucial for improving women's health.

Keywords: STIs, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, Multiplex-Real Time PCR, symptomatic women

Резюме

Инфекциите, предавани по полов път (ИППП), представляват предизвикателство за общественото здраве в световен мащаб. Целта на това проучване е да се определи честотата на три полово предавани инфекции при омъжени жени, клиничните характеристики и свързаните с тях рискови фактори. Проведено е кръстосано проучване сред омъжени жени, които са посещавали гинекологичната болница в град Захо, Ирак, от октомври 2021 г. до април 2022 г. Общо 150 висококачествени вагинални тампона са събрани от всяка участничка на възраст между 18 и 48 години (32.64 години ±8,01 SD). От тампоните е извлечена ДНК за определяне на полово предавани инфекции с помощта на Multiplex Real-Time PCR. Честотата на ППИ е 26 от 150 (17.33%). *C. trachomatis* съставляваше 17 (11.33%), *Trichomonas vaginalis* - 7 (4.66%), а *Neisseria gonorrhoeae* - 2 (1.33%). Най-високите нива на *C. trachomatis, T. vaginalis* и *N. gonorrhoeae* са отчетени сред младите възрастови групи. Нивото на образование (p=0.043) и видът на контрацептивите (p=0.01) са идентифицирани като потенциални рискови фактори за *T. vaginalis*. Броят на ражданията (p=0.001) и използването на контрацептиви (p=0.02) са отчетени като основни рискови фактори за *N. gonorrhoeae*. Необичайни изпускания (p=0.004), вагинален сърбеж (p=0.03), болезнен полов акт (p=0.04), генитални язви (p=0.03) и кървене в урината (p=0.001) също са свързани значително с инфекциите. Честотата на полово предаваните инфекции е ниска при пациентите със симптоми,

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но висока сред хората в репродуктивна възраст. Като потенциални рискови фактори са определени нивото на образование, употребата на контрацептиви и броят на ражданията. Ранното откриване на тези фактори е от решаващо значение за подобряване на здравето на жените.

Introduction

Sexually transmitted infections (STIs) continue to be a serious public health issue and cause high morbidity rates worldwide, particularly among women of reproductive age (Mehrabani *et al*., 2014). The most prevalent STIs caused by bacteria and viruses are Human Papilloma Virus and *Chlamydia trachomatis, Neisseria gonorrhoeae,* and *Trichomonas vaginalis* (Kim *et al*., 2015; Daniel *et al*., 2022). According to the World Health Organization (WHO), in 2020, there were 374 million new infections from all regions around the world: 156 million were *T. vaginalis*, 129 million were *C. trachomatis,* and 82 million were *N. gonorrhoeae*. The high prevalence of *C. trachomatis* and *N. gonorrhoeae*, including high rates of asymptomatic infections (50-80% of men and women), and the potential for serious consequences are crucial criteria that support frequent testing for these bacteria (Luppi *et al*., 2011). Untreated genital infections among women may lead to pelvic inflammatory disease (PID), chronic pelvic pain, ovarian tube abscess, perihepatitis infertility, ectopic pregnancy, abortion, and cervical cancer (Hasani *et al.*, 2021). Pregnancy complications can be passed from infected mothers to their fetuses as known as congenital infections (Adachi *et al*., 2016).

The frequent distribution of STIs among women, and the public health costs associated with the difficult diagnosis of such infections have led to the development of rapid, sensitive, and reliable diagnostic methods for the detection of STIs (Greer and Wendel, 2008). It has been previously reported that the most accurate diagnostics for STI detection are nucleic acid amplification tests (NAATs). Compared to previously known diagnostic techniques including culture, antigen detection, and nucleic acid hybridization), NAATs are around 20–30 times more sensitive (Battle *et al*., 2001). In recent times, the multiplex PCR test has become a convenient method for clinicians in various medical fields to detect multiple causative agents simultaneously. The advantage of using the multiplex PCR test is that it is an affordable diagnostic test that enables quicker detection and lowers labor and reagent expenses (Samra *et al*., 2011; Lee *et al*., 2012).

