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Prevalence and Diagnosis of sexually Transmitted Pathogens in A Sample of Iraqi Women: A Molecular Study

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Abstract

Background Sexually transmitted infections (STI), also referred to as sexually transmitted diseases (STD) and venereal diseases (VD), are infections that are commonly spread by sex, especially vaginal intercourse, anal sex and oral sex. Most STIs initially do not cause symptoms. This results in a greater risk of passing the disease on to others. Symptoms and signs of disease may include: vaginal discharge, penile discharge, ulcers on or around the genitals, and pelvic pain.

Objective To detect the two microorganisms (*Gardnerella vaginitis (G. vaginalis)* and *Trichomonas vaginalis (T. vaginalis)*) in the same sample taken from women with genital tract infection by microbiological and molecular methods and to investigate the contributions of some socioeconomic factors and clinical features.

Methods Two hundred samples were collected from females attending the Gynecology out-patient department in the Al-Imamein Al-Kadhimein Medical City and Baghdad Teaching Hospital during the period from May 2014 to April 2015. Based on availability of full clinical information about each patient, high vaginal swabs were taken from females at different ages (15-54 years) representing patients group complaining of abnormal vaginal discharge with or without other symptoms, questionnaire was applied. The two diseases associated with vaginal infection include *G. vaginalis and T. vaginalis.* Each of the vaginal swabs collected was examined microscopically, whilst the remaining was preserved at -20 °C for DNA extracts were analyzed with the real-time poly meres chain reaction (RT-PCR).

Results In RT-PCR, the rate of infection was 120 (60%) *G. vaginalis*, and 34 (17%) *T. vaginalis*. Highest rate of infection in women with *G. vaginalis* was among age group (15-24) years and (25-34) years 38.3%, 35.0% respectively, the lowest rate was among age group (45-54) years 8.3 %. In *T. vaginalis*, the highest rate of infection was among age group (15-24) years 61.7 %, the lowest was among age group (35-44) years 8.8 % and on infection in age 45-54years.

Conclusion The commonest genital tract infections among women were *G. vaginalis and T. vaginalis.* Molecular methods are considered the gold standard for diagnosis, given the excellent sensitivities and specificities in diagnosis. Presence of clinical symptoms helps and lab diagnosis of infection. Vaginal swab samples showed that most common co-infection is between *G. vaginalis* cases and *T. vaginalis*.

Keywords Sexually transmitted infections, *Gardnerella vaginalis*, *Trichomonas vaginalis*, molecular study

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List of abbreviations: *G. vaginalis* = *Gardnerella vaginalis*, PCR = Polymerase chain reaction, STPs = Sexually transmitted pathogens, *T. vaginalis* = *Trichmonas vaginalis*, VEC = Vaginal epithelial cell

Introduction

The normal vaginal ecosystem is a complex micro environment with important interrelationships among endogenous microflora and their metabolic products, estrogen status and pH [(1)]. Lactobacilli maintain the normal vaginal pH (3.8-4.2) by producing lactic acid, stabilizing the vaginal ecosystem and hydrogen peroxide, suppressing the growth of gram-negative and gram-positive facultative and obligate anaerobes. Vaginitis is the inflammation and infection of vagina commonly encountered in clinical medicine. Diverse spectrums of

pathogenic agents were observed in the vaginal micro flora. Of these, *G. vaginalis* and *T. vaginalis* are responsible for majority of vaginal infections in women of reproductive age [1]. Abnormal vaginal discharge, itching, burning sensation, irritation and discomfort are frequent complaints among patients attending obstetrics and gynecology clinics. However, a number of vaginal infections present with few or no symptoms [2].

Vaginal infections are associated with a significant risk of morbidity in women. If untreated they can lead to pelvic inflammatory disease (PID), which can cause long-term sequelae, such as tubal infertility, ectopic pregnancy, reproductive dysfunction. Cervical dysplasia, increased risk of postoperative infection

G. vaginalis is the most common vaginal infection of reproductive age women and is the most frequently cited cause of vaginal discharge and malodor. Vaginal discharge constitutes a considerable problem for many women causing discomfort, anxiety affecting women. Some vaginal discharges are normal and can vary with age, use of contraceptives and menstrual cycle [3].

