

Detection of Interleukin-8, Interleukin-10 and Tumor Necrosis Factor alpha (TNF- α) among pregnant women infected with acute Trichomoniasis

Entisar Mahdi Hamad

Department of Medical Laboratory Technology Diyala, Middle Technical University, Technical Institute, Baquba, Iraq



This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

<https://doi.org/10.54153/sjpas.2024.v6i4.865>

Article Information

Received: 14/04/2024

Revised: 15/05/2024

Accepted: 20/05/2024

Published: 30/12/2024

Keywords:

Trichomoniasis; Infected women; Immunoglobulin's; Cytokines; mmp-9.

Corresponding Author

E-mail:

entisarmahdi198080@gmail.com

Mobile:

Abstract

Trichomoniasis is one of the most common sexually transmitted diseases caused by *Trichomonas vaginalis* parasite, affecting females in particular at different ages. This study was conducted between September 2023 and March 2024 at Al-Batool Teaching Hospital for gynecological examination. A total of fifty high vaginal swab (HVS) and serum samples were collected from symptomatic women of various ages presenting with vaginal itching. High vaginal swabs were obtained from each woman and diagnosed microscopically then immunological test is performed by using the enzyme-linked immunosorbent assay (ELISA) technique. The present study shows that of the total 50 patients, 21 (42%) were infected with *Trichomonas vaginalis*, and 29 (58%) were negative for *Trichomonas vaginalis*. Further, there was a significant increase in the mean of IgM, IgG, MMP-9 and TNF- α in infected women with *Trichomonas vaginalis* that were (8.7 \pm 3.1 ng/dl, 9.01 \pm 1.90 ng/dl, 5.01 \pm 1.74 ng/ml, and (145.808 + 1.81) pg/ml, respectively, as compared with control. While there were no differences in IL-8 and IL-10 levels in infected women compared to control. This study concluded increase IgM, IgG, mmp-9, and TNF- α in infected women with *Trichomonas vaginalis*, while no differences in IL-8, IL10.

Introduction:

Trichomonas vaginalis is one of the crucial flagellate parasites that lacks a cystic stage and exists in only one stage, which is the trophozoite stage [1]. The parasite causes trichomoniasis, which is the most prevalent non-viral sexually transmitted disease in the world, as it affects the urinary and reproductive systems of both males and females [2, 3]. Preterm labor and low birth weight babies are associated with *T. vaginalis* infection [4]. According to the analysis by the World Health Organization, there are about 170 million cases of infection around the world [5], and in 2016 the rate of parasite prevalence reached over 270 million people annually [6]. In Iraq over the last ten years verified the affirmative diagnosis in women who regularly visit government hospitals in Baghdad, Kufa, Najaf, and Mosul [7]. In Ramadi showed that the infection rate was 24.5% among infected women who had symptoms and 13.6% among women who did not have symptoms [8]. Most infected men are usually asymptomatic.

On the other hand, the infected women are suffering from dysuria, cervicitis, and vaginitis related to abnormal vaginal discharge with a foul odor and possible infertility [9, 10]. *Candida* (monilia) or yeasts cause vaginal infection, as well as being present in the vagina of uninfected women [11]. The parasite infects the genital tract in men, particularly the urethra and prostate [12]. In pregnant women, *T. vaginalis* may cause premature birth, abnormal infant weight, and, if not treated, an increased likelihood of transmission from the infected mother to the child [13]. The immunological response of the host, whether active or passive T-cell mediated, plays a significant role in controlling or aggravating trichomoniasis [14]. A low quantity of IL-2 and IFN- γ was observed in both serum and vaginal secretion of mice that were experimentally infected with *T. vaginalis*. The activation of the resident Natural Killer cells by the trichomonads, on the other hand, leads to the synthesis of IFN- γ , IL-10, and IL-8 by macrophages and cytotoxic T cells [15].

The occurrence of trichomoniasis mainly depends on several risk factors, including age, sexual activity, number of sexual partners, promiscuity of a sexual partner, use of drugs, and low socioeconomic conditions [16]. The traditional approach to diagnose the parasite is by directly examining a wet vaginal swab or urine examination under a microscope. The method relies on expertise and is subjective, with an accuracy of less than 60%. The study was limited to women who showed signs of infection. In the current study, we examined the potential immunological effects of the parasite during infection. We determined the levels of immunoglobulin G (IgG), matrix metalloproteinases-9 (MMP-9), Interleukin-8 (IL-8), Interleukin-10 (IL-10), Tumor Necrosis Factor alpha (TNF- α) in the serum of women with trichomoniasis during the acute and chronic phases of the disease.

