

Seroprevalence and risk factors of *Trichomonas vaginalis* among couples in Al-Hamza city-Iraq.

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Abstract:

Background: Trichomoniasis is a sexually transmitted disease. It is a public health risk factor. This disease associated with many sexual diseases and is likely to be a cause of infertility, abortion, and the birth of low-weight babies. The purpose of the study is to establish a database of parasite infection in the city, especially with no previous research on the rate of infection among men.

Materials and method: Two hundred forty serum samples were collected from 120 couples between 18-43 years old, for the period from 2 Jan. 2020 to 25 jun. 2020, all of which were examined by ELISA test to detect immunoglobulin IgM and IgG. All results were analyzed by SPSS 20.

Result: The study recorded a high rate of infection (27.9%). Infection rates in women were higher than men, when it recorded a total infection rate (31.7%), while the infection rate among men was (24.2%). This infection differentiation among residence, education, use a condom, and presence of symptom. Our study recorded association between Trichomoniasis and infertility, abortion, and the birth of low weight children.

Conclusion: The study showed a relatively high incidence rate among couples, perhaps due to the use of the serological method in detection. It is the first study in the city among couples in general and men in particular.

Key words:

Trichomonas vaginalis; Seroprevalence; Couples; Al-Hamza city; Risk factors.

1. Introduction:

Trichomonas vaginalis is a sexual transmit common parasite. It infects Human only, causes trichomoniasis, which is a worldwide disease. This parasites infects females mainly and males to a lesser extent(Hirt & Sherrard, 2015). The parasite is transmitted directly from one host to another during intercourse or indirectly through a medical examination when using a contaminated vaginal scope, as some research indicated that it can be transmitted through contaminated toilets or uses patient tools(Nouraddin & Alsakee, 2015). But it is customary to depend on the survival of the parasite over the humidity of the surrounding environment(Etuketu et al., 2015).

The presence of *T. vaginalis* is attributed to several factors, including the status of normal flora, pH of the vagina, and the concentration of glycogen, in addition to the concentration of the sex hormones (estrogen and progesterone)(Palm et al., 2006). The decrease in the concentrations of estrogen hormones, glycogen, and acidic PH are factors that reduce the rate of infection(Miranda et al., 2014). On the contrary, the activity of the normal flora (*Lactobacillus* bacteria, for instance) maintains the stability of the vaginal PH at (3.5 - 4.5), as well as the bacteria contribute to the stability of hydrogen peroxide and prevent the growth of gram-positive and gram-negative bacteria and thus prevent reproduction or survival of the parasite(Babu et al., 2017).

In most patients, no signs of disease appear, especially in mild or acute infections(Khalaf & Al-asadi, 2010). In chronic infections, women feel abdominal pain, burning during urination, itching, and yellowish-greenish smelly vaginal discharge(Caini et al., 2014). In men, may developed epididymitis urethritis, and prostatitis(Mahmud et al., 2017). *T. vaginalis* infects about 73% of the partners of infected women, and it can remain latent within the genital tract from 3-12 months, so, 70-100% of them do not show signs of disease (Seña et al., 2007). Also, the parasite is characterized by its high ability to resist treatment with metronidazole (Kissinger et al., 2008). The prostate can be considered the storing organ of the parasite in men (Kim et al., 2019).

T. vaginalis engulfs a bacteria in the environment, or it may devour the mucous membrane of the genital tract. Study indicates that there are three basic steps to a mechanism of affection in living tissue. Firstly, crowding parasites as a clump, then attaching the target cells, followed by production of extracellular cytotoxins, which have an effect on the nature of cells(De Miguel et al., 2010). From this, it was found that the adhesion process is considered the most critical step in the statement of intoxication, parasite ability to invade, and grow in cells, because this process is considered the signal directed to the organization of the many genes that are responsible for parasite reformation and production of many Enzymes, which leads to degeneration of cells and inflammation(Rodríguez et al., 2010).

2. Materials and methods:

1.2 Population and the study area:

current study was performed from 2/1/2020 to 25/6/2020. It was included couples between 18-43 years old , whose living in Al-Hamza city of Al-Qadisiyah Governorate - Iraq, located in the southeast of the capital Baghdad, between 31 ° 51 ' N and 45 ° 3'E.

