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Research Article

Immunological Aspects of *Trichomonas vaginalis* Infection in Women Attending Maternity Teaching Hospital and Some Public Health Centers in Erbil Governorate, Northern Iraq

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ABSTRACT

Trichomonas vaginalis infection is one of the sexually transmitted diseases. It is a health problem all over the world, including Iraq. It has also been associated with adverse outcomes of pregnancy and increased risk of HIV. Trichomoniasis typically elicits aggressive local cellular immune responses with inflammation of the vaginal epithelium and exocervix in women and urethra in men. The present study was aimed to assess serum level of interferon gamma (IFN-γ), Interleukin 10 (IL-10), C-reactive protein, antiphospholipid, anticardiolipin antibodies with eosinophil count as well. Four hundred and forty women with ages ranging between 16 and 60 years old (average of 34.2 years) who attended Maternity Teaching Hospital and a number of public health centers in Erbil Governorate were screened for trichomoniasis by direct wet mount preparation and culture technique. Serum IFN-γ, IL-10, antiphospholipid, and anticardiolipin were assessed by enzyme-linked immunosorbent assay. C-reactive protein (CRP) and eosinophil count were assessed by I CROMA and Coulter, respectively. The results revealed that IL-10 level (96.46 ± 1.97 pg/ml vs. 91.86 ± 1.48 pg/ml), eosinophil count ($0.1 \pm 0.07 \times 10^3$/μL vs. $0.1 \pm 0.01 \times 10^3$/μL), and CRP concentration (2.55 ± 0.74 mg/l vs. 2.27 ± 0.37 mg/l) were non-significantly ($P > 0.05$) changed in infected women in comparison with negative control group. However, serum IFN-γ level (484.83 ± 38.35 pg/ml vs. 372.15 ± 9.49 pg/ml) was significantly ($P < 0.05$) elevated in infected women in comparison to the control group. The results also revealed significant increase ($P < 0.05$) of antiphospholipid immunoglobulin G (IgG) antibodies (4.75 ± 0.3 U/ml vs. 3.79 ± 0.11 U/ml), anticardiolipin IgG, and anticardiolipin IgM antibodies in the sera of infected women, but antiphospholipid immunoglobulin M (IgM), were non significantly ($P > 0.05$) altered in response to *T. vaginalis* infection.

Keywords: Anticardiolipin antibodies, antiphospholipid antibodies, C-reactive protein, cytokines, IL-10, interferon gamma, *Trichomonas vaginalis* infection, trichomoniasis

INTRODUCTION

Trichomoniasis is the most prevalent non-viral sexually transmitted infection worldwide, caused by an anaerobic, parasitic, flagellated protozoan, and *Trichomonas vaginalis*. This infection is not a potentially dangerous disease that can go undiagnosed for years and is often passed on by an asymptomatic carrier.[1] Humans are the only known host, the trophozoites transmitted principally through vaginal sexual intercourse and rarely through fomites. It is very successful as a pathogen causing roughly the same number of sexually transmitted diseases as *Chlamydia trachomatis*, the most prevalent sexually transmitted bacterial pathogen.[2] Worldwide, the prevalence of *T. vaginalis* varies from 2% to >50% depending on region, country, gender, and demographics of the population specifically evaluated.[3] An estimated 180 million infection acquired annually worldwide.[4] It is emerging as a serious reproductive tract pathogen, mainly affecting minorities and people living in poor or disadvantaged communities. *T. vaginalis* is listed as one of the five neglected parasitic infections in the United States.[5]

Nearly half of all women with *T. vaginalis* are asymptomatic. Signs of infection in symptomatic women include vaginal discharge, odor, edema or erythema, and colpitis macularis (Strawberry cervix) which is characterized by punctate hemorrhagic lesions, vulval irritation, and inflammation. Other complaints include dysuria, a yellowish-green frothy discharge, pruritis, dyspareunia, and lower abdominal pain.[2,6]

The infected males are usually asymptomatic and infection is usually self-limiting, and thus, diagnosis is often difficult.

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The general infection areas include urethra, external genitalia, prostate, and epididymis.[7] It is also a known leading cause of prostatitis and urethritis.[8] Furthermore, serious complications may be associated with trichomoniasis such as premature rupture of the placental membranes, premature labor, and low birth weight in pregnant women infection, infertility, cervical cancer, pelvic inflammatory disease, birth outcomes, and increase in human predisposition to immunodeficiency virus (HIV), its transmission, and acquisition.[9,10] Infection with *T. vaginalis* is associated with higher genital HIV-1 levels. The treatment of women infected with *T. vaginalis* results in a 4.2-fold reduction in the quantity of HIV-1 in vaginal secretions.[9] Some recent studies have shown that trichomoniasis may be considered as one of the predisposing factors for some human disease such as prostate and cervical cancer.[11] The outcome of infection with *Trichomonas* may be due to genetic variability of the isolates and the host immune response.[12]

In Iraq, several studies have been conducted in respect to *T. vaginalis* epidemiology and diagnosis.[13-15] However, little is known concerning the immunological aspects of trichomoniasis among women in Erbil. The present study was aimed to assess some immunological parameters including interferon gamma (IFN-γ), Interleukin 10 (IL-10), C-reactive protein, antiphospholipid and anticardiolipin antibodies, and eosinophil count as well.

