ISSN: 0067-2904

Some Clinical and Inflammatory Aspects of *Trichomonas vaginalis* Infection among Women with Pelvic Inflammatory Diseases

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Received: 12/4/2021 Accepted: 17/6/2021

Abstract

Infection with *Trichomonas vaginalis* and its relation to some diseases have lately had much attention. The objective of this study was to assess the infection rate of *T. vaginalis* and its relation to pelvic inflammatory diseases, infertility, and vaginosis. The study also assessed some demographic, clinical, and immunological parameters in women infected with *T. vaginalis*. The study included 160 non-pregnant married women who attended some private clinics and public hospitals in Baghdad from October 2020 to February 2021. All participants had symptoms of vaginal discharge only or vaginal discharge with lower abdominal pain. The participants were divided into an infertility group (n = 61) and fertility group (n = 99). The participants were also divided into Pelvic Inflammatory Disease (PID) group (n=41) and non-PID group (n=119). All participants underwent vaginal examination. Vaginal swabs were taken from all participants for *T. vaginalis*, bacteria, yeast, clue cell, pH, and vaginal leukocyte examinations. Sera were also taken to measure both IL-1α and IL-8 using sandwich ELISA technique to compare them with apparently healthy control subjects who had no vaginal discharge. The study revealed that the total infection rate of *T. vaginalis* was 14.37%. The results showed no significant relations between *T. vaginalis* positive status and each of age, infertility, vaginosis, contraceptive use, and high BMI. While a significant relation (P=0.03) was noticed between *T. vaginalis* positive status and PID. Women with PID exhibited a higher infection rate with *T. vaginalis* (24.39%) versus non PID women (10.92%). High clue cells number was not significantly related to *T. vaginalis* positive status, only if both PID and vaginosis were considered in the statistical analysis. In addition, high leukocytes count was not related to *T. vaginalis* positive status. While abnormal vaginal pH was significantly (P=0.01) related to *T. vaginalis* positive status. Finally, the levels of both IL-1α and IL-8 were noticed to be declined in women who had *T. vaginalis* infection, although they were not declined significantly. This study gives an insight about some clinical aspects of *T. vaginalis* infection among PID women. *T. vaginalis* is highly predictable in women with PID. Consequently, attention and more investigation would be needed on this topic in Iraq.

Keywords: *Trichomonas vaginalis*; Infertility; Pelvic inflammatory disease

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بعض المظاهر السريرية والالتهابية للخمج بالمشعرات المهبلية لدى النساء المصابات بأمراض التهاب الحوض

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الخلاصة

الاهتمام بالعلاقة بين الخمج بطفيلي داء المشعرات المهبلية وبعض الامراض الاخرى زاد مؤخرا. الهدف من هذا البحث هو تقييم معدل الخمج بهذا الطفيلي وعلاقته بالتهابات الحوض, العقم, والتهابات المهبل. كما تضمن هذا البحث تقييم بعض العوامل الديموغرافيه, السريرية و المناعية. اشتملت هذا الدراسة على 160 من النساء البالغات المتزوجات من غير الحوامل اللاتي راجعن بعض العيادات الخاصة والمستشفيات العامة في بغداد ضمن الفترة ما بين تشرين الاول من العام 2020 الى شباط من العام 2021. جميع المشاركات كان لديهن افرازات مهبلية او افرازات مهبلية مصاحبة لآلام في اسفل البطن. تم تقسيم المشاركات في الدراسة الى مجموعة النساءالعقيمات (العدد61) ومجموعة النساء غير العقيمات (العدد=99). كما تم تقسيم المشاركات الى مجموعة المصابات بالتهاب الحوض (العدد 41) ومجموعة النساء غير المصابات بالتهاب الحوض (العدد=119). تم اجراء الفحص السريري المهبلي وأُخذت مسحات مهبلية من جميع المشاركات وذلك للتحري عن كل من طفيلي المشعرات المهبلية, البكتيريا, الخمائر , الخلايا الطلائية المهبلية, الاس الهيدروجيني وكريات الدم البيض في المهبل. كما جُمعت عينات مصل الدم منهن للتحري عن كل من انترلوكين−1 الفا وانترلوكين−8 ومقارنتهما مع نفس الانترلوكينات لمجموعة من النساء الاصحاء اللاتي لا تبدو عليهن اي اعراض تذكر ولايعانين من افرازات مهبلية. اظهرت النتائج ان معدل الخمج بالمشعرات المهبلية كان 14.73% لدى جميع المشاركات. كما اظهرت النتائج عدم وجود علاقة معنوية ($P>0.05$) بين الفحص الموجب لطفيلي المشعرات المهبلية وكل من العمر , العقم, التهاب المهبل, أستخدام موانع الحمل وارتفاع مؤشر الكتلة الحيوية. بينما ارتبط الفحص الموجب لطفيلي المشعرات المهبلية معنوياً ($P=0.03$) مع التهابات الحوض. كانت معدلات الخمج بالطفيلي عالية لدى النساء المصابات بالتهابات الحوض (24.39%) مقارنةً مع النساء اللاتي لم يكونن مصابات بالتهابات الحوض (10.92%). لم ترتبط النسب المرتفعة للخلايا الطلائية المهبلية مع الحالة الموجبة لداء المشعرات المهبلية الا في حال أُخذت كل من التهابات الحوض والمهبل بعين الاعتبار عند التحليل الاحصائي. ولم يرتبط العدد المرتفع لكريات الدم البيض المهبلية معنوياً ($P>0.05$) مع الفحص الموجب لطفيلي المشعرات المهبلية. بينما ارتبط الاس الهيدروجيني غير الطبيعي في المهبل بشكل معنوي ($P=0.01$) مع الحالة الموجبة لطفيلي المشعرات المهبلية. اظهرت النتائج ايضا انخفاضاً في مستويات كل من انترلوكين−1 الفا و انترلوكين−8 لدى المصابات بطفيلي المشعرات المهبلية رغم ان الانخفاض لم يكن معنوياً. قدمت هذه الدراسة نظرة حول بعض الجوانب السريرية للخمج بطفيلي المشعرات المهبلية بين النساء المصابات بمرض التهاب الحوض. كما يمكن ان يكون الخمج بهذه الطفيليات متوقعاً و بدرجة عالية عند النساء المصابات بمرض التهاب الحوض. وبالتالي ، ستكون هناك حاجة إلى مزيد من الاهتمام والبحث حول هذا الموضوع في العراق.

1. Introduction

Trichomonas vaginalis (*T. vaginalis*) is a protozoan parasite of the human urogenital tract that causes trichomoniasis [1]. Women's trichomoniasis symptoms range from asymptomatic to serious vaginitis, whereas men are most likely asymptomatic trichomoniasis carriers [2]. *T. vaginalis* infection is the most common non-viral sexually transmitted infection in the world. Nearly 90% of these infections, according to the World Health Organization (WHO),

occurred among people living in resource-limited settings [3]. *T. vaginalis* is more prevalent than *Chlamydia trachomatis* and *Neisseria gonorrhoeae,* [4]. With no surveillance programs, the epidemiology of *T. vaginalis* is not completely known. It is known, however, to vary greatly by population and geography [1]. Trichomoniasis can increase the risk of getting or spreading other sexually transmitted infections. *T. vaginalis* has been shown in some studies to increase vulnerability to bacterial vaginosis and induce genital inflammation, all of which increase the risk of HIV infection or transmission to a sexual partner [6]. *T. vaginalis* infection was found to be related to infertility and PID [7, 8]. *T. vaginalis* infection may cause infertility because of endometritis, salpingitis, and atypical pelvic inflammatory symptoms [9].

On other hand, many studies suggest that colonization of *T. vaginalis* is increased in the presence of bacterial vaginosis-defining phenomena, such as elevated amine production, loss of facultative lactobacilli, and increased pH [10]. *T. vaginalis* can establish a symbiosis with some kinds of bacteria implicated in bacterial vaginosis [11]. The presence of such kind of bacteria has been demonstrated in trichomonad isolates from different geographical areas. Even though *T. vaginalis* and some kinds of bacteria are able to induce disease independently in the vagina, their association has been shown to have important consequences for the pathogenicity of each of them [12]. *T. vaginalis*-associated inflammation is thought to be primarily caused by a cell-mediated immune response [13]. Neutrophils, the most inflammatory cells in the immune system, are abundantly recruited to the vagina during *T. vaginalis* infection. Neutrophils are also the most abundant immune cell type in the blood [14] and since they extravasate from the blood, they are the first cells recruited to the site of most infections. While within the infected tissue, their effector functions destroy pathogens effectively and eventually limit their spread [15].