2.2 Samples and study community:

Current study included examination of 120 couples, to detection their infection with *T. vaginalis*, and evaluation risk factors that associated with. According to the recommendations of the Ethics Committee at Science College - Al- Qadisiyah university, 5 mm of blood from each one was drawn, samples were left until coagulated, thereafter, it placed in a centrifuge at 3000 rpm for five minutes, then, the serum was withdrawn by a pipette and placed in tubes kept at -20 °C(Adjei et al., 2019).

3.2 Detection of anti-*Trichomonas* antibodies by indirect ELISA.

Specific immunoglobulins of *T. vaginalis* in the blood was detected by commercial kit from Bio-Rad Company (UK)(Bedair & Ali, 2020), according to the manufacturer's instructions, in brief :

1. Coating of the high binding, 96-well, flat bottom ELISA plate wells with 100 µl of the Ag solution diluted in 1x Coating Buffer.
2. The plate was covered and incubated at 4°C overnight in the dark.
3. The solution was discarded and the plate was washed 4 times with 1x Wash Buffer, with agitation to ensure thorough washing.
4. Blocking solution, 150 µl, was added to each coated well and incubated at 37°C for 90 minutes, then washed 4 times with agitation.
5. Serum samples (Primary detection antibody) of 100 µl were added to each well then incubated for 1 hour at 37°C. Next, they were washed 4 times with agitation to get rid of any non-specific binding.

6. HRP: IgM or IgG (enzyme conjugated secondary Ab) of 100 μ l diluted in PBS was added to all wells and incubated at 37°C for 1 hour then washed 4 times as mentioned before.
7. Substrate of 100 μ l was added to all wells in the dark and incubated for 10 minutes or less until blue color development.
8. Stop solution of 50 μ l (0.2M H₂SO₄) was added and gentle tapping was applied to ensure mixing thoroughly.
9. The blue colored substrate turned yellow immediately, and the absorbance was read within few minutes at 450 nm. All reagents were at room temperature before routine-work.

4.2 statistical analysis:

The Chi-square value, SD, and in this study was calculated by SPSS software version 20 (SPSS, Chicago, IL, USA).

3. Results:

1.3. Patient Characteristics :

The study included examination of 120 couples in Hamza district, Iraq, aged between (18-43) years old, divided into five age groups (Table 2). As well as divided by residence, culture, sexual diseases, use of condoms, the appearance of symptoms, and intercourse with more partners (Table 3), (Table 4).

2.3. Epidemiology and seroprevalence:

The study recorded a high rate infection (27.9%), when was confirmed infection (67) out of (240). (14.2%) of the samples indicated an acute infection, when IgM was present in (34) samples, while the presence of IgG in (24) samples at a rate (10%) was indicated chronic infection. Also, the study recorded presence of IgM and IgG together at (3.75%) and (9) samples (Table 1).

3.3 Prevalence by gender:

The study recorded higher infection rates in women than men, when it recorded a total infection rate (31.7%) in women, while the infection rate among men was (24.2%). These infections were more significant (Table 2).

4.3 Risk factors among women.

Table(3) shows some of the risk factors among women in this study. The highest incidence was in the group (28-32) years (10.8%), followed by the group (23-27) years by (10%). According to the residence, rural areas were the highest incidence with (18.3%). Women with primary school achievement scoring higher (12.5%). Infection in women that didn't use Contraceptive was (29.17%) and (26.67%) among women who hadn't symptoms of this disease. (25.8 %) of women did not have other sexual diseases, while (11.6%) were Infertile. (5%) were had an abortion and (1.6%) birthed low weight baby.

5.3 risk factors for men.

Table (4) recorded the risk factors among men. The highest rate of infection was in the group(23-27) years (10.83%), followed by group (28-32) with a rate (67.67%). The population of rural areas is higher infection rate (15.8%) than urban, while patients with less culture were more affected (12.5%). The infection rate among men who do not use condoms (20.8%), and symptoms of the disease disappeared at (19.17%), while (10.83%) had other sexual diseases. (17.17%) had sex with a more one partner, and (4.17%) were infertile.

4. Discussion:

Our study indicated a high rate of infection with *T. vaginalis* when recorded a total infection rate (27.9%), and (31.7%) among women. This result was less than what (Merdaw et al., 2015) reached when it was recorded (34.41%) among women in Baghdad, and (Jarallah, 2013) when scored (55.65%) among women in Basra-Iraq. It is higher than (Taher et al., 2018) when scored (14.3%) in Najaf-Iraq, and (Nouraddin & Alsakee, 2015) (2.73%) Among women in Erbil-Iraq, in Iran, (Ghobahi et al., 2019) scored a total infection rate (2.6%).