The protozoa *T. vaginalis* is a sexually transmitted parasite causing vulvovaginitis characterized by intense frothy yellow-greenish vaginal discharges, irritation and pain in the vulva and dysuria [4,5].

Recent studies have shown that polymerase chain reaction (PCR) increases the rate of *G. vaginalis* and *T. vaginalis* detection in mucocutaneous swabs. Although earlier PCR techniques were laborious, expensive and prone to contamination, newly developed real time PCR assays are fully automated [6]. This is a valuable feature for the use of PCR in routine clinical practice.

The objectives of this study were (1) to study the prevalence of two pathogens (*G. vaginalis* and *T. vaginalis*) in women with lived partner age (15-54) years by microbiological and molecular methods and to investigate the contributions of some socioeconomic factors and clinical features, (2) to study the association between *G. vaginalis* and *T. vaginalis* by real time-PCR.

Methods

Vaginal swabs collection

High vaginal swabs were collected from two hundred samples, women patients (symptomatic and asymptomatic) within reproductive age who attending the Gynecology Outpatient Department in the Al-Imamein Al-Kadhimein Medical City and Baghdad Teaching Hospital during the period from May 2014 to April 2015. Based on availability of full clinical information about each patient, high vaginal swabs were taken from females at different ages from 15-54 years. representing patients group complaining of abnormal vaginal discharge with or without other symptoms.

This research under went to the terms of ethical considerations and in accordance with the form prepared for this purpose by the Committee of Ethical Standards in the Collage of Medicine / University of Al-Nahrain.

Full information history was taken directly from the patient and information was arranged in an informative clearly detailed formula sheet.

Isolation of microorganisms

A-*Macroscopic examination*:

vaginal swabs were observed in terms of consistency, color, odor, viscosity and presence of blood and mucus and testing for pH.

B-*Microscopic examination*:

Microscopic examination of the vaginal discharge wet smears, were conducted immediately after collection of specimens at the respective clinics. All further processing of specimens was done in the laboratory.

1-Wet-mount preparation:

A wet-mount preparation is obtained by diluting the vaginal discharge with one or two drops of normal saline solution and placing it on a slide with a cover slip. Slide is examined microscopically using low power and high power of several fields for motile trichomonads. Microscopic examination of wet-mount preparation also detects "Clue cell"

which are vaginal epithelial cells that are coated with the coccobacilli.

2-Gram stain:

Vaginal smears were prepared by transferring vaginal secretions to glass slides. The slides were air-dried, heat-fixed and Gram-stained. Smears were examined under oil immersion (XlOO objective) and quantitated for the presence of "clue" cells, Gram-variable bacilli, *G. vaginalis*-like organisms.

3-Vaginal WBCs counts:

Vaginal WBCs were quantified after visualization of a minimum of fine fields (range 5-15 based on whether there was a paucity of WBCs present) under light microscopy at X 400. Vaginal WBCs count were routinely categorized into either ≤ WBCs in all visualized fields (representing minimal or no inflammation) or ≥ 5 WBCs in at least one field visualized (considered elevated and more suggestive of significant inflammation).

4-Culture:

Specimen was culture immediately on the laboratory media by rolling the cotton swab on one side of the plate then streaked by standard streaking method. The inoculated plates were incubated as follows:

1-One blood ager plate, MacConkeys ager plate, these were incubated aerobically at 37 °C (24-48 hr) and anaerobically by using anaerobic Jar at 37 °C for (24-48 hr).

2-Modified protease peptone ager plate, this medium was shown to increase the recovery of vaginal bacteria, the plate inoculated sealed and anaerobically at 37 °C for (6-7) days.

5-Biochemical test: Oxidase test, catalase test.

6-Molecular study.

(Sacace ™ Biotecnologies) is a single iplex RT-PCR kit Reagents and contents of kit, for the direct and qualitative detection of *G. vaginalis* and *T. vaginalis*.