MATERIALS AND METHODS

Patients and Study Setting

T. vaginalis infection was screened in 440 women with ages ranging between 16 and 60 years old (average of 34.2 years) who attended Maternity Teaching Hospital and a number of public health centers in Erbil Governorate, Northern Iraq, over a period of 10 months from September 2012 to July 2013. As a control group, 32, age matched, women who were apparently healthy with no history or clinical evidence of other diseases and who revealed negative results for trichomoniasis by both direct wet mount and culture technique, were enrolled as negative control group. Both patients and healthy women were interviewed personally before collection of blood sample and vaginal swab. For each subject, a well-structured questionnaire was filled carefully and included patient name, mobile number, age, address, residency, occupation, marital status, educational level, gestation, and symptoms or history of symptoms (vaginal discharge, fever, and cervix), menstrual cycle, history of allergy or allergic condition, and history of other diseases.

Specimen Collection

High vaginal swabs

For each subject, two high vaginal swabs were collected under aseptic condition using a sterile cotton swab. One kept in 2 ml saline solution and used for direct microscopic examination, while the second swab was kept in 2 ml of culture medium which was used for the recovery of *T. vaginalis*.

Blood sample collection and processing

Blood samples were collected from the patients for the assessment of immunological parameters (IL-10, IFN-γ, anticardiolipin antibodies, antiphospholipid antibodies, C-reactive protein (CRP), and eosinophil count). An amount of 5 ml of peripheral blood was collected from each subject by venipuncture. Half of the blood was centrifuged for serum separation, aliquot and kept at −40°C, and the rest was used as whole blood for quantitative determination of eosinophils by fully automated hematology coulter.

Detection of *T. vaginalis*

T. vaginalis infection was detected initially by direct wet mount preparation, in which one of the duplicate vaginal swabs was used to make a direct smear on a glass slide with a drop of normal saline, the slide was initially scanned at ×100, looking for motile trichomonads and then at ×400 to confirm the motility, flagella movement, and morphologic features of the organism.

The high vaginal swabs were also cultivated on Trichomonas Medium (Oxoid, UK) supplemented with 8% heat-inactivated horse serum (Oxoid, UK) following the manufacturer's instructions. To suppress bacterial and fungal growth, 500 μg/ml of streptomycin (Cox pharmaceutical LTD, UK) and 100 μg/ml of chloramphenicol (OXOID, UK) were added to the complete medium. The pH of complete working medium was adjusted using 1 N HCl and the working medium was sterilized using millipore filter unit (0.2 μm). Inoculated cultures were incubated at 37°C and followed up, microscopically for the presence of motile trophozoites at 24, 48, and 72 h of incubation.[14]

Quantitative Estimation of Human IL-10 by Enzyme-linked Immunosorbent Assay (ELISA)

The OmniKine™ Human IL-10 and IFN-γ ELISA Kits (Assay biotech, USA) were used for quantitative estimation of IL-10 and IFN-γ in the sera of the subjects in accordance with the leaflet provided by the manufacturer.

Quantitative Estimation of High Sensitive CRP (hs-CRP) in the Sera of the Studied Groups

hs-CRP was estimated in the sera of the studied groups using i-CHROMA™ hs-CRP kit (BodiTech Med Inc., UK). The procedure was done according to the instructions provided by the manufacturer.

Quantitative Estimation of Cardiolipin Antibody Immunoglobulin G (IgG) and Immunoglobulin M (IgM)

Anticardiolipin antibodies (IgG and IgM) and antiphospholipid antibodies (IgG and IgM) were quantitatively estimated using ELISA kits (AESKU Diagnostics, Germany) and following the instructions provided by the manufacturer.

Ethical Consideration

This study was approved by Medical Ethics Committee in the College of Medicine, Hawler Medical University, Erbil, and an informed consent was taken from each recruited woman.

Statistical Analysis

The association of two categorical variables or difference between two proportions was assessed by Chi-square test.

Analysis of quantitative data was estimated using Student's *t*-test and ANOVA. Calculation of means and standard deviation was made for different parameters. $P \leq 0.05$ was considered statistically significant.