Materials And Methods

This study was conducted between September 2023 and March 2024 at Al-Batool Teaching Hospital for gynecological examination. A total of fifty high vaginal swabs (HVS) and serum samples were collected from symptomatic women of various ages presenting with vaginal itching. High vaginal swabs were obtained from each participant women. Exclusion criteria included women with chlamydial infection or other medical conditions such as diabetes, rubella, autoimmune diseases, blood and circulatory disorders, hormonal disorders, or other infectious diseases.

Samples examination

For the microscopic examination of vaginal swab samples, two distinct staining techniques were employed to identify *T. vaginalis*. Immediately following collection, each swab sample was placed on a sterile glass slide and mixed with appropriate staining solutions. The preparation was then covered with a slip and examined under a light microscope using a 40 \times objective lens. This method enables the detection motile and teardrops which are considered hallmark of *T. vaginalis*. The samples were directly examined after collection for assurance of optimal identification the parasites [17].

ELISA test

The enzyme-linked immunosorbent assay (ELISA) was utilized to evaluate the serum concentrations of immunoglobulin M (IgM), immunoglobulin G (IgG), MMP-9, IL-18, IL-10,

and TNF- α . The antibodies for IgM, IgG, IL-8, IL10, TNF- α , and MMP-9 were precoated on the plates of the ELISA. The chromogenic response in substrate solutions is related to the concentration of IgM, IgG, IL-8, IL10, TNF- α , and MMP-9 after putting the samples on plates. The cessation of the process was achieved through the introduction of a stop solution, followed by the quantification of absorbance at a wavelength of 450 nm.

The level of IgM and IgG were measured by using (DRG, Germany). The level IgM, IgG, IL-8, IL10, TNF- α , and MMP-9 were measured in serum by using (Sunlong, China).

Statistical analysis

The SPSS application, version 20, was utilized to perform analysis on all of the data. We utilized a t-test in order to compare the mean levels of cytokines between the patients and the control group. The levels of cytokines that were tested are shown as (Mean \pm SD). For every test, we determined the significance level to be 0.05 ($P \leq 0.05$).

Results and Discussion

The present study shows that of the total 50 patients, 21 (42%) were infected with *T. vaginalis* and 29 (58%) were negative for *T.vaginalis*, as shown in Table (1).

Table 1: Distribution of not-infected and infected cases with *T. vaginalis* parasite

Study groups	Number	Percentage
Not infected	29	58
Infected	21	42
Total	50	100

This study shows significant increase the mean of IgM in infected women with *T. vaginalis* as compared with control that were (8.7 \pm 3.1, 5.9 \pm 0.5ng/dl) respectively. In addition, increased IgG in infected women with *T. vaginalis* as compared with control that were (9.01 \pm 1.90, 6.33 \pm 1.69ng/dl) respectively, at p-value <0.05, as shown in Table (2).

Table 2: Immunoglobulin level in infected and non- infected women with *T.vaginalis*

Parameters	Not infected(control)	Infected(patients)	P-value
IgM (ng/dl)	5.9 \pm 0.5	8.7 \pm 3.1	0.03
IgG(ng/dl)	6.33 \pm 1.69	9.01 \pm 1.90	0.007

This study shows a significant increase in MMP-9 in infected women (5.01 \pm 1.74) ng/ml, as compared with control (1.34 \pm 0.98) ng/ml at p-value (<0.05). Furthermore a significant increase in TNF- α in infected women (145.808 \pm 1.81) pg/ml, as compared with control (70.09 \pm 0.64) pg/ml at p-value (<0.05). While no differences in IL-8 and IL-10 levels in infected women (4.056 \pm 1.41, 92.46 \pm 1.97) pg/ml respectively, and non-infected women that were (3.918 \pm 0.47, 89.75 \pm 1.38) pg/ml respectively at p-value (>0.05).