Inflammatory cytokines secreted at the site of *T. vaginalis* infection, such as IL-1, TNF, and IL-8, are thought to play a role in neutrophil extravasation from the blood into the tissue [16]. In Iraq, there have been many studies that reported the infection rates of *T. vaginalis* among women in Dohuk, Mousl, Baghdad, and Basra (5.4%, 15.5%, 16.6%, and 13%, respectively) [17-20]. However, little is known regarding the association between *T. vaginalis* infection with infertility, pelvic inflammatory disease, and vaginosis. Therefore, the purpose of this study was to evaluate the clinical features of *T. vaginalis* infection among women with infertility, pelvic inflammatory disease, and vaginosis.

2. Materials and Methods

2.1. Study design and subjects

Non pregnant married women (n=160) who attended some gynecology private clinics and public hospitals in Baghdad from October 2020 through February 2021 and who had symptoms of vaginal discharge only or of vaginal discharge with lower abdominal pain were included in this study. The mean age of the participants was 31.56 ± 7.7 years (range: 16-54 years). Gynecologists conducted routine gynecological examinations after informing participants about the purpose of the test and obtaining a signed consent document from each participant. The participants were divided into two groups: Infertile (n = 61) and fertile (n = 99). The participants were also divided into PID group (n=41) and non-PID group (n=119). A diagnosis of the PID was made by gynecologist, depending on the presenting symptoms of vaginal discharge, lower abdominal pain, lower abdominal tenderness, and cervical motion tenderness [21].

2.2 Vaginal examination

A gynecologist used a sterile metal speculum without lubricate to examine each woman's vagina for vaginal discharge characteristics (color, consistency, and odor). The pH of the vaginal fluid was then measured by placing a pH paper against the lateral wall [22].

2.3 Sample collection

Samples were taken from the posterior fornix using sterile cotton swabs. The vaginal swabs were then inoculated into a tube containing approximately 2 ml of saline and examined within 10 minutes to identify the motile trichomonod [23]. Five millilitres of the collected blood sample was placed into a gel tube, centrifuged, and the serum was stored at -20 °C for further immunological examination.

2.4 Laboratory examinations

Gram stain of the vaginal discharge smear was examined microscopically using a 100 x oil-immersion objective lens. The numbers of clue cells and normal epithelial cells were estimated [24]. Whiff test was performed to detect the occurrence of bacterial vaginosis or trichomonod in vaginal discharge. A drop of 20% potassium hydroxide (KOH) was mixed with some vaginal discharge. A fishy odor was generated as a positive result. Then the slide was examined microscopically to identify the presence of budding yeast or pseudohyphae. Smears with yeast vaginosis usually have thick, white, so-called cottage cheese discharge with normal pH, without abnormal odour, and with negative Whiff test result [25]. The motile trophozoites of *T. vaginalis* were examined using saline wet mounts, followed by morphological identification on Giemsa stained smears [23]. The diagnosis of bacterial vaginosis was performed by means of the Amsel's method [26], which is based on the presence of three or more of the following criteria: clue-cells in the Gram stain, pH $\geq$ 4.5, positive Whiff test, and thin and homogeneous vaginal discharge. Clue cell number was considered as high when it recorded >20% of epithelial cells per field, while pH was considered as abnormal if its value was $\geq$ 4.5 [28].

2.5 Vaginal leukocyte count

Leukocyte quantification was determined in wet mount under 400x field by the observation of 10 nonadjacent microscopic fields. Vaginal leukocyte counts were considered as high at > 6 cells/high-power field on wet mount [28].

2.6 Immunological tests

Both IL-1α and IL-8 were measured using sandwich enzyme-linked immunosorbent assay (ELISA) kits based on the manufacturer's instructions (BioSource, San Diego/ USA). Samples were screened in a 96-well plate for some women included in this study (*T. vaginalis* positive samples and *T. vaginalis* negative samples). Plates were read at 450 nm by ELISA reader. The control group included 15 serum samples of apparently healthy women with no vaginal discharge or any known illness.

2.7 Statistical Analysis

Data analysis was achieved by SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The data were assessed by chi-square test and ANOVA. P values < 0.05 were considered statistically significant.

3.Results

3.1 Infection rates of *T. vaginalis*

The total infection rate of *T. vaginalis* in the surveyed population was 14.37%, where 23 out of 160 participants were microscopically positive for *T. vaginalis* (Figure 1).

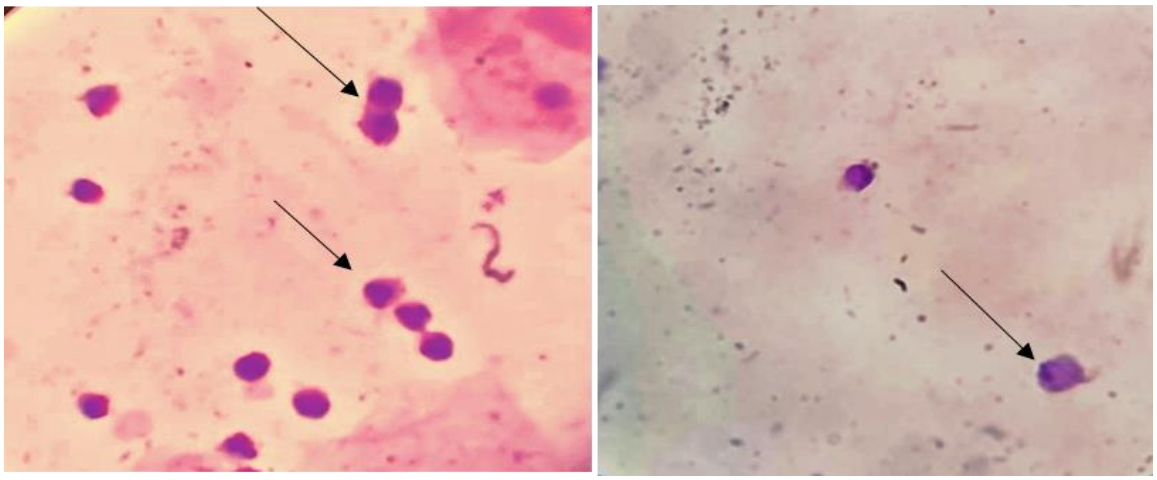

Figure 1- Trichomonas vaginalis was detected microscopically in the vaginal discharge samples by staining with Giemsa stain (100x)

No significant differences were noticed between different age groups regarding infection rate of *T. vaginalis*, although the age group of ≥ 45 years old showed the highest infection rate (44.44%) compared to other age groups (table 1). The results also showed that *T. vaginalis* infections were not found to be related to infertility; no significant differences were noticed in the infection rate of *T. vaginalis* between infertile and fertile groups. While PID was found to be related significantly (P=0.03) to *T. vaginals* infection rate. Regardless infertility status, the majority of *T. vaginalis* infection rates were reported in PID women (24.39%) versus non-PID women (10.92%). The results indicated a significant relation between PID and Trichomoniasis only in fertile women (P=0.02); higher infection rates were noticed in those women who were PID and fertile (27.58%) compared with non-PID and fertile group (10%). While no significant relation was indicated between PID and Trichomoniasis among infertile women, although the infection rate was higher in PID-infertile women versus non-PID-infertile women. The results also showed no significant association between vaginosis (bacterial or yeast vaginosis) with the occurrence of *T. vaginalis*, although women with bacterial vaginosis had the highest (16.27%) infection rate compared with yeast vaginosis group, yeast - bacterial coinfection group, and no-vaginosis group. The results also showed that the use of contraception methods among surveyed women was not significantly related to *T. vaginalis* infection. Convergent infection rates were noticed in both women with contraceptive use (14.03%) and women with no contraceptive use (14.56%). The results revealed no significant relation between high body mass index (BMI) and the occurrence of Trichomoniasis, although high infection rate was observed in high BMI-group (obese and overweight) (15.21%) versus normal BMI group (12.19%).

3.2 Clue cells, vaginal pH, and vaginal leukocytes

High clue cell count was noticed in 28.75 % (46/160) of the total studied population. Women with a positive result of *T. vaginalis* test were more likely to have a high clue cell count (43.47%) than those with a negative result (26.27%). High clue cell count was recorded in the majority (62.5%) of infertile *T. vaginalis* +ve women, while other groups (infertile *T. vaginalis* –ve, fertile *T. vaginalis* +ve, and fertile *T. vaginalis* –ve) were less likely to have high count. There was no significant correlation between high clue cell count in the vagina and positive result of *T. vaginalis* in infertile and fertile groups. While, high clue cell count was more likely to be associated with *T. vaginalis* and vaginosis. The majority of women with PID *T. vaginalis* +ve with vaginosis (71%) and non-PID *T. vaginalis* +ve with vaginosis

(60%) had high clue cell count. There were significant differences (P=0.00018) among these groups (table 2).