Very limited STI surveillance reports among women are published in the Kurdistan Region of Iraq, especially in Zakho City (Naqid *et al*., 2019; Naqid *et al*., 2020; Haydar and Naqid, 2022; Haydar and Naqid, 2023). Early and accurate detection of STIs is crucial for effective treatment and prevention of further transmission. Multiplex-real-time PCR assays have emerged as a reliable and cost-effective diagnostic tool for the simultaneous detection of multiple pathogens. The use of multiplex PCR for the detection of STIs has not been evaluated thoroughly in our region. In this study, we aimed to investigate the prevalence of three common STIs - chlamydia, gonorrhea, and trichomoniasis among symptomatic women using a multiplex-real-time PCR assay. Additionally, we examined the clinical characteristics and associated risk factors for these infections. The findings of this study could contribute to the development of targeted prevention and intervention strategies for reducing the burden of STIs among women.

Materials and Methods

Design of study

This cross-sectional study was performed among married women attending the Obstetrics and Gynaecological Hospital at Zakho City, Kurdistan Region-Iraq from October 2021 to April 2022. During this period, a total of 150 subjects with symptoms of vaginitis and cervicitis were collected and the age of participants ranged from 18-48 years with an average rate of 32.64 years (±8.01 SD).

Data collection setting

From each participant, data was collected using a designed structured questionnaire including sociodemographic, and clinical characteristics of participants (Haydar and Naqid, 2022). Demographic characteristics such as age, educational levels, residency, smoking, contraceptive use, recurrent infection, and past medical history, while the clinical data includes symptoms with the presence of vaginal discharges, color of discharges, and pH of the vagina. The married women presented with vaginal discharge, vaginal itching, painful intercourse, or painful urination were considered symptomatic patients.

After physical and gynecological examination, high vaginal swabs (HVS) were immediately collected from each subject using insertion of a sterile unlubricated speculum into the vagina. The swabs were obtained from the endocervix and the posterior vaginal fornix and then directly collected into transport media (Citoswab, China) for DNA extraction according to the manufacturer's instruc-

tions. Married women who were >18 years old and agreed to participate were included in the present study.

DNA extraction and Multiplex Real-Time PCR

A Ribo-Sorb (Sacace ™ Biotechnologies, Italy) nucleic acid extraction kit was used to isolate and purify RNA/DNA from vaginal swabs. Briefly, swabs were vortexed for 15 seconds speed followed by extraction of RNA/DNA from 100 µL of transport medium (TM-RT), and all samples were subjected to extraction with a single elution buffer volume of 50 µL.

The qualitative detection of *T. vaginalis, N. gonorrhoeae*, and *C. trachomatis* is performed using a Multiplex real-time PCR kit (Sacace™ Biotechnologies, Italy) following the manufacturer's instructions. The PCR amplification reaction was performed in a total volume of 25 µL, each reaction contains 10 µL DNA sample and 15µL RT-PCR master mix reagents (PCR-mix-1-FRT and 2-FRT, and TaqF DNA Polymerase). The amplification program used for real-time PCR following the manufacturer's instructions is presented in (Table 1). The Rotor Gene-q Series Software (Real-Time PCR) program was used for the analysis of data.

Ethics

The protocol and procedure of this study were approved by the ethical committee of the Shekhan Technical College of Health at Duhok Polytechnic University's Scientific Committee, Iraq (Reference number: 15092021-9-4). All participants were voluntary and informed consent was obtained from each subject before sampling

Analysis of Real-time PCR data analysis

PCR amplifications were performed using multiplex real-time PCR according to the fluorescence channel summarized in Table 2. The result was an interpretation as the following: *T. vaginalis* DNA was detected if its Ct value was detected in the FAM/Green channel. *N. gonorrhoeae* DNA was detected if its Ct value was detected in the JOE/Yellow channel. *C. trachomatis* DNA was detected if its Ct value was detected in the ROX/Orange channel. For all Pathogens, the fluorescence curve should cross the threshold line (0.1) in the region of exponential fluorescence growth in Fig. 1.

