Comparison of Interleukin-13 and 15 Levels in Bacterial and Trichomonas Infection Isolated from Women of Childbearing and Menopausal Age

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KEYWORDS

ABSTRACT

Background: Trichomonas vaginalis is the causative agent of trichomoniasis, one of the most common sexually transmitted parasitic diseases worldwide. T. vaginalis primarily replicates in the epithelium during the initial infection of the genital mucosal surface. Innate immune cells and inflammatory reactions cause the release of anti-trophozoite cytokines. Trichomoniasis typically elicits aggressive local cellular immune responses with inflammation of the vaginal epithelium and exocervix in women and the urethra in men. T. vaginalis secretes a large number of cysteine proteinases (CPs) that break down various immunoglobulin types, enabling the parasite to evade the antibody response.

Aim of the study: To assess the effect of trichomoniasis on some hematological markers, and serum IL-13 and IL-15 levels in women of childbearing age.

Materials and Methods: This study was conducted in Kirkuk Governorate between November 10, 2023, and March 15, 2024. The total number of married, non-pregnant, and menopause women under study was 100, whose ages ranged from 18 to 59 years. A vaginal discharge specimen was collected from each participant to identify motile Trichomonas vaginalis through wet-mount microscopy. Blood samples were obtained for hematological assessment and immunological laboratory tests.

Results: Of the 100 women in the study, 10 individuals (10%) had an infection with Trichomonas vaginalis. The study found significant differences in blood components between the T. vaginalis positive and negative groups, with the T. vaginalis positive group having higher average WBC levels, a lower lymphocyte count, and a higher eosinophil count. The study found no significant difference in IL-13 and IL-15 concentrations between the T. vaginalis positive and negative groups

Conclusions: The T. vaginalis positive and negative groups did not significantly differ in their levels of IL-13 or IL-15, but they did differ in their immune cell counts. Specifically, the T. vaginalis positive group had a significant higher average level of white blood cells (WBC) and eosinophils but a lower mean lymphocyte count than the T. vaginalis negative group.

1. Introduction

Trichomonas vaginalis is a protozoan that generally has an anaerobic metabolism and is usually pyriform, although it can also occasionally be amoeboid. It infects the genitourinary tract epithelium (**1**). T. vaginalis is the causative agent of human trichomoniasis. It causes a wide range of clinical symptoms, from mild cervicitis and urethritis to severe vulvovaginitis. Trichomoniasis increases the risk of pelvic inflammatory disease, infertility, bad pregnancy outcomes, and transmission of the human immunodeficiency virus (**2, 3**). The incidence of T. vaginalis infection has historically been linked to a number of characteristics, including younger ages, sexual activity, having more sexual partners, using condoms, having other STIs, social demographics, and low economic conditions (**4,5**).T. vaginalis primarily replicates in the epithelium during the initial infection of the genital mucosal surface, which stimulates innate immune cells and inflammatory reactions to release anti-trophozoite cytokines (6). Innate immunity, which is mediated by myeloid cells, natural killer cells, innate lymphoid cells, the complement system, and defensins, is the first line of defense for the host during an infection **(7).** Cell-mediated immunity is considered to be the main cause of inflammation linked to T. vaginalis (8).

The mucosal immune system is the first stage of defense against pathogenic organisms in the female reproductive tract (9). 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-presenting cells (APCs.) (10).

Although bacterial vaginosis (BV) is a major source of vulvovaginal symptoms globally, it is limited to an asymptomatic condition in at least half of the cases (6). In contrast to vaginal infections caused by a single pathogen, bacterial infections (BV) are actually understood to be a collection of common

clinical signs and symptoms that can be triggered by a wide variety of bacterial species and communities (11, 12). Various bacterial species exhibit varying associations with presenting signs and symptoms, which accounts for a significant degree of variability in clinical presentation (13). The most frequent reason for abnormal vaginal discharge in women of reproductive age is a vaginal dysbiosis called bacterial vaginosis (BV) (14).

Materials & Methods:

Between November 10, 2023, and March 15, 2024, this study was done. One hundred non-pregnant married, and postmenopausal, women, ages 18 to 59, were included in the study. These women were chosen from among those who attended the Gynecology and Pediatric Hospital, Kirkuk General Hospital, and Azadi Teaching Hospital.

Using sterile vaginal swabs, a specimen of vaginal discharge were taken from the posterior vaginal fornix following the patient's placement in a lithotomy position and the vagina's opening with a sterile speculum. In order to identify motile Trichomonas vaginalis using a wet mount smear, the swab tube was filled with about one milliliter of normal saline to preserve the parasite's life and boost its moisture content in order to examine it under a microscope within an hour.

Blood samples were collected from the participants using a disposable 10-milliliter syringe. The blood was then divided into two sterile test tubes: one held 2.5 ml of blood mixed with anticoagulant EDTA for hematological evaluation by automated blood cell counting analyzer, and the other held 5 ml of blood for immunological analysis. After incubating at room temperature for fifteen minutes, the second tube underwent two ten-minute centrifugation cycles at 3000 rpm. The clear serum, aspirated from the top of the gel tube, was transferred to new sterile test tubes. Subsequently, these tubes were stored at -20°C for immunological analysis to estimate human interleukin-13 and 15 using the enzyme-linked immunosorbent assay (ELISA) technique.