Abnormal vaginal pH was detected in 48.17% (66/137) of the total tested population. A significant relation between the presence of *T. vaginalis* infection and abnormal vaginal pH was found. Most (65.2%) of the women who had positive *T. vaginalis* results were most likely to have abnormal vaginal pH, while only 37.2% of the women that had negative *T. vaginalis* results showed abnormal vaginal pH. Abnormal vaginal pH was also noticed in the majority of infertile-*T. vaginalis* +ve (75%) and fertile *T. vaginalis* +ve (60%) groups. No significant correlation was noticed between abnormal vaginal pH and the presence of *T. vaginalis* among fertile and infertile women. On the other hand, the results indicated a highly significant relation (P=0.00081) between abnormal vaginal pH and vaginosis, regardless of the *T. vaginalis* positive status. The majority of all vaginosis groups had abnormal vaginal pH (table 3).

High vaginal leukocyte count was found in 43.75% (70/160) of the total population. The results showed that *T. vaginalis* positive rate was not associated significantly with high vaginal leukocyte number, although 52.17 % of *T. vaginalis* positive women had high vaginal leukocyte number, versus 43.28% of *T. vaginalis* negative women who had high vaginal leukocyte number. No significant relation between the presence of *T. vaginalis* and high vaginal leukocyte number was found in both infertile and fertile women, although 50% and 53.3% of infertile *T. vaginalis* +ve and fertile *T. vaginalis* +ve had high vaginal leukocyte numbers, respectively. No significant relation was noticed between positive results of *T. vaginalis* and high vaginal leukocyte number among PID groups (table 4)

Table 1- Prevalence of *T. vaginalis* among PID, infertile, fertile, age, BMI and vaginosis groups for women infected with *T. vaginalis*

Group	N	n(%)*T. vaginalis* positive women	X^2 (P-value)
All	160	23 (14.37%)	-
18-24	26	3 (11.53%)	7.37 (0.06)
25-34	79	9 (11.39%)	
35-44	46	7 (15.21%)	
45≥	9	4 (44.44%)	
Infertile women	61	8 (13.11%)	0.127 (0.7)
Fertile women	99	15 (15.15)	
PID women	41	10 (24.39%)	4.49 (0.03)*
Non PID women	119	13 (10.92%)	
PID-fertile women	29	8 (27.58%)	4.9 (0.02)*
Non-PID fertile women	70	7 (10%)	
PID-infertile women	12	2 (16.6%)	0.124 (0.7)
Non-PID infertile women	49	6 (12.24%)	
Bacterial vaginosis	43	7 (16.2%)	0.127 (0.7)
Yeast vaginosis	43	5 (11.62%)	
Yeast- bacteria coinfection	34	5 (14.7%)	
No-vaginosis	40	6(15%)	
Women with contraceptive use	57	8 (14.03%)	0.008 (0.9)
Women without contraceptive use	103	15(14.56%)	
Obese & overweight group	119	18(15.21%)	0.2 (0.6)
Normal BMI group	41	5 (12.19%)	

Table 2- percentage of cases with high clue cell count among women with *T. vaginalis*, PID and vaginosis groups

Group	N	n(%) High clue cells	X^2 (P-value)
All	160	46 (28.75%)	-
T.vaginalis (+) women	23	10 (43.47%)	2.8 (0.9)
T.vaginalis (-) women	137	36 (26.27%)	
Infertile *T.vaginalis* (+) women	8	5 (62.5%)	6.43 (0.09)
Infertile *T.vaginalis* (-) women	53	17 (32.07%)	
Fertile *T.vaginalis* (+) women	15	5 (33.3%)	
Fertile *T.vaginalis* (-) women	84	19 (22.6%)	
PID *T.vaginalis* (+)with vaginosis	7	5 (71%)	28.361 (0.00018)*
PID *T.vaginalis* (-)with vaginosis	20	6 (30%)	
PID *T.vaginalis* (+)without vaginosis	3	0 (0%)	
PID *T.vaginalis* (-) without vaginosis	11	0 (0%)	
Non PID *T.vaginalis* (+) with vaginosis	10	6 (60%)	
Non PID *T.vaginalis* (-) with vaginosis	83	27 (32.5%)	
Non PID *T.vaginalis* (+) without vaginosis	3	0(0%)	
Non PID *T.vaginalis* (-) without vaginosis	23	0(0%)	

Table 3- Percentage of cases with pH among women with *T. vaginalis*, PID and vaginosis groups

Group	N	n(%)Abnormal vaginal pH>4.5	X^2 (P-value)
All	137	66 (48.17%)	-
T.vaginalis (+) women	23	15 (65.2%)	6.36 (0.01)*
T.vaginalis (-) women	137	51 (37.2%)	
Infertile *T.vaginalis* (+) women	8	6 (75%)	6.5 (0.057)
Infertile *T.vaginalis* (-) women	53	22 (41.5%)	
Fertile *T.vaginalis* (+) women	15	9 (60%)	
Fertile *T.vaginalis* (-) women	84	29 (34.5%)	
PID *T.vaginalis* (+) with vaginosis	7	5 (71%)	24.83 (0.00081)*
PID *T.vaginalis* (-) with vaginosis	20	14(70%)	
PID *T.vaginalis* (+) without vaginosis	3	2(66.6%)	
PID *T.vaginalis* (-) without vaginosis	11	5(45.4%)	
Non PID *T.vaginalis* (+) with vaginosis	10	6(60%)	
Non PID *T.vaginalis* (-) with vaginosis	83	29(34.9%)	
Non PID *T.vaginalis* (+) without vaginosis	3	3(100%)	
Non PID *T.vaginalis* (-) without vaginosis	23	3(13%)	

Table 4- Percentage of cases with vaginal leukocytes count among women with *T. vaginalis*, PID and vaginosis groups

Group	N	n(%)High vaginal leukocytes count	X^2 (P-value)
All	160	70 (43.75%)	
T.vaginalis (+) women	23	12 (52.17%)	0.7(0.3)
T.vaginalis (-) women	137	58 (43.28%)	
Infertile *T.vaginalis* (+) women	8	4 (50%)	
Infertile *T.vaginalis* (-) women	53	18 (33.9%)	3.2(0.3)
Fertile *T.vaginalis* (+) women	15	8 (53.3%)	
Fertile *T.vaginalis* (-) women	84	40(47.61%)	
PID *T.vaginalis* (+) with vaginosis	7	5 (71%)	
PID *T.vaginalis* (-) with vaginosis	20	10 (50%)	
PID *T.vaginalis* (+) without vaginosis	3	2 (66.6%)	
PID *T.vaginalis* (-) without vaginosis	11	8 (72.7%)	
Non PID *T.vaginalis* (+) with vaginosis	10	4 (40%)	11.69 (0.1)
Non PID *T.vaginalis* (-) with vaginosis	83	35 (42.1%)	
Non PID *T.vaginalis* (+) without vaginosis	3	1(33.3%)	
Non PID *T.vaginalis* (-) without vaginosis	23	5(21.7%)	

3.3 IL-1α and IL-8 levels in the studied population

The results show that IL-1α level was relatively low in both *T. vaginalis +ve* and *T. vaginalis* -ve groups of infected women compared with the control group. The mean concentrations of IL-1α were 8.7653 ± 2.44 and 9.4119 ± 2.99 pg /ml in the positive and negative groups, respectively, while the value in the control group was equal to 8.208 ± 2.34 pg/ml. No significant differences were noticed between the groups regarding IL-1α (Figure 2).

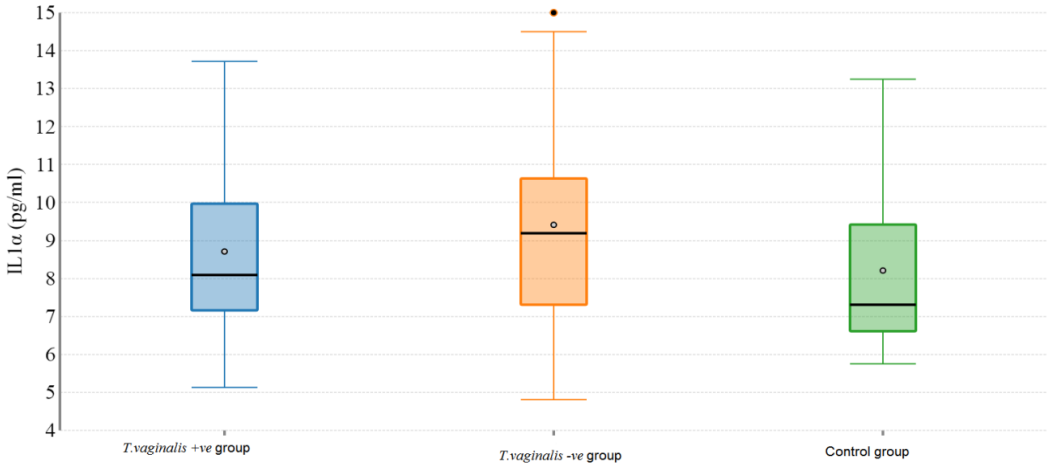

Figure 2-IL-1α level in *T. vaginalis* positive and negative groups.