RESULTS

Out of 440 vaginal discharge samples, 14 (3.18%) revealed positive culture for *T. vaginalis*; however, the number of positive cases was only 12 (2.73%) when direct wet mount technique was used [Table 1]. Thus, considering culture technique as a gold standard for the diagnosis, the sensitivity (78.5%) of direct wet mount technique was lower than that revealed by cultivation. The 14 positive samples were subjected to immunological parameters.

Table 2 illustrates the mean concentration of IL-10, IFN-γ, and CRP in addition to eosinophil count in women with trichomoniasis and women of healthy control group. IFN-γ level (484.83 ± 38.35) was significantly elevated in the sera of women with trichomoniasis when compared with those of healthy control group (372.15 ± 9.49). However, IL-10 level, CRP concentration, and eosinophil count were non-significantly ($P > 0.05$) altered in response to *T. vaginalis* infection.

The mean concentration of serum antiphospholipid antibodies and anticardiolipin antibodies for both infected women and control group revealed a significant increase ($P < 0.05$) in antiphospholipid IgG, anticardiolipin IgG, and anticardiolipin IgM. However, the level of antiphospholipid IgM was non-significantly altered ($P > 0.05$) [Table 3].

DISCUSSION

CRP is a known marker of systemic inflammation.[16] In the current study, the level of CRP was non-significantly altered in infected women when compared with the control. This finding was inconstant with that obtained by Shaker and Hussein[17] who revealed that the level of serum CRP was significantly elevated in women with trichomoniasis. Vaginal bacterial, fungal, and parasitic coinfection significantly change the serum levels of many immunological parameters including CRP and cytokines.[18]

The results revealed non-significant changes in the eosinophil count in both infected and non-infected control groups. This result was in agreement with that obtained by Al-Gazali *et al*.[19] Eosinophilia is a common feature of allergic conditions and parasitic infections, in particular, helminthic infection. Allergens and also excretory-secretory products of helminths activate Th2 arm of the immune system through which IL-5 is expressed and this later is an eosinophil growth factor.[20] In trichomoniasis, T helper type 1 (Th1) is more likely activated since it could be explained by a significant level of IFN–γ obtained in this study in women with trichomoniasis.

IFN-γ level was significantly increased in response to *T. vaginalis* infection, but no significant change was observed in IL-10 level. Controversial studies have been published concerning cytokines profile that may be induced in response to *T. vaginalis* infection. Some previous studies *in vitro* revealed that exposure to *T. vaginalis* stimulated IL-8 secretion in human monocytes,[21] neutrophils,[22] and

Table 1: *Trichomonas vaginalis* infection among women using direct wet mount and culture techniques

Diagnostic method	Number of examined	Number of positive samples (%)	Number of negative samples (%)
Direct wet mount preparation	440	11 (2.5)	429 (97.5)
Culture	440	14 (3.2)	426 (96.8)
χ^2=0.371	df=1	*P*=0.686	

Table 2: Immunological parameter in women with trichomoniasis and control groups

Immunological parameter	Infected women (*n*=12) (Mean±SE)	Control group (*n*=30) (Mean±SE)	*t*-test	*P* value
IL-10 (pg/ml)	96.46±1.97	91.86±1.48	1.86	>0.05
IFN-γ (pg/ml)	484.83±38.35	372.15±9.49	2.85	<0.05
CRP (mg/L)	2.55±0.74	2.27±0.37	0.33	>0.05
Eosinophil (10^3/μL)	0.1±0.07	0.1±0.01	0.49	>0.05

CRP: C-reactive protein, IFN: Interferon gamma, IL-10: Interleukin 10

Table 3: Serum level of antiphospholipid and anticardiolipin antibodies in women with trichomoniasis and control group

Autoantibody	Infected women (*n*=12) (Mean±SE)	Control group (*n*=30) (Mean±SE)	*t*-test	*P* value
Antiphospholipid antibody (U/ml)				
IgG	4.75±0.3	3.79±0.11	2.91	<0.05
IgM	3.004±0.45	2.61±0.11	0.82	>0.05
Anticardiolipin antibody (GPL/ml)				
IgG	3.32±0.31	2.42±0.07	2.75	<0.05
IgM	2.89±0.46	1.85±0.07	2.2	<0.05