The principle, procedure, of (DNA extraction, real-time PCR, nano drop and agarose electrophoresis) preparation sample, and interpretation of the results were as same as those in *G. vaginalis* and *T. vaginalis* determination method.

Statistical analysis

The Statistical Analysis System- SAS, program was used to effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage in this study.

Results

Demographic characteristics of population:

Of the 200 women examined, the age of the study population ranged from 15-54 years, Women aged 15-24 years had significantly higher prevalence of infection 67 (33.5%) than other age classes (Table 1). The lowest percentage of women aged participating were in the age group 45-54 year 10 (5%) statistically significant association between age of subject and occurrence of vaginal infection.

The study revealed that women with primary education have the highest number and percentage 63 (31.5%), while low percentage of infection was seen in higher educational level 17 (8.5%). Based on socioeconomic status, the moderate socioeconomic status had the highest rate 77 (38.5%) followed by low status 62 (31%).

In rural residence the infection rate 66 (33%) was significantly higher than infection rate in urban 90 (45%).

In parity, the infection percentage was increased in multipara status had the highest rate 85 (42.5%) followed by unipara status 49 (24.5%), and finally the nullipara status 22 (11%).

Table 1. Variable factors of sexually transmitted infections according to patients with questionnaire in the present study

Variables		No. of test cases	Positive cases No (%)	X^2	P value
Age group (year)	G1: 15-24	85/200	67 (33.5%)	9.725	0.0001**
	G2: 25-34	60/200	53 (26.5%)		
	G3: 35-44	30/200	26 (13.0%)		
	G4: 45-54	25/200	10 (5.0%)		
Education levels	Illiterate	54/200	44 (220%)	8.136	0.0026**
	Primary	72/200	63 (31.5%)		
	Secondary	42/200	32 (16.0%)		
	Higher	32/200	17 (8.5%)		
Socioeconomic classes	Low	75/200	62 (31.0%)	9.022	0.0008**
	Moderate	90/200	77 (38.5%)		
	High	35/200	17 (8.5%)		
Location	Urban	115/200	90 (4.5%)	4.317	0.0474*
	Rural	85/200	66 (33.0%)		
Parity	Nullipara	40/200	22 (11.0%)	8.166	0.0064**
	Unipara	65/200	49 (24.5%)		
	Multipara	95/200	85 (42.5%)		

* (P<0.05), ** (P<0.01)

Clinical Features of sexually transmitted disease:

Presence of clinical symptoms helps in the diagnosis of infection. The symptoms experienced by 156/200 infected women participating in this study are summarized in table (2).

Women with malodor signs represent the highest percent of cases 47/200 (23.5%). Followed by profuse discharge and vulvar itching 45/200 (22.5%), 26/200 (13%) respectively while joint pain, dysuria and cervical abnormalities were represented signs 20/200 (7.5%), 15/200(7%), 12/200 (4.5%) respectively.

In this study, all vaginal swab samples were examined in the laboratory by microscopic examination (Wet mount preparation and Gram's stain) for detection of (clue cell, *T. vaginalis* trophozoites), 95/200 (47.5%) samples found to be positive for *G. vaginalis* by Gram stain and 25/200 (12.5%) were found to be positive *T. vaginalis* mount preparation in wet as shown in table (3), figures (1), (2) and (3).

This study showed that *G. vaginalis* was isolated from (50%) of infection cases using culture method.

The sexually transmitted pathogens identified from patient group by RT- PCR

The PCR amplification were performed using RT-PCR. The detection of appropriate channels was used as follow channel (FAM) for detection *G. vaginalis* and *T. vaginalis*, channel (CY3) for internal control of DNA (ICD). Used for no evidence of inhibition of the amplification in any of the samples, with the internal control of the RT-PCR samples as shown in table (4), Figures (4) and (5) respectively. In Table (4) show that from 200 samples, only 154/200 samples were diagnosis to be infected by RT-PCR. These assays detected 120 (60%) cases of G. *vaginalis*, 34 (17%) cases of *T. vaginalis*.

