

Study of Some Immunological Parameters with Level of Iron in Women Infected with *Trichomonas Vaginalis*

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Abstract

Trichomonas vaginalis is a flagellated protozoan parasite that causes a disease called trichomoniasis in humans. It affects the urogenital tract and is one of the most important causes of non-viral genitourinary sexually transmitted infection (STI). The host's immune response plays a very important role in the onset of pathological symptoms and their differences between infected people. During the period from July to December 2019, 30 samples were collected, 20 of infected women with *T. vaginalis* and 10 samples as a control group from reviews of external women's clinics in Kirkuk city, whose ages ranged between 18-56 years so this study was designed to know the effect of infection with *T. vaginalis* parasite on some immunological parameters such as the role of humoral immune response based on the production of immunoglobulins IgM, IgG, IgA against the parasite and their estimation in serum by using Enzyme Linked Immunosorbent Assay (ELISA) technique as well as estimating the C-Reactive protein concentration as an indication of systemic inflammation and of serum iron level. The results of the current study showed a "significant" increase in the level of immunoglobulins IgM, IgG in serum of women infected with *T. vaginalis* compared to the control group, as the level of IgM (8.2 ± 2.9 ng/dl), (6.3 ± 0.5 ng/dl) Consequently, at the level ($p < 0.05$), the level of IgG was (8.16 ± 2.20 ng/dl), (6.53 ± 1.69 ng/dl) for the infected women and the control group, respectively. As for the level of IgA, the results showed non-significant increase in the affected women compared with control group (8.22 ± 3.1 ng/dl), (7.82 ± 2.8 ng/dl), respectively. The results of estimating the CRP concentration showed a significant increase in the infected women compared to the control group (5.76 ± 1.56 mg/dl), (4.61 ± 0.66 mg/dl), respectively. While the results of measuring the level of iron showed a significant decrease among infected women and the control group (38.79 ± 8.23 mg/dl), (93.83 ± 34.76 mg/dl), respectively. We conclude from the above that infection with *T. vaginalis* stimulates the humoral immune response and affects on immunoglobulins levels, CRP concentration and Iron serum level.

Keywords: *T. vaginalis*, IgM, IgG, IgA, CRP, Iron level.

Introduction

Trichomonas vaginalis It is one of the flagellated protozoan parasite that causes Trichomoniasis by affecting the urinary genital tract in humans and is one

of the most important causes of non- viral genitourinary sexually transmitted infection (STI) with a prevalence rate of 180 million annually according to WHO estimates⁽¹⁾. *T. vaginalis* has only trophozoite stage with a size of (7-32 μ m) and is pear shaped. It possesses 5 flagellates, 4 of which are forward with a fifth flagella that bends back along the undulating membrane and lacks the cyst stage and possesses a central nucleus and Axostyle, Trophozoite is divided by binary fission. The parasite is an aerotolerant anaerobes that dissolves whole carbohydrates into short chains of organic acids Especially (acetate and lactate) Regardless of the presence of oxygen. The parasite produces hydrogen

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molecules in the absence of oxygen, and this reaction occurs in hydrogenosomes that like mitochondria (which are not present) in *T. vaginalis*, which are energy-producing organelles surrounded by two membranes⁽²⁾.

T. vaginalis live in the vagina and urethra of the female and in the prostate, seminal vesicles and urethra in the male. The incubation period ranges from 4-28 days, and the normal acidity of the vagina pH 4.0-4.5 inhibits infection, but when the parasite moves to the vagina, pH will change in the vagina to the alkalinity pH 5-6 due to metabolic processes of the parasite, which promotes the growth and duplication of it⁽³⁾.

As for the clinical symptoms of trichomoniasis, they have a wide range, ranging from asymptomatic in more than 50% of women to moderate and severe infections. The main causes of the different symptoms are due to the virulence factors of the parasite, the genetic difference and the immune response of the host to the parasite⁽⁴⁾. The symptoms are white to green secretions, frothy discharges, mucopurulent accompanied by severe itching and burning in the affected area, and small hemorrhagic spots in the vaginal and cervical mucosa of the uterus known as strawberry cervix⁽⁵⁾.

Innate immunity and adaptive immunity play an important role in developing trichomoniasis, as innate immunity includes many defensive physical barriers that prevent the transmission of the parasite to the genital tract and include epithelial cells, mucous and acidic pH in the vagina which is caused by the Lactic acid produced by commensal bacteria such as *Lactobacillus spp* in healthy women⁽⁶⁾.

Humoral immunity is an important role in the immune response against trichomoniasis. Local and specific antibodies have been established in the serum of patients with the parasite as well as in experimental studies, as a high level of IgM, IgG, IgA⁽⁷⁾ has been observed. During the latter 40 years have been relying on method and techniques to measure the level of the these immunoglobulins and their classes such as radioimmunoassay and ELISA to find out the role of humoral immunity in eliminating pathogens that enter the body⁽⁸⁾.