Statistic

The GraphPad Prism version 8 (San Diego, California, US) was used to evaluate the significant

Table 1. Real-time PCR amplification setup creates a temperature profile on Rotor-Gene q

Steps		Rotor-Gene Qiagen Real-time PCR		
		Temperature (°C)	Time (minute/second)	Cycles repeats
Hold	Initial denaturation	95	15 min	1
	Denaturation	95	5 s	
Cycling 1	Annealing	60	20 s	5
	Extension	72	15 s	
	Denaturation	95	5 s	
Cycling 2	Annealing	60	20 s (Fluorescence detection)	40
	Extension	72	15 s	

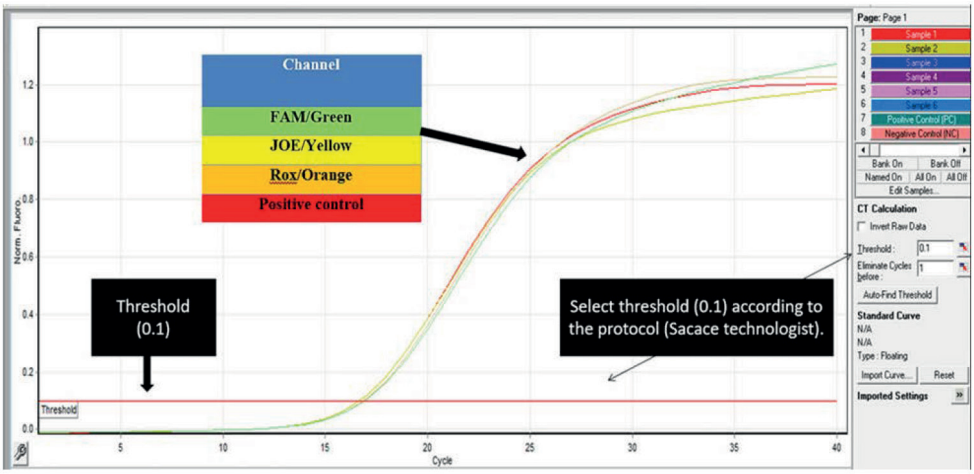

Fig. 1. Fluorescence curve with a cross threshold

differences between variables. Descriptive information was expressed as frequency and percentages. The chi-square test was used to analyze the relationship between *T. vaginalis, N. gonorrhoeae*, and *C. trachomatis* and associated risk factors according to demographic and clinical characteristics. A significant level of p-value <0.05 was considered statistically significant.

Table 2. Infections and internal control DNA product in fluorescence detected channel

DNA amplification product	Fluorescence detected
T. vaginalis DNA	Green: FAM fluorescence channel
N. gonorrhoeae DNA	Yellow: JOE fluorescence channel
C. trachomatis DNA	Orange: ROX fluorescence channel
Internal Control DNA	Cy5 channel

Results

Prevalence of vaginal infections and co-infections using multiplex real-time PCR

Multiplex real-time PCR was used in the present study to diagnose STIs such as *C. trachomatis*, *T. vaginalis*, and *N. gonorrhoeae*. Overall, among 150 subjects, 26 (17.33%) were positive for STIs **(**Table 3**).** Out of 26 positive samples, the rate of *C. trachomatis* was 17 (11.33%), followed by *T. vaginalis* 7 (4.66%) and *N. gonorrhoeae* 2 (1.33%).

Table 3. Prevalence of vaginal infections and mixed infections among married women using Multiplex Real-Time PCR (n=150)

Vaginal infections	Number of positive	Percent
C. trichomatis	17	11.33
T. vaginalis	7	4.66
N. gonorrheae	2	1.33

Risk factors associated with vaginal infections

The risk factors associated with vaginal infections are presented in (Table 3). We found that all studied variables were not a major risk factor for *C. trichomatis* infections (p>0.005) (Table 3). In terms of *T. vaginalis* infection, educational levels (p<0.046) and different types of contraceptives (p=0.01) were significant risk factors for infection (Table 4). Other risk factors including age, residence, contraceptive use, number of births, and history of infection were no significant differences (p>0.005) (Table 3). Regarding *N. gonorrhoeae*, the number of births (p=0.001) and using different types of contraceptives (p=0.02) were reported as significant risk factors, and other studied risk factors were not significant (Table 4).