The results also demonstrated non-significant differences regarding IL-1α levels among fertile (*T. vaginalis* +ve and –ve), infertile (*T.vaginalis* +ve and –ve), and control group.

Nevertheless, infertile *T. vaginalis* -ve group showed the highest mean level of IL-1α (9.8979 ± 3.94 pg/ml) as compared to the other groups (Figure 3).

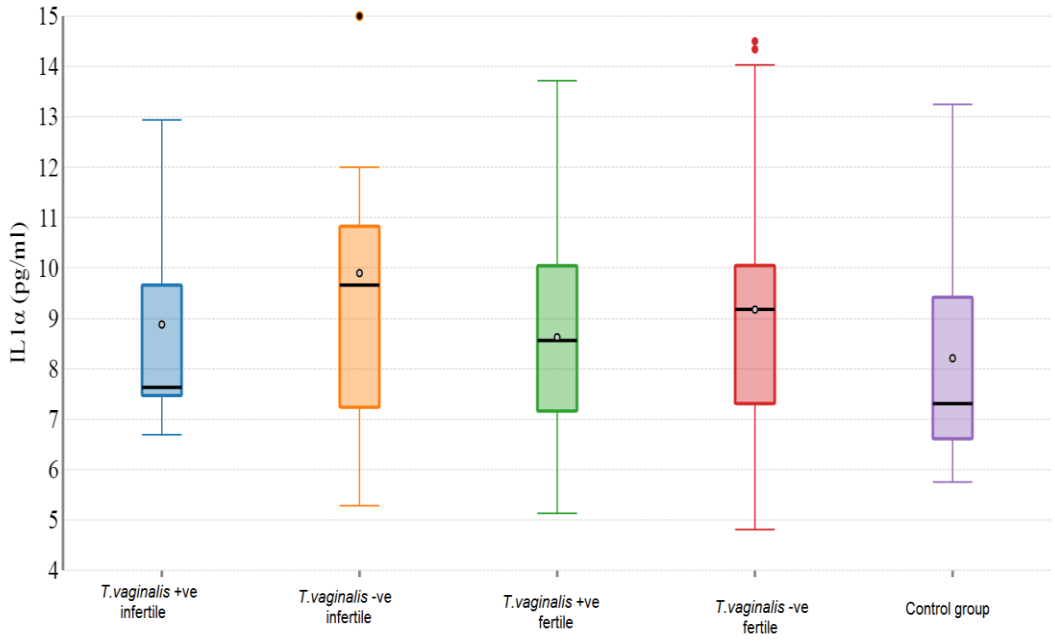

Figure 3-IL-1α level in *T. vaginalis* in the fertile and infertile groups

The PID *T. vaginalis* +ve group showed slightly higher levels of IL-1α (10.374 ± 2.14) pg/ml compared to PID *T.vaginalis* –ve, non PID (*T.vaginalis* +ve and –ve), and control groups (Figure 4), although no significant differences were noticed.

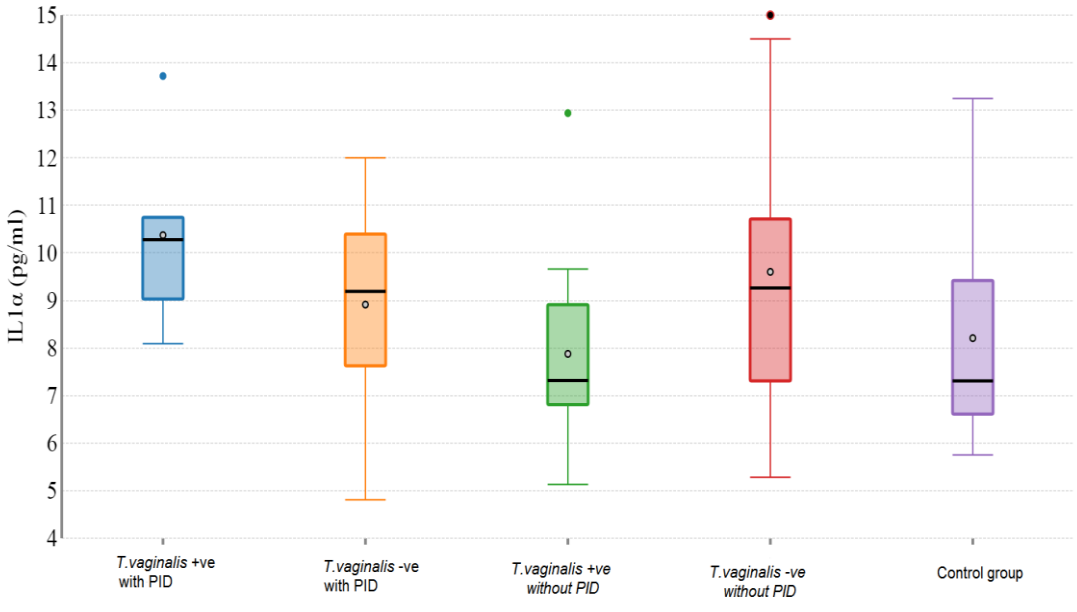

Figure 4-IL-1α level in *T. vaginalis* group in PID and non PID groups

Furthermore, no significant differences were found among different groups (*T. vaginalis* +ve with vaginosis, *T. vaginalis* -ve with vaginosis, *T. vaginalis* -ve without vaginosis) regarding serum IL1α level. The mean levels of IL-1α were 8.9464 ± 2.76, 9.3413 ± 3.22, and 9.6562 ± 2.11 pg/ml, respectively, compared to the control group (8.208 ± 2.34 pg/ml) (Figure 5).

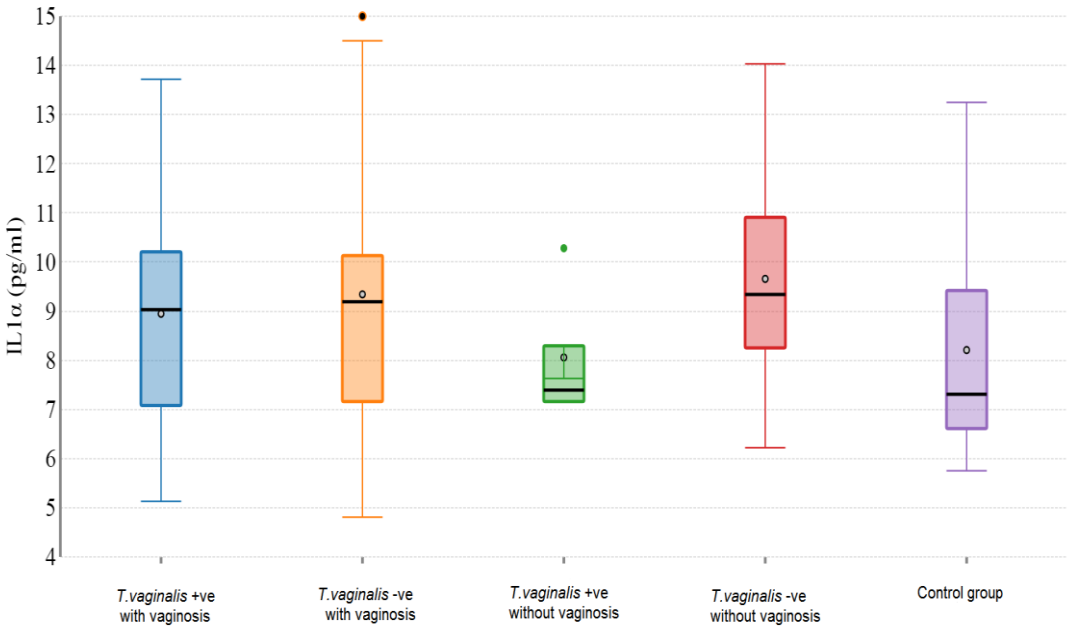

Figure 5-IL-1α level in *T.vaginalis* group with vaginosis and non-vaginosis groups

IL-8 levels were also detected for the same groups. It was noticed that *T. vaginalis* +ve group has interestingly lower level of IL8 (179.5433 ± 49.89 pg/ml) compared to *T. vaginalis* –ve and control groups (266.3247 ± 228.64 and 267.126 ± 153.90 pg/ml, respectively). No significant differences were noticed among the groups (Figure 6).

