

Immunological Study of Women Infected with *Trichomonas vaginalis* Parasite in Baghdad city

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Abstract: *Background:* *Trichomonas vaginalis* is a protozoan parasite that infects the urogenital tract of humans. It is one of the most common causes of non-viral sexually transmitted diseases in the world. Trichomoniasis has important medical, social, and economical implication, with an estimated 180 million infection acquired annually worldwide. *The aim of the study* was to characterize the systemic immune response in women with trichomoniasis as compared with uninfected women, by assess serum concentrations of cytokines, (IL-2, IL-8 and (TNF)-alpha) and (CRP) were compared between infected and uninfected women. *Methods and patients:* the study was carried out during the period from January to may, 2013. A total of 50 cases of infected women with trichomoniasis, these patients have complained of clinical and laboratory diagnosis of *T. vaginalis* were included in this study and 30 healthy women. From infected and healthy women vaginal swab was carefully collected and five ml was the total blood collected from each clinical suspected woman with *T.vaginalis* infection and non-suspected women(as control group). The samples were vaginal swab was collected from patients using two sterile swab s, first swab used for direct microscopic examination, while the second swab was used for osom trichomonas rapid test, then quantify specific cytokine concentration (IL-2, IL-8, TNF- alpha and CRP) in the patient's serum, ELISA kit was used. *Result:* In this study 50 women recorded infected with *T.vaginalis* and 30 healthy women (as control) detected by strip test. The total sera level of IL-2, TNF-Alpha and CRP showed significant difference ($p < 0.05$) between patients and controls respectively 104.50 ± 6.82 , 103.74 ± 6.47 and 1.07 ± 0.05 VS 55.73 ± 3.74 , 60.63 ± 4.12 and 0.406 ± 0.04 . *Conclusion:* there is a significant increase in IL-2, TNF- alpha and CRP concentration in the serum of women infected with *T. vaginalis* in comparison with the controls. This indicates a stimulation of the humoral immune response during the infection with *T. vaginalis*. This may provide directions and insights that could prove critical to the prevention or treatment of this important disease.

Keywords: T.vaginalis, IL2, IL8, TNF, CRP

1. Introduction

Trichomonas vaginalis is a protozoan parasite that infects the urogenital tract of humans. It is one of the most common causes of non-viral sexually transmitted diseases in the world (1). Trichomoniasis has important medical, social, and economical implication (2), with an estimated 180 million infection acquired annually worldwide (3).

The symptoms of trichomoniasis in women range from none at all to a severe acute inflammatory state. Classic signs and symptoms include a purulent, malodorous vaginal discharge with associated pruritus, burning, dysuria and dyspareunia. Physical examination in affected women shows vaginitis and vulvitis, with a frothy, yellow-green mucopurulent discharge (4). However, infections can also lead to severe health complications, such as cervical erosion, premature birth, and infertility in men and women. More recently, infection with this parasite has been associated with increased susceptibility to human immunodeficiency virus, cervical cancer, and aggressive prostate cancer (5), infections are not self-limiting and produce non-ulcerative inflammation of the genital epithelium that can progress to necrosis and hemorrhage (6).

T. vaginalis is known to be a noninvasive microorganism that recruits inflammatory cells to the site of infection following attachment to the surface of the genital tract. Infection typically elicits aggressive local cellular immune responses with inflammation of the vaginal epithelium and exocervix in women and urethra in men(7). large number of human neutrophils have been found in the vaginal discharge of women infected with *T. vaginalis*. In addition, in vitro experiments revealed that the life span of human neutrophils can be prolonged by stimulation with *T. vaginalis*, and that

human neutrophils can produce chemokine IL-8 in response to *T. vaginalis* stimulation (8). in addition, actively rotating *T. vaginalis* harbors and secretes various immunological stimuli. Which may lead to vaginal mucosal inflammation, one study showed that the longevity of human neutrophils is prolonged in response to *T. vaginalis* lysate. Another study shoe that human neutrophils and macrophages stimulated with live *T. vaginalis* produce the chemokine IL-8 and the proinflammatory cytokines tumor necrosis factor- α , IL-6, and IL-1B (9). Other study suggests that Th-1 (IL-2 and IFN) type cytokines might be playing a role in maintaining low levels of infection (10).