Association between vaginal infections and the clinical characteristics of participants

Among positive subjects, abnormal discharges (p=0.004), vaginal itching (p=0.03), painful intercourse (p=0.04), and bleeding in the urine (p=0.001) were significantly associated with *C. trachomatis* (Table 5). The most commonly reported symptoms were bleeding in the urine 11 (39.29%), abnormal discharge 14 (19.18%), painful intercourse 16 (14.95%), and painful urination 7 (14.29%) (Table 5). Regarding *T. vaginalis*, abnormal discharges (p=0.006) and genital ulcers (p=0.03) were positively associated with infection. The most frequently reported symptoms were genital ulcers 5 (10.42%), abnormal discharge 7 (9.59%), painful urination 3 (6.12%), and painful intercourse 6 (5.61%) (Table 5). All the studied clinical symptoms were not significantly associated with *N. gonorrhoeae*, but the most commonly reported symptoms were painful urination 2 (4.08%), bleeding in the urine 1 (3.57%), and abnormal discharge 2 (2.74%).

Relationship between infections and type of vaginal discharge

It was found that the most frequent abnormal color discharge of infected women with *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis* was yellow-green (Table 6). We found that yellow-green discharges were significantly associated with *C. trachomatis* (p=0.001), *N. gonorrhoeae* (p=0.036), and *T. vaginalis* (p=0.001) (Table 4). The most frequent yellow-green discharge of *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis* were 14 (48.28%), 2 (6.90%), and 7 (24.14%), respectively (Table 6). The vaginal pH levels of participants had no significant influence on the rate of infections (Table 6).

Discussion

We measured the prevalence of three sexually transmitted infections (STIs) in 150 symptomatic women at a gynecological hospital in Zakho City, Iraq. This is the first study in our region to concurrently investigate *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis* using multiplex Real-Time PCR. We explored correlations with demographic and clinical characteristics, identifying associated risk factors. The study is crucial for developing targeted prevention and intervention strategies to reduce the STI burden among women in our community.

Our findings showed a low prevalence rate of

Table 4. Risk factors associated with *C. trachomatis, T. vaginalis*, and *N. gonorrhoeae* among married women using RTPCR (n=150)

Variable	*C. trichomatis n (%)*		*p-value*	*T. vaginalis n (%)*		*p-value*	*N. gonorrhoeae n (%)*		*p-value*
	Negative	Positive		Negative	Positive		Negative	Positive	
Age (Year)									
<20	12 (100)	0 (0)	0.06	12 (100)	0 (0)	0.33	12 (100)	0 (0)	0.24
21–30	42 (85.7)	7 (14.3)		45 (91.8)	4 (8.2)		47 (95.9)	2 (4.1)	
31-40	50 (83.3)	10 (16.7)		57 (95)	3 (5)		60 (100)	0 (0)	
41-48	29 (100)	0 (0)		29 (100)	0 (0)		29 (100)	0 (0)	
Level of education									
Illiterate	59 (92.2)	5 (7.8)	0.66	63 (98.4)	1 (1.6)	0.043	64 (100)	0(0)	0.45
Secondary	35 (85.37)	6 (14.63)		40 (97.6)	1 (2.4)		40 (97.6)	1 (2.4)	
High school	23 (85.19)	4 (14.81)		23 (83.2)	4 (14.8)		26 (96.3)	1 (3.7)	
Higher education	16 (88.89)	2 (11.11)		17 (94.4)	1 (5.6)		18 (100)	0 (0)	
Residence									
Urban	92 (88.5)	12 (11.5)	0.98	136 (95.8)	6 (4.2)	0.12	103 (99.1)	1 (0.9)	0.52
Rural	41 (89.1)	5 (10.9)		141 (99.3)	1 (0.7)		45 (97.8)	1 (2.2)	
Number of Birth									
0	21 (87.5)	3 (12.5)	0.91	22 (91.7)	2 (8.3)	0.57	23 (95.8)	1 (4.2)	0.001
1-4	78 (89.66)	9 (10.34)		83 (95.4)	4 (4.6)		86(98.8)	1 (1.2)	
> 4	34 (87.18)	5 (12.82)		38 (97.4)	1 (2.6)		39 (100)	0 (0)	
Contraceptive Use									
Yes	71 (88.8)	9 (11.3)	0.92	74 (92.5)	6 (7.5)	0.12	78 (97.5)	2 (2.5)	0.49
No	62 (88.6)	8 (11.4)		69 (98.7)	1 (14.3)		70 (100)	0 (0)	
Contraceptive Types									
Intrauterine Device	7 (77.8)	2 (22.2)	0.81	9 (100)	0 (0)	0.01	8 (88.9)	1 (11.1)	0.02
Pills	20 (90.9)	2 (9.1)		18 (81.8)	4 (18.2)		22 (100)	0 (0)	
Condom	15 (93.8)	1 (6.3)		16 (100)	0 (0)		15 (93.7)	1 (6.3)	
Natural	29 (87.9)	4 (12.1)		31 (93.9)	2 (6.1)		33 (100)	0 (0)	
Nonusers	62 (88.6)	8 (11.4)		69 (98.6)	1 (1.4)		70 (0)	0 (0)	
History of infection									
Yes	34 (91.9)	3 (8.1)	0.56	36 (97.3)	1 (2.7)	0.91	37 (100)	0 (0)	0.94
No	99 (87.6)	14 (12.4)		107 (94.7)	6 (5.3)		111 (98.2)	2 (1.8)	