IgG: Immunoglobulin G, IgM: Immunoglobulin M

vaginal epithelial cells.[23] It was further demonstrated that the cytokines IL-2 and IFN-γ were secreted by murine lymphocytes infected with *T. vaginalis*. Furthermore, *T. vaginalis* was also stimulated the secretion of GM-CSF but not IL-6 and IL-1β in pregnant women.[24] However, Han *et al.*[25] reported that TNF-α, IL-1β, and IL-6 were significantly expressed by human macrophages on exposure to *T. vaginalis in vitro*. The parasite strain may also be concerned in the immunological studies of trichomoniasis.[25] Malla *et al.*[26] found that IL-2 and IFN-γ were significantly higher in mice infected with *T. vaginalis* isolated from asymptomatic women as compared to isolates from symptomatic women group and in control uninfected animals. In the present study, polymorphonuclear cells that are associated with the infection may be implicated in the induction of significance levels of IFN-γ in *T.vaginalis* infected women in comparison to non-infected control group. Recruitment of polymorphonuclear cells and other inflammatory cells to vaginal milieu in response to *T. vaginalis* infection is induced by a parasite surface adhesion molecules. Fichorova *et al.*[27] have found that *T. vaginalis* lipophosphoglycan (LPG), but not LPG from *Tritrichomonas foetus*, the causative agent of bovine trichomoniasis induced a selective upregulation of chemotactic cytokines by human endocervical, ectocervical, and vaginal epithelial cells, which do not express Toll-like receptor 4/MD2.[27] *T. vaginalis* LPG triggered interleukin 8 (IL-8), which promotes the adhesion and transmigration of neutrophils across the endothelium, and macrophage inflammatory protein 3α, which is a chemoattractant for immune cells and is essential for dendritic cell maturation. These effects were dose dependent that relived in the absence of cytotoxicity and IL-1β release utilizing, in part, a signaling pathway independent from the Toll-like/IL-1 receptor adaptor protein MyD88. This study may also support our finding that CRP was non-significantly changed in response to *T. vaginalis* infection. Despite of the presence of inflammatory cells in the vaginal milieu that is later released as a symptomatic vaginal discharge, CRP concentration was non-significantly changed that may lead to a suggestion that parasite LPG induced inflammatory response in the cervical-vaginal epithelial cells through induction of IL-8 and macrophage inflammatory protein 3α but not TNF-α and IL-1β, two pro-inflammatory cytokines needed for the synthesis of systemic CRP by hepatocytes.[27]

In the present study, there was non-significant difference in IL-10 level between *T. vaginalis* infected women and those non-infected. IL-10 contributes to inhibition of Th1 immunity and favors a T helper Type 2 response.[18,24]

In respect to autoantibodies, the levels of anticardiolipin (IgG and IgM) antibodies and antiphospholipid IgG were significantly elevated in women with trichomoniasis. However, antiphospholipid IgM level was non-significantly elevated in women with trichomoniasis in comparison to uninfected control women. Such elevated levels of these autoantibodies could be explained by the antigenic mimicry between the parasite antigens and host proteins, as cardiolipin and phospholipids are abundant in most cells of multicellular organisms, the former is an important component of the inner mitochondrial membrane, where it constitutes about 20% of the total lipid composition,[27,28] while phospholipids are a class of lipids and are a major component of all cell membranes as they can form lipid bilayers.[29] In respect to our results, the detection of significant titers of anticardiolipin IgG, IgM, and antiphospholipid IgG in the sera of trichomoniasis cases may be explained by the fact that all the infected women were chronic cases as they have been infected weeks before seeking medical advice. That is why IgG antibodies against both cardiolipin and phospholipids were detected in their sera. However, the detected levels are still within the normal range that is referenced by the manufacturer. *T. vaginalis* lack mitochondria instead; it has double membrane-bound organelles, called hydrogenosomes, produced molecular hydrogen. Phylogenetic and biochemical analyses of hydrogenosomes indicate a common origin with mitochondria; however, the identification of hydrogenosomal proteins and studies on its metabolism and chemical composition has been limited.[7] However, the presence of anticardiolipin antibodies in the sera of the patients may reflect the presence of cardiolipin in the membrane of hydrogenosome as it's, phylogenetically, belong to the same origin with the mitochondria. The presence of antiphospholipid antibodies in the sera of the women with trichomoniasis, even low levels, must not be neglected as it can be resulted in undesired, serious consequence in pregnant women. Antiphospholipid syndrome is a leading cause of miscarriage and maternal and fetal morbidity. This condition is characterized by thrombosis and pregnancy loss that occur in the presence of antiphospholipid antibodies.[30]

CONCLUSION

There were no significant differences in CRP level and eosinophil count between infected women and control group. IFN-γ level was significantly elevated in the sera of women with trichomotiasis. The inhibitory cytokine, IL-10 level was non-significantly changed in response to T. vaginalis infection. Trichomoniasis induced significant titers of anti-cardiolipin antibodies (IgG and IgM) and anti-phosplolipid IgG antibodies.

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