Table 2. Frequency of clinical aspects in patient with sexually transmitted in disease

Symptoms and signs	No. of cases	No. of effected cases	(200) %	(156) %	χ^2	P- value
Vulvar itching	45/200	26/45	13	16.66	10.435	0.0001**
Malodor	55/200	47/55	23.5	30.12		
Profuse discharge	53/200	45/53	22.5	28.84		
Joint pain	20/200	15/20	7.5	9.61		
Dysuria	15/200	14/15	7	8.97		
Cervical abnormalities	12/200	9/12	4.5	5.76		

Table 3. Detection of sexually transmitted pathogen (STP) in patients with vaginal infection by microscopic examination

Sexually transmitted pathogen	Positive cases	Infection rate %
G. vaginalis	95/200	47.5
T. vaginalis	25/200	12.5
Total	120	60

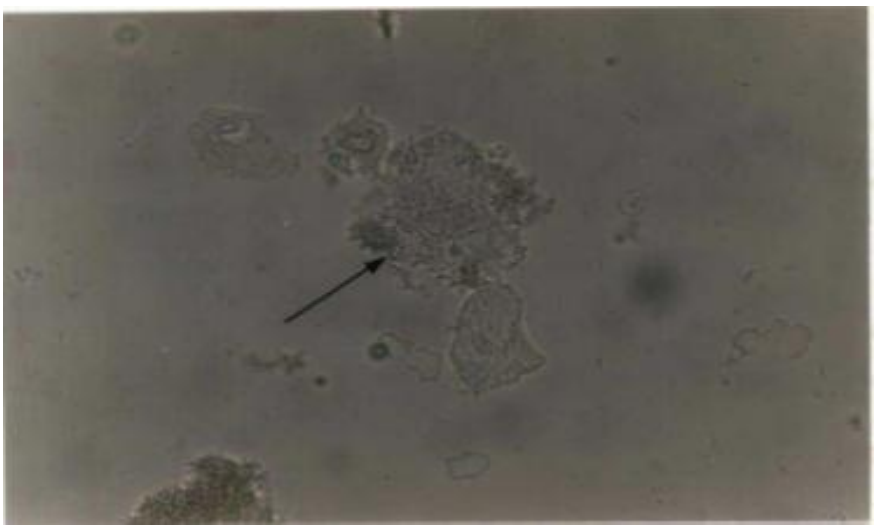

Figure 1. Clue cells as seen on vaginal wet smear microscopic preparation (40X objective)

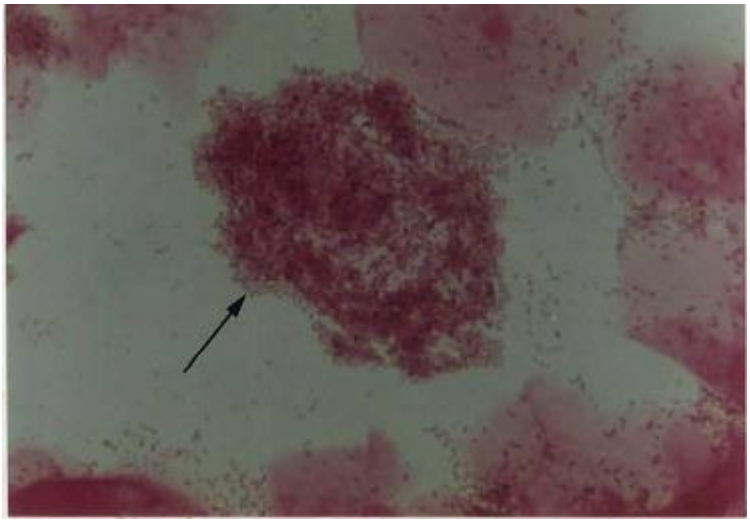

Figure 2. Clue cells as seen on A-vaginal wet smear microscopic preparation B- Gram stained smear with the presence of *G. vaginalis*