three infectious agents with an overall STI rate of 17.33%. Specifically, *C. trachomatis* accounted for 11.33%, *T. vaginalis* for 4.66%, and *N. gonorrhoeae* for 1.33% using RT-PCR. A study in Iraq reported higher infection rates among women with genitourinary tract infections, with *N. gonorrhoea* at 30% and *C. trachomatis* at 22%, using RTPCR (Ali and Shia, 2018). Another recent study in Tehran, Iran focussed on symptomatic women with genitourinary infection and pregnancy-related complications, finding prevalence rates of 11.1%, 13.3%, and 7.2% for *C. trachomatis*, *T. vaginalis, and N. gonorrhoeae*, respectively (Rajabpour *et al*., 2020). In this study, the total prevalence of *C. trachomatis* infections determined by RTPPCR was 11.33%. The prevalence of *C. trachomatis* varies across different studies, with molecular detection in women with genital tract infections reported at 22% in Iraq (Ali and Shia, 2018), 20.2% in Palestine (El Qouqa *et al*., 2009), 11.1-13.8% in Iran (Rashidi *et al*., 2009), 5.2% in Italy (Marcone *et al.*, 2012), 13.5% in India (Dhawan *et al*., 2014), 10.4% in the United Arab Emirates (Mehrabani *et al*., 2014), and 12.7% in Turkey (Tosun et al., 2008). Our results disagree with a previous study conducted in the kingdom which reported an overall prevalence of 19.25 % for *C. trachomatis* infection using Multiplex RT PCR (Ashshi *et al.*, 2015). The differences in infection rates between our study and others could be partially attributed to variations in the study pop-

Table 5. Relationship between *C. trachomatis*, *T. vaginalis*, and *N. gonorrhoeae* and clinical characteristics among married women (n=150)

Variable	*C. trichomatis no. (%)*		*p-value*	*T. vaginalis n (%)*		*p-value*	*N. gonorrehae n (%)*		*p-value*
	Negative	Positive		Negative	Positive		Negative	Positive	
Abnormal discharge									
Yes	59 (80.8)	14 (19.2)	0.004	66 (90.4)	7 (9.6)	0.006	71 (97.3)	2 (2.7)	0.23
No	74 (96.10)	3 (3.90)		77 (100)	0 (0)		77 (100)	0 (0)	
Painful urination									
Yes	42 (85.7)	7 (14.3)	0.42	46 (93.9)	3 (6.1)	0.68	47 (95.9)	2 (4.1)	0.11
No	91 (90.10)	10 (9.90)		97 (96.1)	4 (3.9)		101 (100)	0 (0)	
Vaginal Itching									
Yes	61 (95.3)	3 (4.7)	0.03	62 (96.9)	2 (3.1)	0.69	64 (100)	0 (0)	0.51
No	72 (83.7)	14 (16.3)		81 (94.2)	5 (5.8)		84 (97.7)	2 (2.3)	
Lower abdomen pain									
Yes	73 (91.3)	7 (8.7)	0.31	77 (96.3)	3 (3.8)	0.71	80 (100)	0 (0)	0.22
No	60 (85.7)	10 (14.3)		66 (94.3)	4 (5.7)		68 (97.1)	2 (2.9)	
Genital ulcer									
Yes	44 (91.7)	4 (8.3)	0.58	43 (89.6)	5 (10.4)	0.03	47 (97.9)	1 (2.1)	0.54
No	89 (87.3)	13 (12.7)		100 (98.1)	2 (1.9)		101 (99.02)	1 (0.98)	
Painful intercourse									
Yes	91 (85.1)	16 (14.9)	0.04	101 (94.4)	6 (5.6)	0.67	106 (99.1)	1 (0.9)	0.49
No	42 (97.7)	1 (2.3)		42 (97.7)	1 (2.3)		42 (97.7)	1 (2.3)	
Bleeding in urine									
Yes	17 (60.7)	11 (39.3)	0.001	28 (100)	0(0)	0.34	27 (96.4)	1 (3.6)	0.35
No	116 (95.1)	6 (4.9)		115 (94.3)	7 (5.7)		121 (99.2)	1 (0.8)	