Little evidence regarding the role of immune responses in inducing protection in human trichomoniasis is available. Specific antibody responses to *T. vaginalis* antigens in serum have been reported; however, similar to their local counterparts, the circulating antibody levels also differ and appear to have no function in helping the host to treat the infection and a cell mediated immune response is also evoked. T-cell subsets and cytokines serve a central function as key factors in the regulation of mucosal responses in various parasitic infections (11). Local regulation of CD4+ and CD8 + lymphocytes and the role of Th1/Th2 responses in the genital tract during infection are considered to be crucial for controlling the duration of infection and subsequent pathology(12). Although tumor necrosis factor (TNF)-alpha and interleukins are some of the inflammatory mediators that have a role in the pathogenesis of and the protection against disease. This findings may provide directions and insights that could prove critical to the prevention or treatment of this important disease.

C-reactive protein (CRP) is a marker of systemic inflammation that is frequently elevated in response to infection. The presence of acute inflammation with tissue destruction within the body stimulates its production. Therefore, a positive CRP indicates the presence of an inflammatory process. A recent study indicate the relationship among a sexually transmitted infection, CRP, and preterm birth risk (13).

The objective of this study was to characterize the systemic immune response in women with trichomoniasis as compared with uninfected women, by assess serum concentrations of cytokines, chemokines, (IL-2, IL-8 and tumour necrosis factor (TNF)-alpha) and C-reactive protein (CRP) were compared between infected and uninfected women.

2. Materials and Methods

2.1 Patients and Samples

The study was conducted in the maternity and Children hospital and some of medical centers in Baghdad city, from January to may, 2013,. A total of 50 cases of infected women with trichomoniasis, these patients have complained of clinical and laboratory diagnosis of *T. vaginalis* were included in this study and 30 healthy women.

From infected and healthy women vaginal swab was carefully collected from the posterior vaginal fornix after putting the patient at a lithotomic position and taking swab after opening the vagina by a sterile speculum, the swab are immersed in a tube with 1 ml of a sterile normal saline ,and five ml was the total blood collected from each clinical suspected woman with *T.vaginalis* infection and non-suspected women(as control group) by disposable syringe, the blood samples were drawn in sterile plain tubes and remains for 30 minutes at room temperature. After that the samples were centrifugation at 3000 rpm for 5 minutes to separate the serum and collected in other sterile tubes, each sample of serum was divided into four parts; each of them was kept in deep freeze at -20C°.

2.2 Samples examination

The samples were collected during vaginal examination , vaginal swab was collected from patients using two sterile swab s, first swab used for direct microscopic examination , this swab mixed with drops of normal saline and placed on a slid and examined at (X40) , Positive results were defined as the presence of one or more Trichomonads with characteristic motility jerky movement and morphology (14), while the second swab was used for osom trichomonas rapid test: assay was performed in accordance with manufacturer's for indicated present of *T. vaginalis*.

In order to quantify specific cytokine concentration (IL-2, IL-8 and TNF- alpha) in the patient's serum, ELISA kit (RayBiotech, Inc.) was used. And to quantify specific C-reactive protein (CRP) concentration in the patient's serum, (accent- 200 CRP ultra kit) was used. All assays were performed in accordance with manufacturer's specifications.

2.3 Statistical Analysis

The statistical analysis system SAS -2012 (15), was used to effect of different factors in study parameters. Least Significant Difference- LSD test was used significant compare between means in this study.

3. Result

In this study 50 women recorded infected with *T.vaginalis* and 30 healthy women (as control) detected by strip test. The total level of IL-8 in serum showed no significant difference between patients (107.58±5.72) and control (98.47±7.32). However, the total sera level of IL-2, TNF-Alpha showed significant difference (p<0.05) between patients and controls respectively 104.50± 6.82 and 103.74± 6.47. VS 55.73± 3.74 and 60.63±4.12 in table (1).