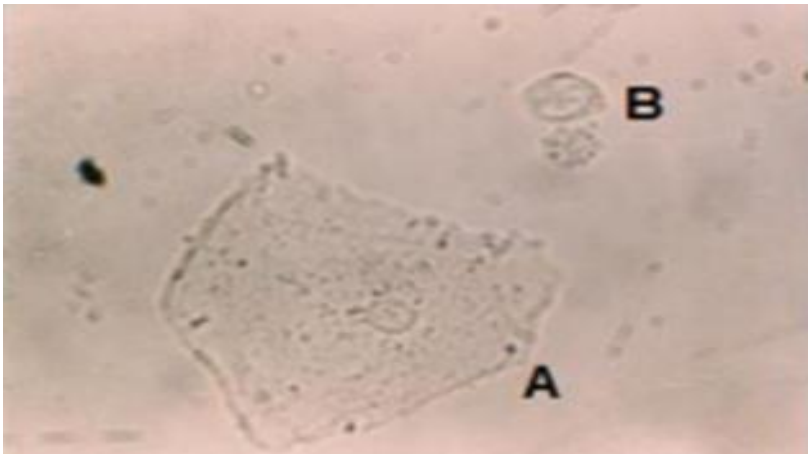

Figure 3. Wet mount preparation under light microscope 40X. A: epithelial cell B: two shapes of *T. vaginalis*

Table 4. The sexually transmitted infection identified from patient group according to type of pathogens by RT- PCR

Sexually transmitted pathogen	Multiplex RT- PCR	Infection rate %
G. vaginalis	120	60
T. vaginalis	34	17
Total	154	77

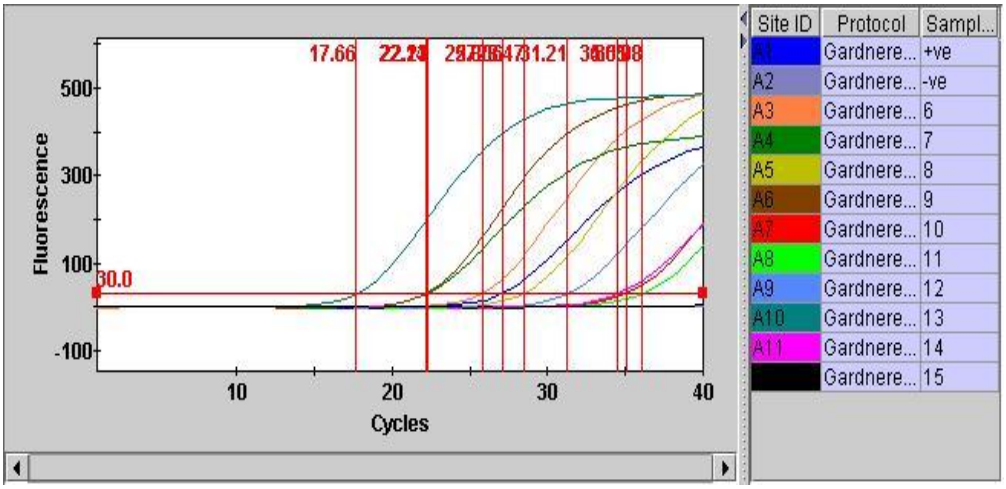

Figure 4. *G. vaginalis* RT-PCR results; positive sample (blue), negative sample (green), each one with internal control