Unfortunately, there are no adequate data on parasite infection among men in Iraq. This study recorded a moderate infection rate (24.2%). While the following incidence rates were recorded in different country (0.5%) (Flagg et al., 2019), (3.7%) (Gaydos et al., 2013), (2,1) (Freeman et al., 2010), (17 and 3%) (J. R. Schwebke & Hook, 2003) and (73. 2%) (Hobbs et al., 2006) .

The difference in infection rates attributed to many factors, including the size and the type of the sample, the period in which the study was conducted, as well as the method adopted for diagnosis and the nature of social traditions prevalent in society such as religion or cultural level (Dahab M. M, 2012). The more restrictions imposed by the Islamic religion on the practice of illegal sex contribute to lower rates of infection (Mahmood et al., 2015), as well as the use of serological methods can give unmeasurable results, especially since the immune objects against the parasite can remain for years (Bowden & Garnett, 2000).

The current study showed an increase in the infection rates in women age group (28-32) years (10.8%), followed by (10%) at the age group (23-27), while the age group (38-43) was the lowest incidence rate (2.5%), the results of this study are consistent with what was recorded by (Hawel, 2018).

Previous studies indicated that the rate of infection increases in men between 30-39 years (Seo et al., 2014), and the rate of infection in elderly men is higher than that of young men (Gaydos et al., 2013), while (Flagg et al., 2019) reported that the highest infection occurs in ages (14-19) years, while (Hobbs et al., 2006), (Daugherty et al., 2019), and (J. Schwebke et al., 2018) recorded the highest rates of injury among ages 20 to 29 years.

The reason for the differences in proportions between the different age groups can be due to several factors, including vaginal PH, the concentration of estrogen and progesterone, as well as recurrent pregnancy and miscarriage and weak body defenses after the menstrual cycle (Alim, 2009). The decrease in the infection in elderly women may be attributed to the rise in the vaginal PH, which will become (6-8), as well as the decrease in the estrogen and progesterone concentration, which does not encourage parasite growth (Johnston & Mabey, 2008), (Bachmann et al., 2011). As for the emergence of high rates of infection in young women, it may be attributed to sexual activity and the high concentration of sex hormones, in addition to the increase in glycogen, which causes the pH to shift to the base (Miranda et al., 2014). (Sutton et al., 2007) added that the high rates of infection in middle-aged women may have been due to previous infections that were cured and the antibodies to the parasite remained. Infected the partner the main cause to infect men.

This study recorded a higher rate of infection in rural areas than in urban areas, and this result is consistent with (Mahmood et al., 2015), (Nu et al., 2015), but did not consistent with (Ghobahi et al., 2019). The study indicated that there is a relationship between general education, academic achievement and parasite infection, as less educated women had higher infection rates, and this corresponds to many studies (Nourian et al., 2013), (Nayer, 2018), while other studies reported that the

relationship between The educational level and the parasite infection is reversible(Malla et al., 2008), while (Buvé et al., 2001)showed that there is no relationship between them.

The absence of health and preventive measures, unsanitary living conditions, in addition to the frequent visits to medical clinics or the infection of the partner, are all contributing factors to the transmission of infection.

The pathological symptoms appear as a result of the parasite's attachment to the epithelial mucous of the vagina, followed by inflammatory cascade, which includes the increase in mast cells (that responsible for many secondary symptoms such as arthritis)(Han et al., 2012).The inflammatory cascade causing the killing of the infected cells in addition to the parasite, thus The emergence of secretions characteristic of the disease(Donders et al., 2013), although many of these secretions may appear as a result of other infections (bacterial for example)(Khamees, 2012), as well, the majority of these secretions may differ according to age, menstrual cycle and Contraceptive use(Haltas et al., 2012).

It seems that the culture of using the female condom among women is little, as the study found that only (10%) of women used a different female condom, despite that, the rate of infection among them was (5%). The reason may be due to the hormonal content of some contraceptives, which contributes to an increase of vaginal PH after this, providing an appropriate environment for the growth of the vaginal *Trichomonas vaginalis*(Allsworth et al., 2009). The use of the intrauterine device IUD can contribute to the transmission of infection, as the thread of the IUD carries micro-organisms at its end, which helps its entry into the genital tract(Sönmez Tamer et al., 2009). On the other hand, a condom represents a barrier that prevents the entry of various micro-organisms and thus reduces infection rates(Crosby et al., 2012).