However, the total sera level of CRP showed significant difference (p<0.05) between patients and controls 1.07±0.05 VS 0.406± 0.04 in table (2).

Table 1: Compare between patients and control in ILS study

Group	No.	Mean ± Standard Error		
		IL-2	IL-8	TNF-alpha
control	30	55.73± 3.74	98.47±7.32	60.63±4.12
patients	50	104.50± 6.82	107.58±5.72	103.74± 6.47
LSD value		18.730*	18.642 NS	15.888*

*(p<0.05)

LSD : Least Significant Difference

Table 2: Compare between patients and control in CRP study

Group	No.	Mean ± Standard Error
		CRP
control	30	0.406± 0.04
patients	50	1.07±0.05
LSD value		0.152*

LSD : Least Significant Difference

4. Discussions

Many local studies indicated comparable incidence of trichomoniasis in our community . This represented an important public health problem, which should be drawn to the attention of the public as well as health authorities. Sexually transmitted diseases are a major global cause of acute illness, infertility, long term disability and death, with severe medical and psychological consequences for millions of men, women and children(16).

The increase in the incidence along the years and in this study may be due to the fact that there is no safe and effective method of prevention of trichomoniasis .Because of the potential side effects and clinical failures associated with the therapy drug of choice, metronidazole and the reemergence of resistant strains novel strategies are needed for the control of *T. vaginalis* infection, including vaccine production (11).

The cytokine responses in various microbial diseases have been reported with varying observations, depending upon distinct patterns of immune responses stimulated by

different microbes and unique mechanisms for evading specific immunity (17). Data from the present study showed highly significant increase in the concentration of IL-2 in serum of patients infected with *T. vaginalis* in comparison with control group, suggesting that Cytokines and chemokines provide a mechanism for initiation, amplification or containment of inflammation during disease status. Experimental studies have revealed that the Th-1 cytokine (IL-2) responses might play a role in the elimination of *T. vaginalis* and thus might be maintaining low levels of infection in asymptomatic infected subjects(18).

In the present study cytokine level TNF-alpha showed highly significant increase in the concentration in serum of patients infected with *T. vaginalis* in comparison with control group. These result agreement with (19) suggest that *T. vaginalis* stimulates human macrophages to produce proinflammatory cytokines, such as TNF-alpha, and NO. In particular, showed that *T. vaginalis* induced TNF-alpha production in macrophages through NO-dependent activation of NF-kappaB, which might be closely involved in inflammation caused by *T. vaginalis*.

Data from the present study showed increase in the concentration of IL-8 in serum and no significant increase in serum of patients infected with *T. vaginalis* in comparison with control group. This is agreement with the study has shown that *T. vaginalis* induces blood monocytes to produce large amounts of bioactive IL-8, mainly by membrane components of *T. vaginalis* (MTV). Monocyte-derived IL-8 induced by MTV was dose and time dependent(20).

Another study showed previously that *T. vaginalis*-induced neutrophil recruitment may be brought about by the IL-8 produced by neutrophils in response to activation by live *T. vaginalis* (1). The elevation of CRP in the serum of infected women with trichomoniasis in this study represents a biologically plausible link between a local vaginal infection and a systemic response.

CRP is an acute-phase protein produced in response to cytokine stimulation and plays a role in a variety of responses to infectious agents. CRP is a known marker of systemic inflammation. Because labor is a systemic process, it may be that there is systemic inflammation in the pathway leading to birth both at term and preterm(13). The presence of increased C-reactive protein in the sera of *T. vaginalis* infected women suggests that the impact of the immunoinflammatory reaction to the parasite exceeds the boundaries of the reproductive tract mucosa (21).

From this present study we concluded that there is a significant increase in IL-2, TNF-alpha and CRP concentration in the serum of women infected with *T. vaginalis* in comparison with the controls. This indicates a stimulation of the humoral immune response during the infection with *T. vaginalis*. This may provide directions and insights that could prove critical to the prevention or treatment of this important disease.

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