Table 3: Levels of MMP-9 in women patients compared to control

Parameters	Not infected(control)	Infected(patients)	P-value
MMP-9 ng/ml	1. 1.34±0.98	5.01 ± 1.74	0.001
IL-8(pg/ml)	2. 3.918± 0.47	4.056± 1.41	0.16
IL-10(pg/ml)	89.75±1.38	92.46±1.97	0.07
TNF-α(pg/ml)	70.09 ± 0.64	145.808 ± 1.81	0.002

Trichomonas vaginalis infection is experienced by women from different countries and ethnicities around the world, because is one of the most prevalent non-viral sexually transmitted diseases. In 2016, Baghdad had the most significant infection rate of *T. vaginalis* at 85.5%[7]. The study done by [18] in Erbil showed the lowest infection rate which was 3.1% in 2015. The high infection rate reported in Baghdad city is attributed to the large female population affected by this parasite infection. Several factors contributed in the transmission of parasites such as lack of health education, and insufficient immunity. As regards parasite, insufficient information about *T. vaginalis* and poor cleanliness[7]. The study done by [19]. In Basra , the prevalence of *T. vaginalis* was 55.4% as well as in Babylon was 50% [20].

The study shows an increase IgM and IgG in infected women as compared with non-infected women, this study agrees with LAZAR and ABASS,(2022) that show an increase IgM and IgG in infected women with *T. vaginalis* as compared with control[21]. Based on the results of a study, it was observed that the concentration of IgG in the bloodstream of females afflicted with a parasite exhibited an elevation compared to the control cohort. This implies that there is a high prevalence of chronic infections, particularly among married women, as a result of repeated infections because they do not seek treatment for them[22]. Furthermore, in addition to the fact that the majority of women who are infected with *T. vaginalis* do not exhibit any symptoms of the infection. To control parasite infection, immunoglobulin, specifically immunoglobulin G, plays a crucial function in humoral immunity and control the parasitic infection[23]. During their research, Nouraddin (2019) reported that women infected with the parasite had more immunoglobulin M (IgM) in their serum. This was compared to the control group, which indicated that acute infection with *T. vaginalis* stimulated the humoral immune response. This is because IgM is an indicator of the acute period of infection.[24].

The current study shows an increase MMP-9 levels in infected women as compared with control. This study agrees with YASIR *et al.*,(2022) reported that the increase in the level of MMP-9 in infected patients suggested that MMP-9 plays an important role in immunity against infection in both males and females infected with *T. vaginalis*. In addition, the level of MMP-9 was increased in females more than in males [25]. This study shows no differences in the level of IL-8 and IL-10 in infected women as compared with control. Kadhum *et al.* (2020) showed that increase IL-8 in infected women [26]. Some studies showed that no differences in IL-10 level [24].]. On the other hand, IL-10 contributes to the inhibition of Th1 immunity and favors a T helper Type 2 response [27, 28].

The study done by HAN *et al.*,(2009) demonstrated an increase in TNF- α levels in infected women compared to the control group. Furthermore, the TNF- α production that *T. vaginalis* caused in the study was significantly reduced by pretreatment with L-NMMA in human macrophages. This finding suggests that nitric oxide plays a significant role in producing TNF- α that *T. vaginalis* induces [29]. The Tv α -actinin2 antigen, which is considered to be one of the most significant *T. vaginalis* antigens, was discovered to be responsible for the generation of cytokines in experimental vaginal epithelial cells. During the initial 16 hours of screening, it was revealed that the levels of IL-10, IL-12, IL-6, and TNF- α were observed to increase. As a cytokine produced by macrophages, T-lymphocytes, basophils, and monocytes, TNF- α is classified as a Th1 response cytokine [30]. One of the most important events that occurs during the beginning stages of immunization to *T. vaginalis* is the production of a type 1 inflammatory cytokine response, which includes TNF- α and IFN- α [24] [31]. Several studies have demonstrated that cytokines play a significant part in the pathophysiology of *T. vaginalis* diseases[9] [32].

Conclusion

This study concluded that the highest percentage of infection within *T. vaginalis* was shown in women. The results revealed that MMP-9 and TNF- α play an important role in immunity against infection in infected women with *T. vaginalis*. In addition, there were no differences in proinflammatory cytokines (interleukin 8 and 10) in *T. vaginalis*-infected women in comparison with non-infected women.

Funding

No funding entity for this research.

Conflicts of interest

There are no conflicts of interest.

References

1. Sood S, Kapil A. An update on Trichomonas vaginalis. Indian Journal of Sexually Transmitted Diseases and AIDS. 2008;29(1):7-14.
2. Newman L, Rowley J, Vander Hoorn S, Wijesooriya NS, Unemo M, Low N, et al. Global estimates of the prevalence and incidence of four curable sexually transmitted infections in 2012 based on systematic review and global reporting. PloS one. 2015;10(12):e0143304.
3. Marquardt WC, Demaree RS, Grieve RB. Parasitology and vector biology. (No Title). 2000.
4. Sutton M, Sternberg M, Koumans EH, McQuillan G, Berman S, Markowitz L. The prevalence of Trichomonas vaginalis infection among reproductive-age women in the United States, 2001–2004. Clinical infectious diseases. 2007;45(10):1319-26.