ulation size, duration, diagnostic methods, sample collection procedure, culture, and geographical distribution. The prevalence of *T. vaginalis* is influenced by various risk factors, such as age, residence, socioeconomic status, education, marital status, contraception method, and drug use (Ton Nu *et al*., 2015). In the present study, *T. vaginalis* prevalence was 4.66 % among married women of reproductive age aligning with findings in Erbil City, Iraq, where rates ranged from 2.73% to 3.2% using different diagnostic techniques (Avreen and Hadi, 2015). Conversely, studies in Turkey (15.37%) (Yazar *et al*., 2021), and Saudi Arabia (28.1%) (Madani, 2006) reported higher rates. This difference between our study and others could be partially due to differences in population size, methods of diagnosis, sample collection, and geographical distribution. Variations in infection rates between studies may also be related to sample size, study duration, socioeconomic level of the community, and the study settings. Rules and habits prohibiting non-marital sexual intercourse; may contribute to the lower prevalence of this protozoan infection in Islamic nations compared to permissive non-Islamic ones.

In our study, the majority of *T. vaginalis* infections occurred in the childbearing age range (20–40 years) but no statistically significant difference was observed among different age groups (p=0.33). Our results are in agreement with studies in Basra and Kirkuk, indicating the highest infection rate in childbearing age (Mahdi *et al*., 2001; Kadir *et al*., 2014). The higher *T. vaginalis* infection rate in younger age groups may be attributed to increased sexual activity and a lack of adherence to safe sex practices (Ambrozio *et al*., 2016). The high level of sexual activity during the childbearing age could result in increased reproductive hormones declining with age progression (Herath *et al*., 2021). Various factors influence infection rates in different age groups, including vaginal pH, estrogen and progesterone hormones secretion for maintaining vaginal pH during the childbearing years, abortion and pregnancy frequency, and the body's immunodeficiency after the menstrual cycle (Deka *et al*., 2021).

Table 6. Relationship between infections, vaginal discharges, and pH level

Variable	*C. trichomatis no. (%)*		p-value	T. vaginalis n (%)		*p-value*	N.gonorrhoeae n (%)		*p-value*
	Negative	Positive		Negative	Positive		Negative	Positive	
Yellow-Green									
Yes	15 (51.7)	14 (48.3)	0.001	22 (75.9)	7 (24.1)	0.001	27 (93.1)	2 (6.9)	0.036
No	118 (97.5)	3 (2.5)		121 (100)	0 (0)		121 (100)	0 (0)	
Whitish									
Yes	57 (95)	3 (5)	0.06	60 (0)	0 (0)	0.99	60 (100)	0 (0)	0.99
No	90 (100)	0 (0)		90 (100)	0 (0)		90 (100)	0 (0)	
Vaginal pH level									
<3.8	86 (90.5)	9 (9.5)	0.52	92 (96.8)	3 (3.2)	0.26	94 (98.9)	1 (1.1)	0.98
>4.5	47 (85.4)	8 (14.6)		51 (92.7)	4 (7.3)		54 (98.2)	1 (1.8)	

According to sociodemographic factors, lower education levels and strong sexual desire have been associated with a higher prevalence of *T. vaginalis* (Bassey *et al*., 2022). However, our study found that higher education levels tended to confer protection against *T. vaginalis*. The infection rates were nearly equal among women with primary education (2.44%), higher education (5.56%), and illiterate individuals (1.56%), compared to a higher rate (14.81%) in high school-educated women. This finding is in agreement with a study conducted in Basra, which reported infection rates of (10.6%), (12.2%) and (18.6%) among low, moderate, and high education levels, respectively (Mahdi *et al*., 2001). Our study also identified a correlation between general education, academic performance, and parasite infection, consistent with previous findings that lower education is associated with higher infection rates (Nourian *et al*., 2013). However, some studies suggest a reversible association between educational level and *T. vaginalis* infection is reversible (Malla *et al*., 2008). This variation could be attributed to the absence of health and preventative measures, living conditions, clinic visits, and partner infection among different studies. While our study showed a lower *T. vaginalis* infection rate in rural areas (2.17%) compared to urban areas (5.77%), this difference was not statistically significant (p=0.12). This contrasts with studies in Iraq and Iran reporting higher infection rates in rural areas (Ghobahi *et al*., 2019; Al-Ardi, 2021). Discrepancies in results may be attributed to variations in sample size, urban lifestyles, international travel, multiple sexual partners, behavior, and economic and environmental factors in developing countries.

Abnormal vaginal discharge, a prevalent clinical symptom in our study, showed statistical significance (p=0.006). A high percentage of cases exhibited abnormal discharges, with yellow-green discharge significantly associated with *T. vaginalis* (p=0.001). Our results are in accordance with a study, reporting a high prevalence rate (81.57%) of yellow to green discharge (Al-Mamoori *et al*., 2020). The elevated occurrence of yellow or yellow-green discharge may be linked to intense vaginal infection and a substantial number of trophozoites, observed during long-term patient evaluations. In cases with minimal parasite presence, vaginal discharge appeared normal; however, the color shifted to yellowish and greenish with increased *T. vaginalis* numbers.

In the present study, a multiplex real-time PCR assay identified *C. trachomatis* in women aged 21–30 years and 31–40 years, with a prevalence rate of 14.29% and 16.67%, respectively. However, no significant association was found between different age groups (p=0.06). This result is in agreement with studies in Africa, reporting a 10.3% *Chlamydia* infection rate among women aged between 15-24 years (Torrone *et al.*, 2018). In India, another study with an average participant age of 29.7 years found a 73% rate in the 15–34 age group (Sood *et al*., 2012). The age distribution in our study, especially the highest positivity in the 31–40 age group, differs from most studies, possibly due to a later onset of sexual activity in our population. A correlation exists between the age of sexual activity onset and *C. trachomatis* infection (Mawak *et al*., 2011). Considering the high risk in young women, the CDC recommends routine screening for all sexually active women under 25 due to behavioural characteristics and increased exposure to cervical columnar epithelium, enhancing the organism's ability to infect new cells.

In our study, education level did show statistical significance (p=0.66). This aligns with other

studies, where education level was not a significant factor in *C. trachomatis* infection (Casillas-Vega *et al.*, 2017). The accessibility of comprehensive knowledge on STD prevention through information technologies and the internet might contribute to this trend (Hu *et al*., 2021). The most prevalent clinical symptom among women in our study was abnormal vaginal discharge, with a significant prevalence of 19.18% (p=0.004). This is consistent with another study, which reported 91.75% of patients in India presenting with vaginal discharges (Sood *et al*., 2012). Additionally, another study found excessive vaginal discharge in 49.6% of C. trachomatis-infected women (Nessa *et al*., 2004) and another reported a 33.3% positivity for C. trachomatis among women complaining of vaginal discharge (Mohan and Borthakur, 2015).

In conclusion, the prevalence of STIs among married women in this study was lower in symptomatic patients compared to other studies elsewhere that used Multiplex PCR. High infection rates were reported among the reproductive age group, with factors such as educational levels, contraceptive methods, and the number of childbirths identified as potential risk determinants for STIs. Positive associations were found between STI infections and symptoms such as abnormal vaginal discharges, vaginal itching, painful intercourse, bleeding in the urine, and genital ulcers. Early detection of risk factors leading to STI infections is crucial for improving women's health. Further studies are necessary to assess the adverse reproductive outcomes associated with STIs in the Kurdistan region of Iraq.

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