C-Reactive Protein is a protein produced from the liver to the blood circulation and stimulates its production by many cytokines such as IL6 and TNF α and plays an important role in the immune response to pathogens that enter the body and is considered as an

indication of systemic infections and evidence of the acute phase and severity of infection, also in chronic infection but to a lesser degree than in acute infections, and vaginitis which caused by *T. vaginalis* is a form of systemic inflammation response⁽⁹⁾.

Iron is an essential ingredient for living organisms, including humans and parasites, because it is necessary for biological processes including oxygen transport, DNA formation, and electron transport⁽¹⁰⁾. Also, some parasites such as *Trichomonas vaginalis*, *Giardia lamblia* and *Entamoeba histolytica* need iron in high concentrations ranging from (5-200 μ m) to their growth compared to other prokaryotic and eukaryotic organisms.

Iron is very necessary in the pathogenesis of *T. vaginalis* as it contributes to cytoadherence in the surface of the epithelial cells of the vagina and regulates the genetic expression of adhesion proteins and its role was confirmed by culture experiment to find the relationship between the virulence of *T. vaginalis* and the role of iron in that⁽¹¹⁾.

Therefore, this study was designed to know the effect of parasite infection on some immunological parameters such as immunoglobulins IgM, IgG, IgA, CRP concentration, iron level in infected women and comparison with control group.

Materials and Method

During the period from July to December 2019, 30 samples were collected, 20 of infected women with *T. vaginalis* and 10 samples as a control group from reviews of external women's clinics in the Kirkuk city, whose ages ranged between 18-56 years and vaginal swabs were taken from posterior vaginal fornix and put it on tube Contains 1ml of normal saline and samples were examined within few minutes with a wet mount examination. A drop of the mixture was placed on a clean slide, then a cover slip was placed on it and examined under 100X and 400X magnification forces to observe a pear shaped trophozoite with jerky motility⁽¹²⁾.

Five milliliters of blood samples were collected from infected women with *T. vaginalis* and control group and serum was obtained by making a centrifuge 3000 rpm for 3 minutes and keeping serum by freezing one section at -20°C to measure the CRP concentration as well as the iron level and the other section -70°C to measure The level of immunoglobulins IgM, IgG, IgA for both groups.

The level of immunoglobulin M (IgM) was measured in serum of infected women and the control group by using (Human IgM ELISA Kit), the principle of this method is based on Sandwich ELISA from (Bethyl Laboratories, Inc. USA) and the attached instructions have been followed by the manufacturer.

The level of immunoglobulin G (IgG) was measured in serum for infected women and the control group by using (Human IgG ELISA Kit), the principle of this method is based on Sandwich ELISA from (Bethyl Laboratories, Inc. USA) and the attached instructions have been followed by the manufacturer.

The level of immunoglobulin A (IgA) was measured in the serum for infected women and the control group by using (Human IgA ELISA Kit), the principle of this method is based on Sandwich ELISA from (Bethyl Laboratories, Inc. USA) and the attached instructions have been followed by the manufacturer.

CRP concentration was measured by (CRP Direct Latex Reagent kit) from (LINEAR CHEMICALS, Barcelona, Spain) and the instructions included with the kit were followed.

The iron level was measured by Iron Colorimetric Assay kit (Ferrozine method) from (LINEAR CHEMICALS, Barcelona, Spain) and the instructions included with the kit were followed. Furthermore, this study was approved and conducted by the Scientific Committee at the College of Nursing/University of Kirkuk/Iraq.

Statistical Analysis: Statistical analysis was performed using (t-test) under a significant level (0.05)⁽¹³⁾.

Results and Discussion

1. Estimation of immunoglobulin levels IgM, IgG and IgA: Table 1. shows the level of immunoglobulins in the serum of women infected with parasite compared to the control group, as the results of the current study showed a significant increase in the level of IgM in the serum of women with *T. vaginalis* compared to the control group (8.2 ± 2.9 ng/dl), (6.3 ± 0.5 ng/dl), respectively. This finding is consistent with ⁽¹⁴⁾ who, through their studies, found a higher IgM level in the serum of mice infected with the parasite compared to the control group, also⁽¹⁵⁾ found through their study a rise in the IgM level

in the serum of women infected with the parasite and comparison with the control group indicating that acute infection with *T. vaginalis* stimulate the humoral immune response because IgM is indicator of acute period of infection. In addition, this leads to knowledge of insights and trends for preventing parasite infection or developing treatment ⁽¹⁶⁾.

The results of the current study also showed a significant increase in the level of IgG in the serum of women infected with *T. vaginalis* compared to the control group (8.16 ± 2.20 ng/dl), (6.53 ± 1.69 ng/dl). This result is consistent with ⁽¹⁴⁾ who found, through their studies, a high level of IgG in the serum of mice infested with the parasite compared to the control group as well as with ⁽¹⁷⁾ who demonstrated through their study a rise in the level of IgG in the serum of women infected with parasite compared to the control group, which indicates that chronic infections are common especially in married women due to repeated infection and leave it without treatment. Moreover, to the fact that most of the infected women are asymptomatic when infected with *T. vaginalis*. In addition to what has been mentioned, the role of immunoglobulin, especially IgG, has a significant role in humoral immunity to control the parasitic infection⁽¹⁸⁾.