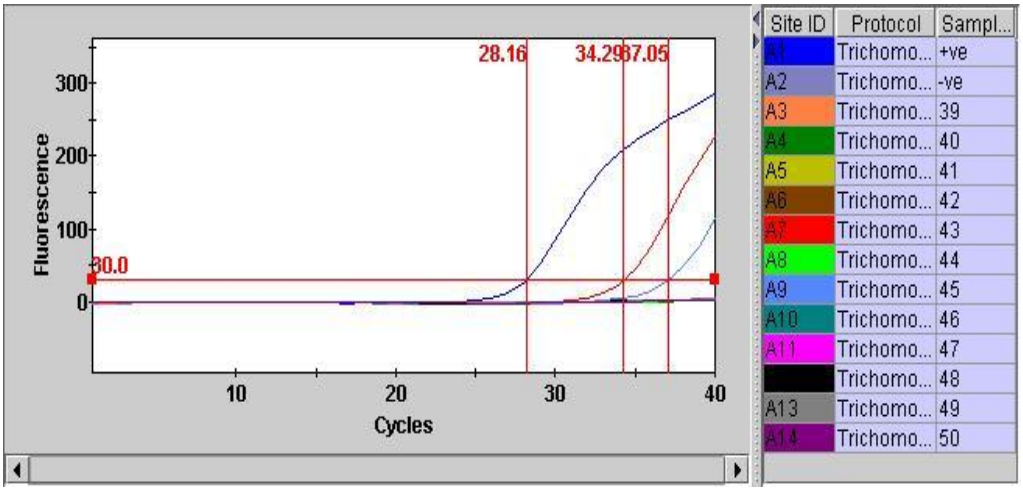

Figure 5. *T. vaginalis* RT-PCR results; positive sample (blue), negative sample (green), each one with internal control

Types of vaginal infection in relation to age group

The age groups that were subjected to this study ranged from 15-54 years. Table (5) showed highly significant relationship between age and *G. vaginalis* and *T. vaginalis* infection.

Highest rate of infection in women with *G. vaginalis* was among age group (15-24) years and (25-34) years 38.3%,35.0% respectively, the lowest rate was among age group (45-54) years 8.3 %.

In *T. vaginalis*, the highest rate of infection was among age group (15-24) years 61.7%, the lowest was among age group (35-44) years 8.8 % and on infection in age 45-54years.

Mix vaginal infection (co-vaginal infection)

Diagnosis of 120 *G. vaginalis* infections in vaginal swab samples and 34 cases for *T. vaginalis* by multiplex RT-PCR showed that Most common co-infection is between *G. vaginalis* cases and *T. vaginalis* were 30/156 (19.23%) cases of co-infection, in the present

study as is illustrated in Table (6). Statistically, there were highly significant differences among co-infection cases.

Table 5. Types of genital tract infection in relation to age groups

Sexually transmitted pathogen		Age groups / Year 15-24	25-34	35-44	45-54	Total	P value
G. vaginalis	No.	46	42	22	10	120	0.0001 **
	%	38.3	35.0	18.3	8.3	100	
T. vaginalis	No.	21	10	3	-	34	0.0001 **
	%	61.7	29.4	8.8	-	100	
Total	No.	67	52	25	10	154	
	%	43.5	33.8	16.23	6.5	100	

Table 6. Mixed of vaginal infection

Mixed infection	Positive cases	% from total	Negative cases	Total
G. vaginalis* + *T. vaginalis	30	19.48	124	154
Age groups	15-24	25-34	35-44	30
Positive cases	14	13	3	
Color of discharge	Yellow or green	White	Gray-White	30
Positive cases	13	17	-	
Consistency of discharge	Flocculent	Thin homogenous	Crude	30
Positive cases	-	30	-	

Discussion

Of the 200 women examined, the age of the study population ranged from 15-54 years, Women aged 15-24 years had significantly higher prevalence of infection 67 (33.5%) than other age classes. The lowest percentage of women aged participating were in the age group 45-54 year 10 (5%); there was statistically significant association between age of subject and occurrence of vaginal infection. This result agreement with Hassan et al. (2005) in Basrah [8], who showed that the highest percentage of infection occurred (47.2%) at the same age group and disagreement with other studies like Bahram et al. (2009) in Iran [9]. In Vietnam it was 8.7% (4/64) [10], while in Turkey, 10% (2/20) menopausal vaginal infection patients was recorded [11].

Women in the age groups (15-24), (25-34), (35-45) years had the highest prevalence of infection compared with older age category in a study of women according to the pH level, vaginal discharges were varied with age, use of contraceptives, menstrual cycle and with the estrogen level, douching used. Sexually transmitted pathogens (STPs) regard as the most serious causative agent for vaginal infection. However, while vaginal infection is

possible increased prevalent in reproductive ages due to biological factors such as age, hormonal changes, cervical ectopic [12-14].