The vagina represents a medium in which many pathogens grow(Khamees, 2012). These pathogens may be transmitted to the partner or vice versa through sexual contact (Ambrozio et al., 2016). Many pathogens affect the reproductive system that are associated with infection with trichomoniasis (Haltas et al., 2012), as these pathogens contribute to changing the environment PH, thus facilitate the growth and reproduction of the parasite (Alim, 2009). On the other hand, the analysis of red blood cells and the mucous epithelial layer by *T. vaginalis* contributes to breaking down the barrier that prevents the entry and proliferation of other pathogens(Nd & Sa, 2020),(Mavedzenge et al., 2010). The parasite also acts as an agent that transmits these pathogens (Chang et al., 2016). The relationship between parasite infection and HIV is very clear, as the parasite infection is two to three times more prevalent among HIV-infected women than in healthy women (McClelland et al., 2007).

It was not clear that there was an association between trichomoniasis and recurrent miscarriage, but high incidence levels remained. Adverse public health manifestations, the parasite *T. vaginalis* does not participate in miscarriage factors but may cause premature labor (Minkoff et al., 1984). While other studies have indicated the relationship between infection with the parasite and the birth of low-weight or premature rupture of membrane(Schwandt et al., 2008),(Carter & Whithaus, 2008), (Cotch et al., 1997). Other studies attribute the cause of taking anti-parasite drugs such as metronidazole or excessive use of antibiotics(Jarallah, 2013).

The current study revealed a high rate of infection among infertile women and men. These results are consistent with the findings of (el-Sharkawy et al., 2000) where he attributed the reason to “the increase in C3 & C4, IgA and the increase in prolactin”. (Kaya et al., 2015)goes on to state that the potent immune action and the production of cytokine and reactive nitrogen, in addition to the cysteine

protease(CP30) secreted by the parasite, all contribute to the destruction of the mucous membrane of the vagina, and the immune cells in it.

The presence of the pathogen in the semen affects the effectiveness of the sperm and its ability to fertilize the egg (Harold Moreno, 2014). On the other hand, infected with *T. vaginalis* shows abnormal sperm motility, high semen viscosity, and change in head or sperm flagellate tropism after infection with the parasite. *T. vaginalis* adhesion affects sperm motility and subsequently “phagocytosis, lysis, and digestion of sperm cells”(Midlej & Benchimol, 2010).

Conclusion:

Trichomoniasis affects men and women of different ages, and the disease is associated with the conditions of sanitary protection, the use of condoms, and the concentration of sex hormones in women. The disease has a clear relationship with infertility or abortion and the birth of low-weight children. Since the disease is transmitted sexually, so intercourse with more than one partner is a major cause of infection and its spread.

Table (1): Seroprevalence of *T. vaginalis*.

Total Samples	Positive				Negative (%)	P. value
	IgG (%)	IgM (%)	IgG+IgM (%)	Total positive(%)		
240	24(10)	34(14.2)	9(3.75)	67(27.9)	173(72.1)	0.1

Table (2): men and women infection.

parameters	women	Men
Total	120	120
IgG(%)	13 (10.8)	11(9.2)
IgM(%)	19 (15.9)	15(12.5)
IgG+IgM(%)	6 (5)	3 (2.5)
Total positive	38 (31.7)	29 (24.2)
Negative	82(68.3)	91 (75.8)
P.value	0.01*	

Table (3): Risk factors of T. vaginalis in women.