The results of the current study also showed a significant increase in the level of IgA in the serum of women with *T. vaginalis* compared to the control group (8.22 ± 3.1 ng/dl), (7.82 ± 2.8 ng/dl), respectively, and this result is consistent with ⁽¹⁸⁾ as well as with ⁽¹⁷⁾, As our study showed that there are no significant differences between the patients and the control group, and the reason for this result is that IgA rises in acute cases of the disease and returns to its normal level in chronic cases, and because most of the cases with *T. vaginalis* are chronic cases as mentioned Above. As the immune response depends on humoral immunity and begins as a local inflammation at the place of invasion and pathogen presence, WBC is attracted to the site of infection and produces cytokines, which in turn stimulate the production of specific immunoglobulins, especially IgA, which is considered one of the main media specialized in host defenses present in the secretions. The proteinase secreted by the parasite *T. vaginalis* is excreted in the vaginal and serum secretions and reduces the level of immunoglobulins over time and this effect on humoral immunity is one of the most important reasons for the difficulty in forming a vaccine against *T. vaginalis* ⁽¹⁹⁾.

Table 1. Immunoglobulins levels in women infected with *T. vaginalis* and control group

Mean ± SD (ng/dl) Control group n=10	Mean ± SD (ng/dl) Patients n=20	Immunoglobulins levels In serum
6.3±0.5	8.2±2.9	IgM
6.53±1.69	8.16±2.20	IgG
7.82±2.8	8.22±3.1	IgA

2. C-Reactive Protein concentration: The results of the current study showed a significant increase in the CRP concentration in the serum of women with *T. vaginalis* compared to the control group (5.76 ± 1.65 mg/dl), (4.61 ± 0.66 respectively) (Table 2) and this is consistent with ⁽²⁰⁾. The high concentration of CRP in the serum in women with the parasite represents a strong link between localized vaginal infection and the response of the body's systems to infection, as CRP is produced from the liver in large quantities and transmitted to blood circulation and rises in bacterial, fungal and parasitic infections and is considered as an indication of the presence of infections in the body, it is not A diagnostic test, but it is used to link signs and symptoms.

Infection with *T. vaginalis* stimulates the cellular immune response, as the parasite causes vaginitis in females and urethritis in the male. The high CRP in the serum of infected women indicates that the reactions of the immune response to the parasite have exceeded the boundaries of the mucosal genital tract in the host⁽⁸⁾.

Table 2. CRP concentration in women infected with *T. vaginalis* and control group

Mean ± SD mg/dl	Number of samples	CRP Concentration Group
5.76±1.56	20	Patients
4.61±0.66	10	Control

3. Serum Iron level: The results of the current study showed a significant decrease in the iron level in the serum of women with *Trichomonas vaginalis* compared with the control group (38.79 ± 8.23 mg/dl), (93.83 ± 34.76 mg/dl), respectively (Table 3), and this result is compatible with⁽²¹⁾. Note that the normal level of serum Iron level is (50-170 mg/dl), and the reason for this is that iron is a key component in pathogenesis of the parasite as the level of adhesion to human cervical cell line, or what is called Hella cells, changes with iron levels, because *T. vaginalis* isolates from an iron-rich medium show a high level

of adhesion and the reason is because the parasite has developed many mechanisms for adhesion, including Lipophosphoglycan, which is a major adhesion factor for parasite. In addition to Adhesion Proteins (AP), Fibronectin binding protein (FN), Laminine –binding protein, these proteins have the ability to interact with the parasite through their own receptors on its surface. Moreover, the presence of four types of important proteins in the adhesion process, namely (Ap33, Ap23 Ap51 Ap65), which iron plays a very important role in the process of gene expression and translation of these proteins⁽²²⁾. There are many experiments proven by scientists⁽²³⁾ on the role of iron in the pathogenicity of the parasite, as they cultured *T. vaginalis* in the Trypticase yeast media (TYM) which provided with iron, While the parasite's isolates from an iron-deficient medium failed to produce any pathogenicity⁽²⁴⁾. The source of the iron for the parasite comes from hemolysis of red blood cells from lesions formed by the parasite in addition to iron from the menstrual blood cycle⁽²⁵⁾.

Table 3. Serum Iron level in women infected with *T. vaginalis* and control group

Mean±SD mg/dl	Number of samples	Serum Iron level Group
38.79±8.23	20	Patients
93.83±34.76	10	Control

In conclusion, infection with *T. vaginalis* affects on some immunological parameters levels which indicate the role of humoral immune response against trichomoniasis, also the infection decreases serum iron level due to important role of iron in pathogenicity and cytoadherence of *T. vaginalis*.

Conflict of Interests: The authors of this paper declares that he has no financial or personal relationships with individuals or organizations that would unacceptably bias the content of this paper and therefore declare that there is no conflict of interests.

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