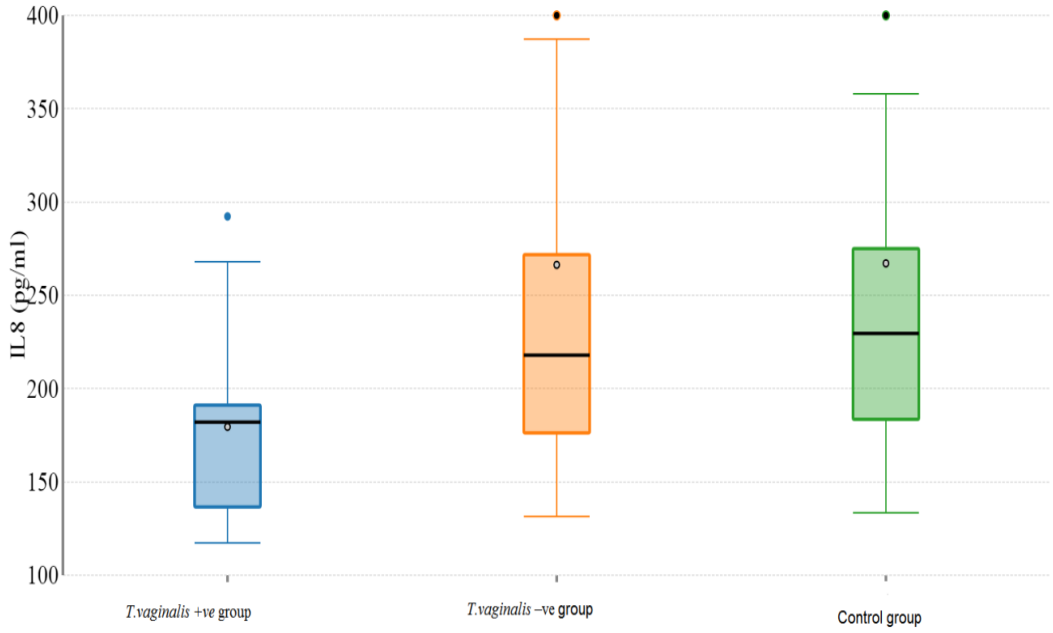

Figure 6-IL-8 level in *T. vaginalis* positive and negative groups

Similar results of low IL-8 levels were observed in both *T. vaginalis*+ve infertile and *T. vaginalis* +ve fertile groups (156.352 ± 19.91 and 189.339 ± 57.42 pg/ml, respectively), versus a high level of IL-8 in the control group (267.126 ± 153.90 pg /ml). On the other hand, a high level of IL-8 was noticed in the *T. vaginalis*-ve infertile group (316.4316 ± 382.70 pg/ml). The statistical analysis showed no significant differences among groups **(**Figure 7).

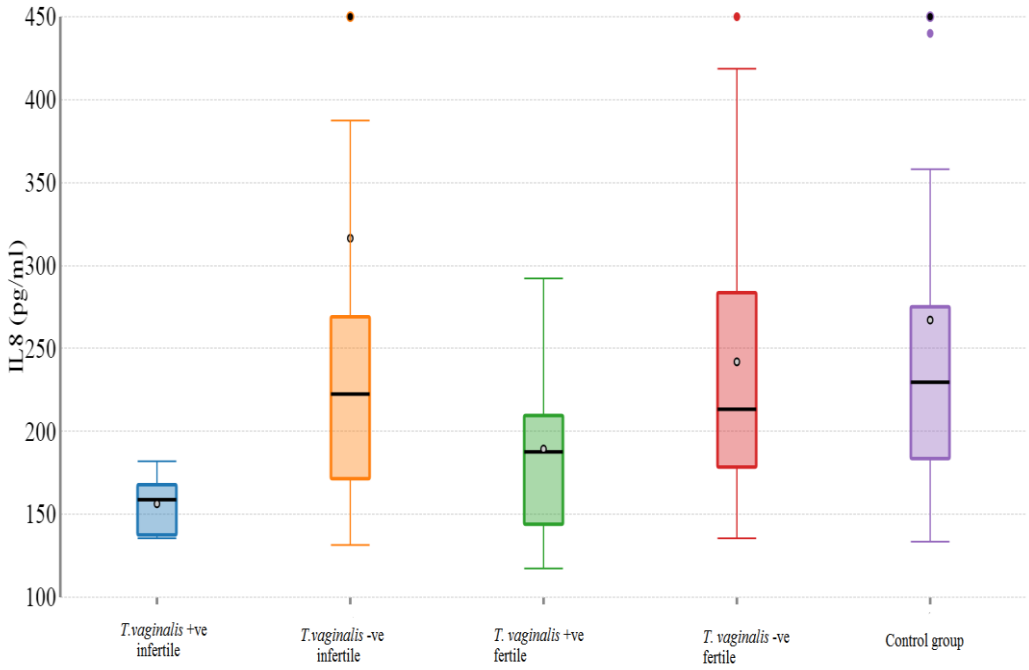

Figure 7-IL-8 level in *T. vaginalis* in the fertile and infertile groups

Low levels of IL-8 were found in both PID *T. vaginalis* +ve (179.448 ± 29.62 pg /ml) and non-PID *T. vaginalis* +ve (177.791 ± 59.04 pg /ml) groups, versus a higher level in the control group (267.126 ± 153.90 pg /ml). However, the level of IL-8 was slightly increased in non-PID *T. vaginalis* -ve group (284.9014 ± 265.79 pg /ml). No significant differences were noticed among these groups (Figure 8**).**

The results also illustrated lower concentrations of IL-8 in both *T. vaginalis* +ve with vaginosis group and *T. vaginalis* +ve without vaginosis groups (179.4636 ± 47.56 and 179.7625 ± 63.85 pg/ml, respectively). While the level of IL-8 was slightly increased in *T. vaginalis* -ve with vaginosis group (269.4613 ± 258.25). compared to the control groups. (267.126 ± 153.90) pg /ml. The statistical analysis indicated no significant differences among groups (Figure 9).

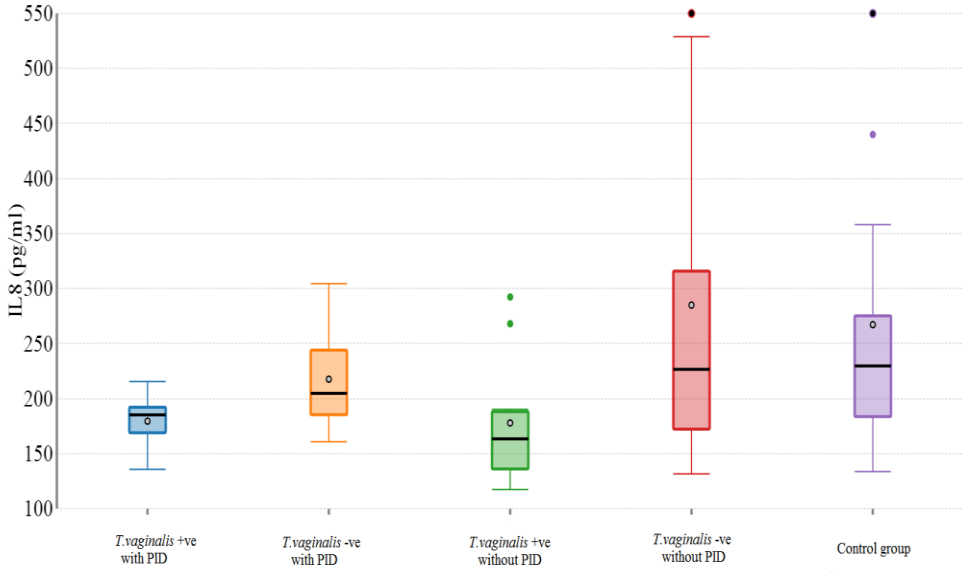

Figure 8-IL-8 level in *T. vaginalis* group in PID and non PID groups

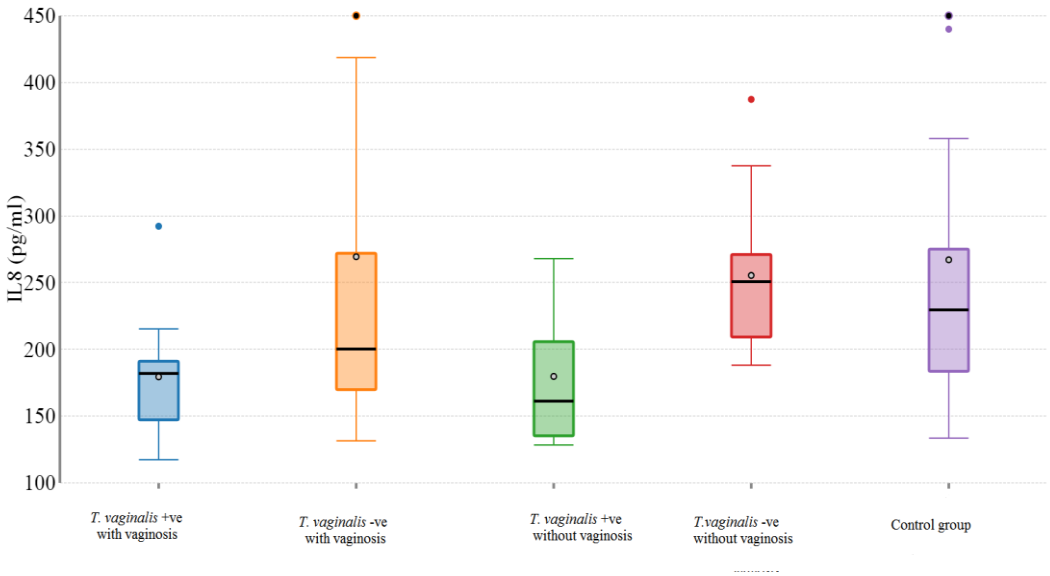

Figure 9-IL-8 level in *T. vaginalis* group with vaginosis and non-vaginosis groups

3. Discussion

4.1 Infection rate of *T. vaginalis*

T. vaginalis belongs to the group of neglected parasitic infections (NPIs) which has been causing sexually transmitted diseases worldwide [7]. Accurate diagnosis and effective control management of sexually transmitted infections represent important strategies for the prevention, since the parasite causes adverse health problems that include infertility, HIV infection, and pelvic inflammatory disease [30]. In the current study, the total infection rate of *T. vaginalis* was 14.73%, which is considered as a high infection rate. This value is higher than that obtained by an earlier study [17], which showed 5.4% infection rate among 425 women in Dohuk province, north Iraq. However, it is lower than the value reported by another study [18], which showed 15.5% infection rate among 440 women in Mousl province, north Iraq. In addition, it was lower that the 16.6% value reported among 114 women in Baghdad province, central Iraq. The variations in infection rates of *T. vaginalis* among different studies are likely due to some influences, including sample size, diagnostic method used, living circumstances, socio-economic conditions, and immunological standings [31]. Moreover, *T.vaginalis* infection depends on many other factors, including age, sexual activity, association of other STIs, and period of menstrual cycle [32]. No significant differences were noticed among different age groups regarding infection rate of *T. vaginalis*, although high infection rates were noticed among elder women (age group ≥ 45 years). This result was compared with some other studies [33, 34] which showed that the prevalence of *T. vaginalis* infection did not differ by age category. Furthermore, the results agree with the findings of Ginocchio *et al*. [29] who reported that the prevalence of the infection was higher in women ≥ 40 years old. While Sutton *et al*. [36] showed a higher rate (2.8 %) of infection among young women with the age of 18–25 years and above in the population. However, the parasite infected women in all reproductive-age and the prevalence of the infection is unknown [35, 36].

No significant relation was noticed between infertility and infection with *T. vaginalis* in the current study, although some investigators showed that *T. vaginalis* infection likely caused compromising in tubal patency in women, that may have led to infertility [8]. This result disagrees with El-Shazly *et al*., 2001, who showed that *T. vaginalis* plays an important role in female infertility [37].

On other hand, there are limited studies on *T. vaginalis* infection and its correlation with PID. None has looked into the association between trichomoniasis and histologically confirmed PID [8]. In the current study, trichomoniasis was significantly associated with pelvic inflammatory disease. High incidence of the infection was reported in women with PID group versus non PID group. This association may be occurring due to untreated *T. vaginalis* infection or as a result of co-infection with other SIDs [29]. These results agree with that stated by Cherpes *et al.* [35] who reported a significant correlation between acute endometritis and fallopian tube abstraction with *T. vaginalis* infection, HSV-2, and other bacterial vaginosis conditions. These results also agree with those reported by Trigg *et al*. [38], Moodley *et al*. [39] and Paisarntantiwong *et al.* [40]. who stated the association of PID with sexually transmitted infection, including *T. vaginalis* infection.

The results showed that the association of the PID with *T. vaginalis* infection was higher among fertile women compered to infertile women.

Usually, the fertile are less likely to make screening and attend gynecological clinic, or they might have asymptomatic infection or fail to have successful treatment [21]. In addition, some fertile women are less likely to use contraceptive methods. This explanation agrees with Baeten *et al.* [41], who stated that there was association between women who used contraceptive pills and the increased risk of acquiring pelvic inflammatory disease, chlamydia, and vaginal candidiasis. Also, there was an association between PID and using intrauterine device [27]. Moreover, using birth control, like oral contraceptive, was reported to confer a protective barrier against *T. vaginalis* infection, although it was not protective against bacterial and yeast vaginosis [42]. In this current study, no significant association was found between contraceptive uses and low infection rate of *T. vaginalis*. This result agrees with that of Mahdi *et al*. [20] who found that *T. vaginalis* infection was not associated with contraceptive use. On the other hand, our finding disagreed with some other studies. This disagreement is likely due to differences in the age groups, sample size, or association of *T. vaginalis* with other sexually transmitted infections. It was noticed from the results of the current study that there was no infection (0%) among the use of condom. This agrees with Holmes *et al*. [43] who reported that the use of condoms may be associated with protecting women against *T. vaginalis* and other sexually transmitted infections.

The results of the current study showed that higher infection rate was noticed among women with high BMI compared with women who had normal BMI, although no statistical association was noticed. The high infection rate of *T. vaginalis* among women with high BMI can be due to the role of specific factors, such as obesity-associated hormonal and metabolic dysfunctions and dietary habits; all these factors can be correlated indirectly to STI [44]. This finding agrees with Lokken *et al.* [44] and DeMaria *et al.* [45] who stated that women with higher BMI were less likely to be diagnosed with STD than those with a normal BMI. Our results are in contrast to the results of Leech *et al.* [46] and Kershaw *et al*. [47] who found that women with high body mass index were more likely to have STI than those with normal BMI.

4.2 Clue cells, vaginal pH, and vaginal leukocytes

Lactobacilli, as normal flora, stabilize vaginal environment and maintain a normal vaginal pH (3.8 to 4.2) by generating lactic acid [48]. *T. vaginalis* and bacterial vaginosis infections are associated with elevated pH, to values higher than 4.5, and clue cell count to higher than 20% per one field of microscopic examination at 40X magnification. These factors can be used as diagnostic features to detect the infection [49, 50]. The results of the current study showed significant elevation of both pH value and clue cell count among women with *T.vaginalis*. These findings agree with the findings presented by Van Der Pol *et al*. [51], who identified clue cells in 11.5% of women infected with *T. vaginalis*. These results also agree with the same study regarding pH, which confirmed the significant difference between elevated pH

and high clue cell count in women with *T. vaginalis* and vaginosis infection as compared to women with no infection. The current results showed high values of both vaginal pH and clue cells among PID women compared with non PID women. These results can be due to the decline in *Lactobacillus* numbers, which may contribute to overgrowth of some pathogenic bacteria, resulting in the subsequent development of PID [52]. On the other hand, no significant correlation was noticed between high leukocyte count and *T. vaginalis* positive status, although about half of the *T.vaginalis* +ve women and the majority of the PID positive women had high leukocyte count, regardless of *T. vaginalis* status. These results are likely due to the infection and inflammation of the upper genital tract that characterize women who had PID [53]. This finding agrees with that of an earlier work [54], which found no correlation between vaginal leucocytosis and vaginal infections. Another study showed that secretory leukocyte protease inhibitor (SLPI) concertation was lower in women with *T. vaginalis* infection [28]. However, there are many factors that might elevate leukocyte count among women, which include viral infection and urinary tract infection; some were not considered in this study.

4..3 IL-1 and IL-8

The virulence factors of *T. vaginalis* are responsible for the evasion of the host immunity. The interaction between the parasite and inflammatory response remains unclear. Although the host may have developed antibodies, but reinfection may still occur [28]. The results of the current study showed low level of IL-1α in *T. vaginalis* infected women compared with the control group. This finding may indicate that *T. vaginalis* cytoadherence induced cytokine modulation and down-regulated the expression of proinflammatory response [55]. Low level of IL-8 was also noticed in *T.vaginalis* +ve women compared with the control group; this may be due to the possibility that *T. vaginalis* generates an immunosuppressive response in monocytes, macrophages, and dendritic cells to evade the host immunity [56]. These results agree with those of Shaker and Hussein [57], who reported a low level of IL-8 among *T. vaginalis* positive women. Moreover, this result agrees with the same study regarding the non-significant differences between the infected and control groups. These results also agree with other investigators, including Shaio *et al.* [58] who reported an approximately 55% reduction in the half-life of IL-8 mRNA in monocytes culture treated with membrane *T. vaginalis* components. However, our results disagree with some other studies, such as that of Kucknoor *et al*. [59] and Ali [60]; they found a higher level of IL-8 in both acute and chronic *T. vaginalis* infections. *T. vaginalis* releases extracellular exosome-like vesicles and other factors that play important roles in immunomodulation of some interleukins in response to infection [5]. This fact can explain the decrease in Both IL-1α and IL-8 in our study population. The finding of the current study provided some insights about some clinical aspects of *T. vaginalis* infection among infertile and PID women. *T. vaginalis* is highly predictable in women with PID. Consequently, attention and more investigation would be required on this topic in Iraq.

References

[1] Kissinger, Patricia. "*Trichomonas vaginalis*: a review of epidemiologic, clinical and treatment issues," *BMC infectious diseases*, vol. 15, no. 1, pp. 1-8, 2017.

[2] Meites, Elissa, Charlotte A. Gaydos, Marcia M. Hobbs, Patricia Kissinger, Paul Nyirjesy, Jane R. Schwebke, W. Evan Secor, Jack D. Sobel, and Kimberly A. Workowski. "A review of evidence-based care of symptomatic trichomoniasis and asymptomatic *Trichomonas vaginalis* infections," *Clinical infectious diseases*, vol. 61, no. suppl_8 pp. S837-S848, 2015.

[3] World Health Organization. *Global incidence and prevalence of selected curable sexually transmitted infections-2008*. World Health Organization, 2012.

[4] Hammond, T. L., G. D. Hankins, and R. R. Snyder. "Transvaginal-peritoneal migration of *Trichomonas vaginalis* as a cause of ascites. A report of two cases." *The Journal of reproductive medicine*, vol. 35, no. 2, pp.179-181, 1990.

[5] Olmos-Ortiz, L. M., M. A. Barajas-Mendiola, M. Barrios-Rodiles, L. E. Castellano, S. Arias-Negrete, E. E. Avila, and P. Cuéllar-Mata. "*Trichomonas vaginalis* exosome-like vesicles modify the cytokine profile and reduce inflammation in parasite-infected mice." *Parasite immunology*, vol. 39, no. 6, pp. e12426, 2017.

[6] Thurman, Andrea R., and Gustavo F. Doncel. "Innate immunity and inflammatory response to *Trichomonas vaginalis* and bacterial vaginosis: relationship to HIV acquisition." *American journal of reproductive immunology*, vol. 65, no. 2, pp. 89-98, 2011.

[7] Mielczarek, Ewelina, and Joanna Blaszkowska. "*Trichomonas vaginalis*: pathogenicity and potential role in human reproductive failure." *Infection*, vol. 44, no. 4, pp. 447-458, 2016.

[8] Wiringa, Ann E., Roberta B. Ness, Toni Darville, Richard H. Beigi, and Catherine L. Haggerty. "*Trichomonas vaginalis*, endometritis and sequelae among women with clinically suspected pelvic inflammatory disease." *Sexually transmitted infections*, vol. 96, no. 6, pp. 436-438, 2020.

[9] Tsevat, Danielle G., Harold C. Wiesenfeld, Caitlin Parks, and Jeffrey F. Peipert. "Sexually transmitted diseases and infertility." *American journal of obstetrics and gynecology*, vol. 216, no. 1, pp. 1-9, 2017.

[10] Rezk, Mohamed, Tarek Sayyed, Alaa Masood, and Ragab Dawood. "Risk of bacterial vaginosis, *Trichomonas vaginalis* and Candida albicans infection among new users of combined hormonal contraception vs LNG-IUS." *The European Journal of Contraception & Reproductive Health Care*, vol. 22, no. 5, pp. 344-348, 2017.

[11] Henriquez, Fiona L., Ronnie Mooney, Timothy Bandel, Elisa Giammarini, Mohammed Zeroual, Pier Luigi Fiori, Valentina Margarita, Paola Rappelli, and Daniele Dessì. "Paradigms of protist/bacteria symbiosis affecting human health: *Acanthamoeba* species and *Trichomonas vaginalis*." *Frontiers in Microbiology*, vol. 11, pp. 3380, 2020.

[12] Dessì, Daniele, Valentina Margarita, Anna Rita Cocco, Alessandra Marongiu, Pier Luigi Fiori, and Paola Rappelli. "*Trichomonas vaginalis* and *Mycoplasma hominis*: new tales of two old friends." *Parasitology*, vol. 146, no. 9, pp. 1150-1155, 2019.

[13] Rein, Michael F., James A. Sullivan, and Gerald L. Mandell. "Trichomonacidal activity of human polymorphonuclear neutrophils: killing by disruption and fragmentation." *Journal of Infectious Diseases*, vol. 142, no. 4, pp. 575-585, 1980.

[14] Rosales, Carlos. "Neutrophil: a cell with many roles in inflammation or several cell types?" *Frontiers in physiology*, vol. 9, pp. 113, 2018.

[15] Kolaczkowska, Elzbieta, and Paul Kubes. "Neutrophil recruitment and function in health and inflammation." *Nature reviews immunology*, vol. 13, no. 3, pp. 159-175, 2013.

[16] Bhakta, Suhani B., Jose A. Moran, and Frances Mercer. "Neutrophil interactions with the sexually transmitted parasite *Trichomonas vaginalis*: implications for immunity and pathogenesis." *Open biology*, vol. 10, no. 9, pp. 200192, 2020.

[17] Al Saeed, W. M. "Detection of *Trichomonas vaginalis* by different methods in women from Dohok province, Iraq." *EMHJ-Eastern Mediterranean Health Journal, vol. 17, no.9, pp. 706-709, 2011*.

[18] AAl-Daheen, Ghada, Nawfal Y Al-Dabbagh, and Haytham M Al-Habib. "The prevalence of *Trichomonas vaginalis* in association with other micro-organisms among women with vaginal discharge in Mosul." *Annals of the College of Medicine, Mosul*, vol. 31, no. 1, pp. 37-44, 2005.

[19] Bedair, Nada H., and Hayder Z. Ali. "Comparison of Trichomoniasis Diagnosis by Microscopic Methods and Indirect ELISA Technique in a Sample of Iraqi Women." *Iraqi Journal of Science*, pp. 742-748, 2020.

[20] Mahdi, N. K., Z. H. Gany, and M. Sharief. "Risk factors for vaginal trichomoniasis among women in Basra, Iraq." *EMHJ-Eastern Mediterranean Health Journal, vol. 7, no. 6., pp. 918-924, 2001*.

[21] Soper, David E. "Pelvic inflammatory disease." *Obstetrics & Gynecology*, vol. 116, no. 2, pp. 419-428, 2010.

[22] Scamell, Mandie, and Mary Stewart. "Time, risk and midwife practice: the vaginal examination." *Health, risk & society*, vol. 16, no. 1, pp. 84-100, 2014.

[23] Domeika, Marius, L. Zhurauskaya, Alevtina Savicheva, Natalia Frigo, Evgenij Sokolovskiy, Anders Hallén, Magnus Unemo, R. C. Ballard, and EE SRH Network. "Guidelines for the laboratory diagnosis of trichomoniasis in East European countries." *Journal of the European Academy of Dermatology and Venereology*, vol. 24, no. 10, pp. 1125-1134, 2010.

[24] Rotimi, V. O., Z. Yakubu, O. O. Abudu, and T. O. Banjo. "Direct Gram's stain of vaginal discharge as a means of diagnosing bacterial vaginosis." *Journal of medical microbiology*, vol. 35, no. 2, pp. 103-106, 1991.

[25] Ponka, David, and Faisal Baddar. "Microscopic potassium hydroxide preparation." *Canadian Family Physician*, vol. 60, no. 1, pp. 57-57, 2014.

[26] Amsel, Richard, Patricia A. Totten, Carol A. Spiegel, Kirk CS Chen, David Eschenbach, and King K. Holmes. "Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations." *The American journal of medicine*, vol. 74, no. 1, pp. 14-22, 1983.

[27] Kessel, Elton. "Pelvic inflammatory disease with intrauterine device use: a reassessment." *Fertility and sterility*, vol. 51, no. 1, pp. 1-11, 1989.

[28] Huppert, Jill S., Bin Huang, Chen Chen, Hassan Y. Dawood, and Raina N. Fichorova. "Clinical evidence for the role of *Trichomonas vaginalis* in regulation of secretory leukocyte protease inhibitor in the female genital tract." *The Journal of infectious diseases*, vol. 207, no. 9, pp. 1462-1470, 2013.

[29] Ginocchio, C. C., K. Chapin, J. S. Smith, J. Aslanzadeh, J. Snook, C. S. Hill, and Charlotte A. Gaydos. "Prevalence of *Trichomonas vaginalis* and coinfection with Chlamydia trachomatis and Neisseria gonorrhoeae in the United States as determined by the Aptima *Trichomonas vaginalis* nucleic acid amplification assay." *Journal of clinical microbiology*, vol. 50, no. 8, pp. 2601-2608, 2012.

[30] Workowski, Kimberly A., and Stuart M. Berman. "Centers for Disease Control and Prevention sexually transmitted disease treatment guidelines." *Clinical infectious diseases*, vol. 53, no. suppl_3, pp. S59-S63, 2011.

[31] Nu, Phuong Anh Ton, Vu Quoc Huy Nguyen, Ngoc Thanh Cao, Daniele Dessì, Paola Rappelli, and Pier Luigi Fiori. "Prevalence of *Trichomonas vaginalis* infection in symptomatic and asymptomatic women in Central Vietnam." *The Journal of Infection in Developing Countries*, vol. 9, no. 06, pp. 655-660, 2015.

[32] Adegbaju, A., and O. A. Morenikeji. "Cytoadherence and pathogenesis of *Trichomonas vaginalis*." *Sci. Res. Essay*, vol. 3, no. 4, pp. 132-138, 2008.

[33] Allsworth, Jenifer E., Jane Alyce Ratner, and Jeffrey F. Peipert. "Trichomoniasis and other sexually transmitted infections: results from the 2001–2004 NHANES surveys." *Sexually transmitted diseases*, vol. 36, no. 12, pp. 738, 2009.

[34] Tompkins, Erin L., Thomas A. Beltran, Elizabeth J. Gelner, and Aaron R. Farmer. "Prevalence and risk factors for *Trichomonas vaginalis* infection among adults in the US, 2013–2014." *PloS one*, vol. 15, no. 6, pp. e0234704, 2020.

[35] Cherpes, Thomas L., Harold C. Wiesenfeld, Melissa A. Melan, Jeffrey A. Kant, Lisa A. Cosentino, Leslie A. Meyn, and Sharon L. Hillier. "The associations between pelvic inflammatory disease, *Trichomonas vaginalis* infection, and positive herpes simplex virus type 2 serology." *Sexually transmitted diseases*, vol. 33, no. 12, pp. 747-752, 2006.

[36] Sutton, Madeline, Maya Sternberg, Emilia H. Koumans, Geraldine McQuillan, Stuart Berman, and Lauri Markowitz. "The prevalence of *Trichomonas vaginalis* infection among reproductive-age women in the United States, 2001–2004." *Clinical infectious diseases*, vol. 45, no. 10, pp. 1319-1326, 2007.

[37] El-Shazly, A. M., H. M. El-Naggar, M. Soliman, M. El-Negeri, H. E. El-Nemr, A. E. Handousa, and T. A. Morsy. "A study on *Trichomoniasis vaginalis* and female infertility." *Journal of the Egyptian Society of Parasitology*, vol. 31, no. 2, pp. 545-553, 2001.

[38] Trigg, Bruce G., Peter R. Kerndt, and Getahun Aynalem. "Sexually transmitted infections and pelvic inflammatory disease in women." *Medical Clinics of North America*, vol. 92, no. 5, pp. 1083-1113, 2008.

[39] Moodley, Prashini, David Wilkinson, Cathy Connolly, Jack Moodley, and A. Willem Sturm. "*Trichomonas vaginalis* is associated with pelvic inflammatory disease in women infected with human immunodeficiency virus." *Clinical Infectious Diseases*, vol. 34, no. 4, pp. 519-522, 2002.

[40] Paisarntantiwong, Rita, Susan Brockmann, Lorraine Clarke, Sheldon Landesman, Joseph Feldman, and Howard Minkoff. "The relationship of vaginal trichomoniasis and pelvic inflammatory disease among women colonized with Chlamydia trachomatis." *Sexually transmitted diseases*, vol. 22, no. 6, pp. 344-347, 1995.

[41] Baeten, Jared M., Patrick M. Nyange, Barbra A. Richardson, Ludo Lavreys, Bhavna Chohan, Harold L. Martin Jr, Kishorchandra Mandaliya, Jeckoniah O. Ndinya-Achola, Job J. Bwayo, and Joan K. Kreiss. "Hormonal contraception and risk of sexually transmitted disease acquisition: results from a prospective study." *American journal of obstetrics and gynecology*, vol. 185, no. 2, pp. 380-385, 2001.

[42] Barbone, Fabio, Harland Austin, William C. Louv, and W. James Alexander. "A follow-up study of methods of contraception, sexual activity, and rates of trichomoniasis, candidiasis, and bacterial vaginosis." *American journal of obstetrics and gynecology*, vol. 163, no. 2, pp. 510-514, 1990.

[43] Holmes, King K., Ruth Levine, and Marcia Weaver. "Effectiveness of condoms in preventing sexually transmitted infections." *Bulletin of the World Health Organization*, vol. 82, pp. 454-461, 2004.

[44] Lokken, Erica M., Barbra A. Richardson, John Kinuthia, Khamis Mwinyikai, Amina Abdalla, Walter Jaoko, Kishorchandra Mandaliya, Juma Shafi, and R. Scott McClelland. "A prospective cohort study of the association between body mass index and incident bacterial vaginosis." *Sexually transmitted diseases*, vol. 46, no. 1, pp. 31, 2019.

[45] DeMaria, Andrea L., Jonathan M. Lugo, Mahbubur Rahman, Richard B. Pyles, and Abbey B. Berenson. "Association between body mass index, sexually transmitted infections, and contraceptive compliance." *Journal of Women's Health*, vol. 22, no. 12, pp. 1062-1068, 2013.

[46] Leech, Tamara GJ, and Janice Johnson Dias. "Risky sexual behavior: a race-specific social consequence of obesity." *Journal of youth and adolescence*, vol. 41, no. 1, pp. 41-52, 2012.

[47] Kershaw, Trace S., Anna Arnold, Jessica B. Lewis, Urania Magriples, and Jeannette R. Ickovics. "The skinny on sexual risk: the effects of BMI on STI incidence and risk." *AIDS and Behavior*, vol. 15, no. 7, pp. 1527-1538, 2011.

[48] Ahmet, A. L. İ. M., Ali Çetin, and Çağlar YILDIZ. "Evaluation of vaginal flora and susceptibility test of microorganisms in reproductive-age women with or without vaginitis in primary care settings." *Cumhuriyet Tıp Dergisi*, vol. 31, no. 2, pp. 116-121, 2009.

[49] Neal, Chemen M., Lauren H. Kus, Linda O. Eckert, and Jeffrey F. Peipert. "Noncandidal vaginitis: a comprehensive approach to diagnosis and management." *American journal of obstetrics and gynecology*, vol. 222, no. 2, pp. 114-122, 2020.

[50] Mittal, Vineeta, Amita Jain, and Yashodhara Pradeep. "Development of modified diagnostic criteria for bacterial vaginosis at peripheral health centres in developing countries." *The Journal of Infection in Developing Countries*, vol. 6, no. 05, pp. 373-377, 2012.

[51] Van Der Pol, Barbara, James A. Williams, Donald P. Orr, Byron E. Batteiger, and J. Dennis Fortenberry. "Prevalence, incidence, natural history, and response to treatment of *Trichomonas vaginalis* infection among adolescent women." *The Journal of infectious diseases*, vol. 192, no. 12, pp. 2039-2044, 2005.

[52] Gradison, Margaret. "Pelvic inflammatory disease." *American family physician*, vol. 85, no. 8, pp. 791-796, 2012.

[53] Mitchell, Caroline, and Malavika Prabhu. "Pelvic inflammatory disease: current concepts in pathogenesis, diagnosis and treatment." *Infectious Disease Clinics*, vol. 27, no. 4, pp. 793-809, 2013.

[54] Larsson, Per-Göran, and Jens-Jörgen Platz-Christensen. "Bacterial vaginosis and the vaginal leucocyte/epithelial cell ratio in women attending an outpatient gynaecology clinic." *European Journal of Obstetrics & Gynecology and Reproductive Biology*, vol. 42, no. 3, pp. 217-220, 1991.

[55] Chang, Jae-Ho, Yong-Suk Ryang, Tomohiro Morio, Sang-Kyou Lee, and Eun-Ju Chang. "*Trichomonas vaginalis* Inhibits Proinflammatory Cytokine Production in Macrophages by

Suppressing NF-κB Activation." *Molecules & Cells (Springer Science & Business Media BV)*, vol. 18, no. 2, 2004.

[56] Fichorova, Raina N. "Impact of T. vaginalis infection on innate immune responses and reproductive outcome." *Journal of reproductive immunology*, vol. 83, no. 1-2, pp. 185-189, 2009.

[57] Shaker, Mohammed Jasim, and Rawaa Abdulkhaleq Hussein. "Immunological study of women infected with *Trichomonas vaginalis* parasite in Baghdad city." *International Journal of Science and Research*, vol. 3, no. 9, pp. 1145-1148, 2014.

[58] Shaio, Men-Fang, Pey-Ru Lin, Jah-Yao Liu, and Kuender D. Yang. "Generation of interleukin-8 from human monocytes in response to *Trichomonas vaginalis* stimulation." *Infection and immunity*, vol. 63, no. 10, pp. 3864-3870, 1995.

[59] Kucknoor, Ashwini S., Vasanthakrishna Mundodi, and John F. Alderete. "The proteins secreted by *Trichomonas vaginalis* and vaginal epithelial cell response to secreted and episomally expressed AP65." *Cellular microbiology*, vol. 9, no. 11, pp. 2586-2597, 2007.

[60] Ali, Hayder Z. "Detection of Pro-Inflammatory IL-8 and IL-12 in Iraqi Women Infected with Trichomoniasis." *Iraqi Journal of Science*, vol. 62, no.2, pp. 449-454, 2021.