5. Johnston VJ, Mabey DC. Global epidemiology and control of *Trichomonas vaginalis*. *Current opinion in infectious diseases*. 2008;21(1):56-64.
6. Menezes CB, Frasson AP, Tasca T. Trichomoniasis-are we giving the deserved attention to the most common non-viral sexually transmitted disease worldwide? *Microbial cell*. 2016;3(9):404.
7. Saheb EJ, Kuba RH, Zghair KH, Mosa IS. A comparison between trichomoniasis Infection and other vaginal infection among females in Baghdad governorate-Iraq. *Iraqi Journal of Science*. 2016:545-51.
8. MITEB MM. ISOLATION AND IDENTIFICATION: University of Kufa; 2000.
9. Zariffard MR, Harwani S, Novak RM, Graham PJ, Ji X, Spear GT. *Trichomonas vaginalis* infection activates cells through toll-like receptor 4. *Clinical immunology*. 2004;111(1):103-7.
10. Mielczarek E, Blaszkowska J. *Trichomonas vaginalis*: pathogenicity and potential role in human reproductive failure. *Infection*. 2016;44:447-58.
11. Organization WH. Report on global sexually transmitted infection surveillance 2018. 2018.
12. Song H-O, Lim Y-S, Moon S-J, Ahn M-H, Ryu J-S. Delayed human neutrophil apoptosis by *Trichomonas vaginalis* lysate. *The Korean Journal of Parasitology*. 2010;48(1):1.
13. Hamilton H, Pontiff KL, Bolton M, Bradbury RS, Mathison BA, Bishop H, et al. *Trichomonas vaginalis* brain abscess in a neonate. *Clinical Infectious Diseases*. 2018;66(4):604-7.
14. Motes HC, Stuart MK. Cytokine array analysis of mediators produced by human macrophages stimulated with *Trichomonas tenax*. *Experimental parasitology*. 2022;242:108382.
15. Ali HZ. Detection of pro-inflammatory IL-8 and IL-12 in Iraqi women infected with trichomoniasis. *Iraqi Journal of Science*. 2021:449-54.
16. Martínez DLC, Barquín LAM. Sexually Transmitted Diseases in Pediatrics. 2022.
17. Hasson KF, Naama JK, Abdullah EE. Detection of *Trichomonas Vaginalis* among women with contraceptive usage in AL-Najaf AL-ashraf city. *University of Thi-Qar Journal Of Medicine*. 2008;2(1):46-50.
18. Nouraddin AS, Alsakee HM. Prevalence of *Trichomonas vaginalis* infection among women in Erbil governorate, Northern Iraq: An epidemiological approach. *European Scientific Journal*. 2015;11(24).
19. Al-Quraishi MA. Epidemiology study of *Trichomonas vaginalis* in Babylon province and the efficiency of mentha spicata leaves extracts in vivo. *Epidemiology*. 2015;4.