The study revealed that women with primary education have the highest number and percentage 63 (87.50%), while low percentage of infection was seen in higher educational level 32 (31.5%). Based on family income, moderate socioeconomic status had the highest rate 77 (38.5%) followed by low status 62 (31%). In Iraq, previous studies in Baghdad and Al-Najaf showed that the uneducated women (illiterates) were more associated with the disease than other age classes [15,16]. The low infection rate was shown in women who have high educational level, and this agree with number of demographic studies, which showed that higher educational level was the lowest level associated with the disease [17].

In rural residence, the infection rate 66 (77.65%) was significantly higher than infection rate in urban 90 (87.26%), this is in agreement with Al-Quraishi in Babylon [18], (probably because of the difference between the city and countryside in lifestyle and vaginal infection mainly affecting people living in poor or disadvantaged communities [18,19]. In parity, the infection percent increase in multipara status had the highest rate 85 (89.47%) followed by unipara status 49 (75.38%), and finally the nullipara status 22 (55%). It appeared that infection with is strongly correlated with reactivation of some agents, abortion, through vaginal delivery and vaginal hygiene practices (such as douching) and type of contraceptive this result agreement with [20].

In the current study, the majority of symptoms were malodor and profuse discharge than the vulvar itching, other symptoms occur with less frequency.

Symptoms alone are not sufficient to make reliable diagnosis of sexually transmitted pathogen. Symptoms in women with malodor signs represents 47 (85.45%). Profuse discharge, vulvar itching, joint pain, dysuria and cervical abnormalities were represented 84.91% (45/53), 57.78% (26/45), 75% (15/20), 93.33% (14/15), 75% (9/12) respectively. It is known that vulvovaginitis is characterized by discharge and strong odor in particular which have bacterial vaginosis while *T. vaginalis* is associated with dysuria and pain.

The pathogenesis of infection leading to the most aforementioned symptoms is somehow obvious; however, the relationship between this infection and symptoms is not so clear. It is believed that the adhesion of STPs to vaginal epithelial cells (VECs) plays an important role in the pathogenesis STPs. Han et al. showed that the inflammatory mediators made by VECs in response to *T. vaginalis* activate and attract masts cells and neutrophils, thus, joint pain, especially in knees and lower back, can be used as indicator for *T. vaginalis* [21-23].

Presence of cervical abnormalities in infected patients agree with the study of Donders et al. 2013 that *T. vaginalis* infection is also associated with the cervical cytological abnormalities [24] and depicted as a risk factor for cervical dysplasia and cancer [25].

In this study, most women who tested positive for the evaluated disorders reported some clinical signs, being discharge the most prevalent one. However, this sign was statistically associated with any infection, unlike what was reported by Gama [5]. No association between the occurrences of strong odor and the three infections was found, as well as no positive correlation for dysuria with vaginitis, corroborating other studies.

The disparity in the prevalence of STPs could be attributed to many reasons; environmental and socio-economic factors, accurate take of sample form patient by gynecologist, diagnostic method, number of tested samples, type of samples and cultural factors.

Diagnosis of *G. vaginalis* were done by demonstration of clue cell by wet mount, gram stain and culture.

The infection rate of *G. vaginalis* wet preparation technique and Gram's stain 95(47.5%), Gram's stain along with culture methods help to demonstrated the causative agent of bacterial vaginosis, this result agrees

with reports done by Amsel et al (1983) [26] whom they reviewed that the prevalence of clue cells on wet mount preparation of *G. vaginalis* discharge and demonstration that there was a strong inverse relationship between the bacterial vaginosis and *G. vaginalis* infection. It is commonly believed as with many studies done by Gardner et al. (1957) [27]. These previous studies found that Gram's stain method represent the optimal laboratory test for of diagnosis of bacterial vaginosis, which is simple to perform, sensitive and specific. All these studies are in agreement with our study that shows *G. vaginalis* is the most prevalent form of vaginal disturbances in women. For *T. vaginalis*, the result in this study was higher than results in different provinces in Iraq. In Baghdad, 7% [28] while in Najaf 10.88% [15].

This study showed that *G. vaginalis* was isolated from (50%) of infection cases using culture method. Isolation of *G. vaginalis* from culture of vaginal swabs are unreliable and should not be utilized for diagnosis because of the associated of various anaerobes and presence of these organisms in normal vaginal flora, this result of positive culture cannot be considered diagnostic for this infection because when the predictive value of positive *G. vaginalis* culture is around (54%) [29].

Vaginal culture is one of the most difficult cultures to be evaluated in a clinical microbiology practice. The necessity of some expensive and complicated processes for diagnosis of some specific agents, age related variability of normal vaginal flora, and failure to make a diagnosis caused by the temporary presence of some pathogens in normal flora can be listed among the probable causes of that problem [30].

Regarding to the RT-PCR test, that from 200 samples, only 156/200 samples were diagnosis to be infected by RT-PCR. This assay detected 120 (60%) cases of *G. vaginalis*, 34 (17%) cases of *T. vaginalis*. PCR is more accurate to detect the STPs. Molecular methods are considered the gold standard for diagnosis, given the excellent sensitivities and specificities in diagnosis. because it is allowed to distinguish between STPs However, new molecular methods such as PCR, qualitative and quantitative real time PCR rapid detection were STDs in comparison to serological methods have been used as common. Moreover, monitoring of DNA level of a pathogen in body fluids can reveal the status of the disease, its response to medication, and its resistance patterns [31,32].

Regarding to age, the study showed that STPs occurred in reproductive age group 15-54 years this may be attributed to the reality that the sexually active woman, menstrual cycle, hormonal factors and use of contraceptive. Women in reproductive aged group were at an increased risk for vaginitis. The highest rate of infection with G.V. among 15-36 years old, this finding is in agreement with other study [32], but in contrast to other study that showed marked increase in the prevalence of *G. vaginalis* with increasing age [33].

They reported high prevalence of *T. vaginalis* among age group 15-24 years of age followed by 25-34 and 35-44 years, the lowest in 45-54 years of age, this is identical with the study of that proved the prevalence of T. vaginalis was higher in women over age 15 years [34,35].

In previous study in Baghdad, almost close percentage for those women 12% (12/100) [36]. *T. vaginalis* found that infection predominantly occurs in age group 15-24 years represent the highest rate and this is in agreement with our study, Omer et al. (1985) in Sudan, found that *T. vaginalis* infection predominantly occurs in age group 16-19 years, this result agreement with our study in which we found that the lowest rate occur in age group 16-27 years [37].

This result may be due to the ability of the parasite to alter at the vaginal environment for its survival. In the present study, the distribution of microorganisms was high among different age groups. A study done by Mahdi et al. showed that the infection rate (12.6%) was found in women of reproductive age. The majority of positive cases were in the age

group 20-40 years, while women near or post-menopause showed low incidence of infection, and this is probably due to absence of suitable environment for growth of *T. vaginalis*. Vaginal infection is considered one of the major feminine health problems [38] from time to time during their reproductive lives due to its strong relationship with the menstrual cycles, birth control methods, aging, medicines, or changes after pregnancy [39,40].

Most common co-infection is between *G. vaginalis* cases and *T. vaginalis* were 30/156 (19.23%) cases of co-infection. Statistically, there were highly significant differences among co-infection cases. All these findings suggest that there are combined infections in patients, which in agreement with our study. Several workers studied the association of *T. vaginalis* with other pathogens many of them agreed that *T. vaginalis* may precipitate other type of infection which may be due to sexual risk behavior. The variable discharges that noticed in this study might be resulted from co-infection of *T. vaginalis* with other microbial pathogens. Many studies found combined infections, found *T. vaginalis* and *G. vaginalis*. *G. vaginalis* was related to concurrent infection with *T. vaginalis*. The highest rate of combined infection was seen in cases infected with both *T. vaginalis* and *G. vaginalis* since both favor the growth in similar environment specially pH >4.5. *T. vaginalis* might be responsible for the change in normal vaginal flora and may therefore precipitate for *G. vaginalis* bacterial vaginosis [41-44].

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Authors Contribution:

Ali conducted the sampling, isolation, and staining, the molecular work and writing the manuscript. Dr. Al-Marsome and Dr. Almoayed supervised the work, edit and finalize the writing of the study.

Conflict of interest

The authors declare no conflict of interest.

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