Variables	Samples(%)	IgM(%)	IgG(%)	Total	P.ratio	P.value
Age						
18-22	15 (12.5)	4 (3.3)	0(0)	4 (3.3)	(0.27)	0.021
23-27	32 (26.67)	7 (5.83)	8 (6.67)	12 (10)	(0.19)	
28-32	34 (28.3)	7 (5.83)	7 (5.83)	13 (10.8)	(0.38)	
33-37	23 (19.17)	5 (4.17)	2 (1.67)	6 (5)	(0.52)	
38-43	16 (13.3)	2 (1.67)	2 (1.67)	3 (2.5)	(0.19)	
Residences						
Rural	68 (56.67)	15 (12.5)	10 (8.3)	22 (18.3)	(0.32)	0.1
Urban	52 (43.3)	10 (8.3)	9 (7.5)	16 (13.3)	(0.31)	
Culture						
Primary	44 (36.67)	10 (8.3)	7 (5.8)	15 (12.5)	(0.34)	0.09
secondary	41 (34.17)	7 (5.83)	6 (5)	12 (10)	(0.29)	
university	35 (29.17)	8 (6.67)	6 (5)	11 (9.17)	(0.31)	
Contraceptive						
Use	10 (8.3)	3 (2.5)	1 (0.83)	3 (2.5)	(0.3)	0.41
doesn't use	110 (91.67)	22 (18.3)	18 (15)	35 (29.17)	(0.32)	
Symptoms						
Yes	22 (18.3)	6 (5)	3 (2.5)	6 (5)	(0.27)	0.38
No	98 (81.67)	19 (15.8)	16 (13.3)	32 (26.67)	(0.32)	
Sexual diseases						
Yes	10 (8.3)	5 (4.17)	4 (3.3)	7 (5.8)	(0.7)	0.35
No	110 (91.67)	20 (16.67)	15 (12.5)	31 (25.8)	(0.28)	
Infertile						
Yes	25 (20.83)	8 (6.67)	8 (6.67)	14 (11.6)	(0.56)	0.16
No	95 (79.17)	17 (14.17)	11 (9.167)	24 (20)	(0.25)	
Abortion						
Yes	13 (10.83)	4 (4)	4 (3.3)	6 (5)	(0.46)	0.38
No	107 (89.17)	21 (17.5)	15 (12.5)	32 (26.6)	(0.3)	
low weight children						
Yes	5 (4.17)	1 (0.83)	2 (1.67)	2 (1.6)	(0.4)	0.46
No	115 (95.83)	24 (20)	17 (14.17)	36 (30)	(0.31)	
Total	120 (100)	25 (20.83)	19 (15.8)	38 (31.6)		

Table (4): Risk factors of T. vaginalis in men.

Variables	Samples(%)	IgM(%)	IgG(%)	Total	P.ratio	P.value
Age						
18-22	13 (10.83)	3 (2.5)	2 (1.67)	4 (3.3)	0.3	0.05
23-27	30 (25)	7 (5.83)	6 (5)	13 (10.83)	0.43	
28-32	35 (29.17)	5 (4.17)	4 (3.3)	8 (6.67)	0.23	
33-37	26 (21.7)	2 (1.7)	1 (0.83)	2 (1.67)	0.077	
38-43	16 (13.3)	1 (0.83)	1 (0.83)	2 (1.67)	0.13	
Residences						
Rural	68 (56.7)	11(9.17)	9 (7.5)	19 (15.8)	0.28	0.03
Urban	52 (43.3)	7(5.83)	5 (4.17)	10 (8.3)	0.19	
Culture						
Primary	29(24.17)	9 (7.5)	6 (5)	15 (12.5)	0.52	0.15
secondary	55 (45.83)	5 (4.17)	4 (3.3)	8 (6.67)	0.15	
university	36 (30)	4 (3.3)	4 (3.3)	6 (5)	0.17	

Use Condom						
Use doesn't use	40 (33.33) 80 (66.67)	3 (2.5) 15 (12.5)	2 (1.67) 12 (10)	4 (3.3) 25 (20.8)	0.1 0.32	0.39
Symptoms						
Yes No	13 (10.83) 107 (89.17)	5 (4.17) 13 (10.83)	2 (1.67) 12 (10)	6 (5) 23 (19.17)	0.46 0.21	0.33
Another Sexual disease						
Yes No	32 (26.67) 88 73. 33)	8 (6.7) 10 (8.3)	5 (4.17) 9 (7.5)	13 (10.83) 16 (13.3)	0.4 0.2	0.06
Infertile						
Yes No	12 (10) 108 (90)	2 (1.7) 16 (13.3)	5 (4.17) 9 (7.5)	5 (4.17) 24 (20)	0.42 0.22	0.36
Multiple partner						
Yes No	33 (27.5) 87 (72.5)	7 (5.83) 11 (9.17)	6 (5) 8 (6.7)	11 (9.17) 18 (15)	0.33 0.21	0.15
Total	120 (100)	18 (15)	14 (11.7)	29(24.17)		

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