20. Al-Mayah QS, Al-Saadi M, Jabbar RN. Trichomonas vaginalis infection as a risk factor for prostate cancer. *Int J Curr Microbiol App Sci.* 2013;2(11):105-13.
21. Lazar LTY, Abass KS. Study of Some Immunological Parameters with Level of Iron in Women Infected with Trichomonas Vaginalis. *Indian Journal of Forensic Medicine & Toxicology.* 2020;14(4):9255-60.
22. Mushrif E, Al-Kuraishi AH, Ad AH. Immunoglobulin levels in serum and cervicovaginal secretions of patients infected with Trichomonas vaginalis. *Al-Nahrain Journal of Science.* 2010;13(2):147-51.
23. Yadav M, Gupta I, Malla N. Kinetics of immunoglobulin G, M, A and IgG subclass responses in experimental intravaginal trichomoniasis: prominence of IgG1 response. *Parasite immunology.* 2005;27(12):461-7.
24. Nouraddin AS, Alsakee HM. Immunological aspects of Trichomonas vaginalis infection in women attending maternity teaching hospital and some public health centers in Erbil governorate, Northern Iraq. *Cihan University-Erbil Scientific Journal.* 2019;3(1):56-60.
25. Yasir AS, Sayal RA, Tikki KA, editors. Immunological and hematological estimation of matrix metalloproteinase-9 (MMP9) level in a serum of female infested through trichomonas vaginalis. *AIP Conference Proceedings; 2022: AIP Publishing.*
26. KADHUM NJ, RAISAN SJ, AL-MAYAH SH. Role IL6, IL8, Hu MIF, NF- κ B-P65 in the development of trichomoniasis. *International Journal of Pharmaceutical Research (09752366).* 2020.
27. González-Fernández D, Pons EdC, Rueda D, Sinisterra OT, Murillo E, Scott ME, et al. C-reactive protein is differentially modulated by co-existing infections, vitamin deficiencies and maternal factors in pregnant and lactating indigenous Panamanian women. *Infectious diseases of poverty.* 2017;6:1-14.
28. Chapwanya A, Usman AY, Irons PC. Comparative aspects of immunity and vaccination in human and bovine trichomoniasis: a review. *Tropical animal health and production.* 2016;48:1-7.
29. Han I-H, Goo SY, Park S-J, Hwang S-J, Kim Y-S, Yang MS, et al. Proinflammatory cytokine and nitric oxide production by human macrophages stimulated with Trichomonas vaginalis. *The Korean journal of parasitology.* 2009;47(3):205.
30. Lee H-Y, Kim J, Ryu J-S, Park S-J. Trichomonas vaginalis α -actinin 2 modulates host immune responses by inducing tolerogenic dendritic cells via IL-10 production from regulatory T cells. *The Korean Journal of Parasitology.* 2017;55(4):375.
31. Sturm-Ramirez K, Gaye-Diallo A, Eisen G, Mboup S, Kanki PJ. High levels of tumor necrosis factor— α and interleukin-1 β in bacterial vaginosis may increase susceptibility to human immunodeficiency virus. *The Journal of infectious diseases.* 2000 Aug 1;182(2):467-73.

32. Al-Mamoori ZZ, Alhisnawi AA, Yousif JJ. Prediction of trichomoniasis in women complaining vaginal discharge by different methods and determine some immunological markers. *Plant Archives*. 2020;20(1):3653-8.

الكشف عن الانترلوكين 8 والانترلوكين 10 وعامل نخر الورم ألفا (TNF- α) بين النساء الحوامل المصابات بداء المشعرات الحاد

انتصار مهدي حمد*

قسم تكنولوجيا المختبرات الطبية دىالى، الجامعة التقنية الوسطى، المعهد التقني، بعقوبة، العراق

الخلاصة:

داء المشعرات هو أحد الامراض الشائعة التي تنتقل جنسياً التي يسببها طفيلي المشعرة المهبليّة، ويصيب الإناث بشكل خاص في اعمار مختلفة. أجريت هذه الدراسة في الفترة ما بين ايلول 2023 واذار 2024 في مستشفى البتول التعليمي لإجراء الفحص النسائي. تم جمع خمسين مسحة مهبليّة اضافة الى عينات مصل الدم من النساء اللاتي يعانين من أعراض الحكة المهبليّة ومن مختلف الاعمار من كل امرأة وتشخيصها مجهرياً ومن ثم إجراء الاختبار المناعي باستخدام تقنية ELISA. اظهرت الدراسة الحالية أنه من إجمالي 50 مريضاً، كان 21 (42%) مصابين بالمشعرات المهبليّة، و29 (58%) كانوا سلبين للمشعرات المهبليّة. علاوة على ذلك، كانت هناك زيادة معنوية في متوسط الاجسام المضادة من نوع IgM و IgG وMMP-9 وTNF- α في النساء المصابات بالمشعرة المهبليّة والتي كانت (3.1 \pm 8.7 نانوجرام/ديسيلتر، 1.90 \pm 9.01 نانوجرام/ديسيلتر، 1.74 \pm 5.01 نانوجرام/مل، و145.808 + 1.81 بيكوغرام / مل) على التوالي، بالمقارنة مع عينات السيطرة. في حين لم يكن هناك فروقات في مستويات IL-8 و IL-10 في النساء المصابات مقارنة بالسيطرة. تم الاستنتاج من هذه الدراسة ان هنالك زيادة في مستويات IgM و IgG و mmp-9 و TNF- α في النساء المصابات بالمشعرة المهبليّة، في حين لا يوجد فروقات في IL-8 و IL-10..

معلومات البحث:

تاريخ الاستلام: 2024/04/14

تاريخ التعديل : 2024/05/15

تاريخ القبول: 2024/05/20

تاريخ النشر: 2024/12/30

الكلمات المفتاحية:

داء المشعرات، النساء المصابات،

المناعية، السيٹوكينات mmp-9

معلومات المؤلف

الايمل: m
الموبايل: