EVALUATION OF B-LYMPHOCYTE MARKER (CD19) AND INTERLEUKIN-4 (IL-4) IN WOMEN INFECTED WITH *TRICHOMONAS VAGINALIS*

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SUMMARY: The aim of this investigation was to evaluate the B-lymphocyte subset (CD-19) and interleukin-4 (IL-4) in women infected with Trichomonas vaginalis. Vaginal swabs, washes and blood specimens were collected from 65 women attending outpatient clinic at Al-Kadhimyia Teaching Hospital in Baghdad suffering from vaginal discharge starting from January 2005, to October 2005. Twenty healthy looking age matched women were also included for control studies. Blood was taken to heparinized tubes and serum was separated. Heparinized blood was used for evaluation of the CD marker, CD-19 using the indirect immunostaining technique. The cytokine IL-4 was evaluated in serum and vaginal washes using the ELISA technique.

Trichomonas vaginalis was isolated from 25 women with a prevalence rate of 38.5%. The results of CD marker showed significant differences between the infected women and controls. There was a significant increase in IL-4 in the infected women.

It was found that this parasite has the ability to stimulate the cell-mediated immunity which eventually led to production of specific immunoglobulin against Trichomonas vaginalis.

Key Words: Trichomonas vaginalis, B-lymphocytes, CD19, IL-4.

INTRODUCTION

Trichomoniasis is a sexually transmitted disease (STD) with public health ramifications (1). It is a ubiquitous protozoal infestation of the lower genitourinary tract in men and women. It is usually encountered during the reproductive years, and its transmission is almost exclusively through sexual intercourse (2). This disease is caused by a flagellated parasite, named *Trichomonas vaginalis*. Approximately 180 million women worldwide may be infected with *T. vaginalis*. Prevalence estimates vary between populations studied, but range from 5-47% in women and 5-29% in men (1).

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Trichomoniasis encompasses a broad range of symptoms from a state of severe inflammation and irritation with a frothy malodorous discharge to a relatively asymptomatic carrier state (3). This disease has also been associated with vaginitis, cervicitis, urethritis, pelvic inflammatory disease (PID), and adverse birth outcomes (1) manifested with preterm rupture of membranes, preterm labor, and low birth weight infants (4). Relative risk of developing invasive cervical cancer (5) and six fold higher probability of infection by human immunodeficiency virus (HIV) (6) are linked to this disease.

The mucosal immune system is the first stage of defense against pathogenic organisms in the female reproductive tract (7). It involves both innate and adaptive

immune responses including humoral and cell-mediated immunity, and evokes lymphocyte effector functions including cytokine production, cytotoxic effects, and antibodies produced after presentation by antigen manufacturing cells (8). The innate immune system plays a particularly crucial role in resistance to a variety of protozoa during the early stages of infection (9). Immune responses to infection with *T. vaginalis* have been described, including specific secretory antibodies in vaginal secretions and (IgM and IgG) antibodies in serum (10).

Little evidence regarding the role of immune responses in inducing protection in human trichomoniasis is available in the literature. 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 (11) and a cell-mediated immune response is also evoked (3). T-cell subsets and cytokines serve a central function as key factors in the regulation of mucosal responses in various parasitic infections (11).

MATERIALS AND METHODS

The study included 65 women in the reproductive age group attending the Obstetrics and Gynecology out-patients department of Al-Kadhimyia Teaching Hospital in Baghdad, Iraq, from January, 2005 till October 2005. Vaginal swabs, vaginal washes, blood samples were taken into heparinized syringes and serum samples were collected from symptomatic patients presenting with vaginal discharge, itching and dysuria.

Three vaginal swabs were collected for wet-smear examination (12), cultivation of *T. vaginalis* using Diamonds' medium and modified Diamonds' broth (13) and the third one was inoculated into culture tubes containing 5 ml of Stuart transport medium (14). On arrival, the swab was inoculated into the following, blood agar, chocolate agar, and MacConky agar for identification of the microorganizm. Finally, the swab was streaked onto a glass slide for Gram staining.

Standardized vaginal rinsing method (during vaginal examination) with 2 ml of 0.9% sterile sodium chloride solution was performed by flushing and re-aspirating the fluid through a sterile Pasteur pipette in the left, central, and right upper vaginal vault (15). The tubes were brought to the laboratory and were centrifuged at 3500 g for 15 minutes at 4ºC and the supernatants were transferred to glass tubes and stored at -20ºC until used for cytokine evaluation (16).

Heparinized blood was processed according to Boyum (17) for lymphocyte separation and indirect immunostaining method for the determination of T-lymphocyte phenotypes.

In order to quantify specific cytokine concentration (IL-4) in the patient's serum and vaginal washes, ELISA kit (MABTECH.AB, Sweden) was used. All assays were performed in accordance with manufacturer's specifications.

Statistical evaluation

Results are presented as means ± standard error and significance was analyzed by Student's t- test. Because of the fact that a large number of cases had concomitant infections with *T. vaginalis* in addition to the presence of the normal flora, the lymphocytes percentage and the cytokine concentration are difficult to interpret as being due to *T. vaginalis* and not the associated existing other microorganisms. Therefore, a cut-off value was calculated for each parameter included in this study according to Al-Murrani *et al.* (18).

RESULTS

Prevalence rate of *T. vaginalis* and other microorganisms

Out of a total of 65 women studied, 25 (38.5%) revealed the presence of T.vaginalis. 18 of these 25 were diagnosed by the wet mount technique (72% sensitivity) and the other 7 were diagnosed using the culture technique. 24 of 25 cases (96% sensitivity) were positive using Diamond's medium and one case diagnosed by the wet mount technique was negative. All cases (100% sensitivity) were positive by culture using the modified Diamonds' broth.

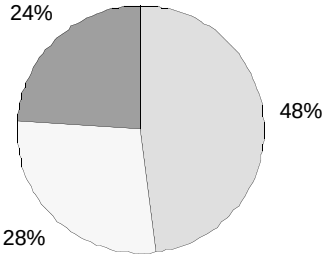
All patients had concomitant infections other than *T. vaginalis*. Out of these 25 patients, 7 (28%) had *Candida* and *Gardnerella vaginalis*, 6 (24%) had *Escherichia coli, Staphylococcus aureus*, group (B) streptococci (*Streptococcus agalactiae*) and *Lactobacillus*. The other 12 patients (48%) displayed the presence of *T. vaginalis* with normal vaginal flora (Figure 1).

Immunostaining of lymphocytes in peripheral blood

The percentage of B-lymphocyte cells (CD19- positive cells) was significantly higher ($P<0.0001$) in women with *T. vaginalis* infection (57.24 ± 0.64%) in comparison with the controls (16.05 ± 0.55%) as shown in Figure 2.

Regarding the calculation of the cut-off value for CD19-positive cells, the value above 59.019% was considered due to *T. vaginalis* and the associated microorganisms (6 cases, 24%) and below that is considered due to *T. vaginalis* alone (19 cases 76%) under 99% confi-

Figure 1: The prevalence rate of *T. vaginalis* infection and other microorganisms. (48% *T. vaginalis* + normal flora), (28% *T. vaginalis* + *Candida albicans* + *Gardnerella vaginalis*), and (24% *T. vaginalis* + *E. coli* + *S. aureus* + group B *Streptococci* and *Lactobacillus*).

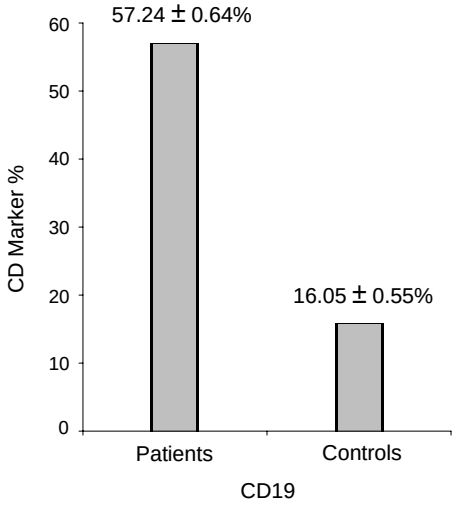


dence interval. When the lower limit is considered 55.461%, 9 cases, 36% are considered absolutely, with no doubt, due to *T. vaginalis* alone. Any value above that is considered due to *T. vaginalis* and associated microorganisms.

Interleukin-4 (IL-4)

There was a significant increase ($p<0.05$) in IL-4 concentration of patients' serum (3.64±0.15 ρg/ml) in comparison to the control group (0.0676±0.0056 ρg/ml). In patients' vaginal wash, the IL-4 concentration was highly significant difference ($p<0.0001$) (0.172±0.015 ρg/ml) in comparison to the control group (0.0362 ± 0.0044 ρg/ml) as shown in Figure 3.

Figure 2: The mean lymphocyte phenotype standard error of peripheral blood lymphocytes of patients infected with *T. vaginalis*.



DISCUSSION

Trichomoniasis is one of the commonest sexually transmitted diseases capable of causing considerable morbidity in infected patients (6). Infection with *T. vaginalis* may show diversities with respect to socio- cultural properties of the communities changing from a country to another from a society to another (5).

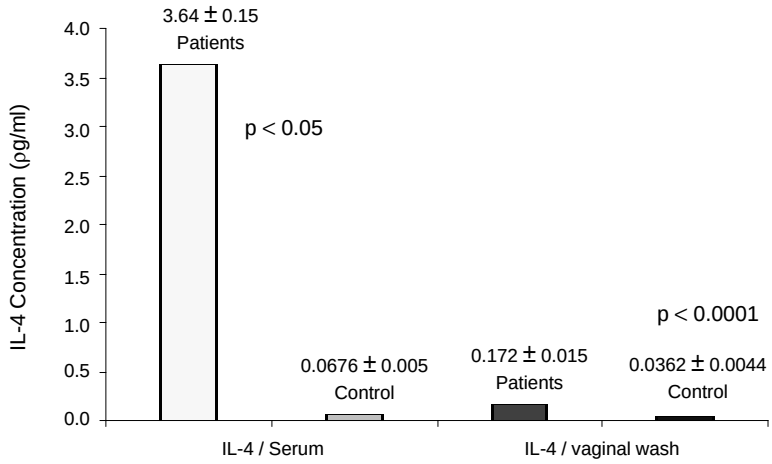
Prevalence rate of *T. vaginalis* obtained in this present study was 38.5%. This result revealed that the incidence of *T. vaginalis* infection is much higher than many other studies reported from different parts of Iraq by many workers. The increase in the incidence along the years and in this study may be due to the fact that there is no safe and effective method of prevention of trichomoniasis (10).

Because of the potential side effects and clinical failures associated with the therapy drug of choice, metronidazole (19,20) and the reemergence of resistant strains (21) novel strategies are needed for the control of *T. vaginalis* infection, including vaccine production (10,22). Another important point worth mentioning is the low socioeconomic and cultural levels of the patients enrolled in the present study. Other studies have also linked the high prevalence rate to this fact (5).

During epithelial cell function, *T. vaginalis* stimulates cytokine production and evokes inflammatory responses (23,24) that recruit a range of neutrophils across the epithelial barrier (25,26). It also releases neutrophil activating factor (27). There is evidence that leukocytes, including lymphocytes and monocytes/macrophages, are present in the vaginal environment (28).

Although a cut-off value was calculated for each parameter included in the present study to eliminate the effect of concomitant infection on the results, studies have indicated that the systemic immune response (serum) is not altered by the concomitant infections. However, the local immune response may be slightly altered. *T. vaginalis* infection with *N. gonorrhoeae* induced an immune response (IL-1 and IL-6) that was the same when *T. vaginalis* was the causative agent of infection alone (29).

Figure 3: The mean IL-4 concentrations (rg/ml) ± standard error of patients' serum and vaginal wash infected with *T. vaginals*.



The present study on trichomoniasis revealed a significant increase in the population of B-cells (CD19- positive cells) in the peripheral blood of women infected with *T. vaginalis* in comparison with the control group. This increase in B-cells population may be due to the fact that there are large numbers of plasma cells of all three major immunoglobulin classes present in case of trichomoniasis (30). The immune enzyme adenosine deaminase, vital for the maturation of T and B lymphocytes (31) was found to be elevated in women with acute *T. vaginalis* infection indicating an enhancement in both, cellular and humoral immune response (32).

Data from the present study showed a significant increase in the concentration of IL-4 in serum and highly significant increase in vaginal wash of patients infected with *T. vaginalis* in comparison with control group. This is in agreement with the experimental trichomoniasis study conducted by Paintlia *et al.* (11) on mice infected with symptomatic and asymptomatic isolates of *T. vaginalis* alone. This increase in the concentration of IL-4 resulted from the induction of T helper 2 (Th2) cells (CD4-positive cells) in response to the infection with *T. vaginalis* (Al-Lihaibi and Juma, unpublished data) and indicating that there is a local immune response to this infection.

These (Th2) cells produce IL-4, IL-5, IL-6 and IL-10 and cooperate with B-cells to generate IgM, IgG, IgA, and IgE responses (33). Immune responses to infection with *T. vaginalis* have been described, including specific secretory antibody in vaginal secretions (34,35) and IgM and IgG antibody in serum (36). Although an association between the presence of local antibody and low parasite counts has been postulated, there is no conclusive evidence to suggest that the presence of IgA antibodies is specifically related to the immune response to *T. vaginalis* (11).

Elevation of cytokines in vaginal washes indicates the presence of local immune response. The local immune response is identified in *T. vaginalis* (22) and other vaginal infections including *Candida albicans* infection (16). The production of any local immune response to genital tract infection raises a question since a mucosally associated lymphoid tissue is not seen in histologic sections, especially in the uterus. Histopathologic studies of reproductive tracts of experimentally infected heifers, however, showed lymphoid accumulations under the uterine surface and glandular epithelium (37-39).

From this present study we concluded that there is a significant increase in the percentage of B-cells (CD19) in peripheral blood in women infected with *T. vaginalis* when compared to the control group and a significant increase in IL-4 concentration in the serum and vaginal washes 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*.

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