Results

Out of the 100 women who participated in the study, 10 (10%) had T. vaginalis. Among these 10 patients, 6 women (60%) exhibited asymptomatic trichomoniasis, while 4 women (40%) presented with flagrant vaginitis, the P value of 0.37346 suggests insignificant association between T. vaginalis positivity and the clinical patterns of trichomoniasis (Table-1)

Table-1: Clinical patterns of trichomoniasis in infected women.

Clinical patterns of trachomoniasis	**T. vaginalis Positive group No.**	**Percentage**	P value
Asymptomatic	6	60%	0.37346
Flagrant vaginitis	4	40%	
Total	10	100%	

T. vaginalis positive group showed a mean IL-13 concentration of 2.27 pg /ml with a standard deviation of 0.817, while the **T. vaginalis** negative group had a lower mean concentration of 1.3 pg /ml and a standard deviation of 0.8. A P value of 0.2947 indicated that there was no statistically significant difference in IL-13 levels between the two groups. On the other hand, the mean

concentration of IL-15 in the T. vaginalis positive group was 2.9 pg /ml, with a standard deviation of 0.88. In comparison, the mean concentration in the **T. vaginalis** negative group was slightly lower at 2.56 pg /ml, with a standard deviation of 0.96, yielding a P value of 0.0868, demonstrating no significant difference between the two groups (Table-2).

Table-2: Comparison the mean of cytokines concentration (Pg / ml) between the T. vaginalis positive group and negative group.

Cytokine	T. vaginalis Positive group n(10)	T. vaginalis Negative group n(90)	P Value
IL - 13 (pg/ml)	2.27 ± 0.817	1.3 ± 0.8	0 .2947
IL - 15 (pg/ml)	2.9 ± 0.88	2.56 ± 0.96	0 .0868

In the examined blood components, the **T. vaginalis** positive group had significantly higher average levels of white blood cells (WBC) compared to the T. vaginalis negative group (8964.2 ± 1197.6 vs. 5634.6 ± 985.4, P = 0.00001). Additionally, the T. vaginalis positive group showed a significantly lower mean lymphocyte count compared to the **T. vaginalis** negative group (1124.3 ± 214 vs. 2044.87 ± 554.7, P = 0.00001). Moreover, the **T. vaginalis** positive group exhibited a significantly higher mean eosinophil count compared to the T. vaginalis negative group (394.6 ± 169.8 vs. 193.2 ± 142.49, P = 0.00001). Therefore, there were statistically significant differences in all three blood components between the **T. vaginalis** positive and negative groups (Table-3).

Table-3: Comparison the mean of blood parameters included in the study between TV positive and negative groups.

Blood Components included in the study	T. vaginalis Positive group n(10)	T. vaginalis Negative group n(90)	P Value
WBC Count /µL	8964.2 ± 1197.6	5634.6 ± 985.4	< 0.00001
Lymphocyte Count/µL	1124.3 ± 214	2044.87 ± 554.7	< 0.00001
Eosinophil Count/µL	394.6 ± 169.8	193.2 ± 142.49	< 0.00001

Discussion

10% of the study population had a T. vaginalis infection. In comparison with the ranges found in prior research, the infection rate was slightly lower than rates of 14.73% in Baghdad (15) and 15.5% in Mosul (16), but it was higher than the 5.4% infection rate in Dohuk (17) and it was still lower than the rates recorded in other studies, which showed 20.49% in Kirkuk province (18) and 41.6% in

Baquba city (19).

Two cytokines were measured in this study, IL-13 and IL-15 (Table-2), in participants with and without T. vaginalis infection. Cytokines are signaling molecules that play a key role in the immune response to infections and can have a significant impact on the outcome of the infection (20).The results showed that there was no statistically significant difference in the levels of IL-13 , IL-15 between the T. vaginalis positive and negative groups. Similarly, these findings may due to that these cytokines do not play a major role in the immune response to T. vaginalis infection, the severity of the infection, and the presence of co-infections that influence the levels of these cytokines. It is worth noting that the immune response to T. vaginalis infection is complex and involves a wide range of cytokines and immune markers.

While this study focused on IL-13 and IL-15, there are many other cytokines that could potentially play a role in the immune response to T. vaginalis. For example, previous researches had shown that T. vaginalis infection can induce the production of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α, as well as anti-inflammatory cytokines such as IL-10 (21, 22, 23, 24.), these cytokines can have a significant impact on the host immune response and the outcome of the infection.

The findings of the study (Table-3) indicate that individuals with *T. vaginalis* infection exhibit significant differences in their blood components compared to those without the infection. Specifically, the T. vaginalis positive group had higher average levels of white blood cells (WBC) and eosinophils, and a lower mean lymphocyte count compared to the T. vaginalis negative group.

The observed elevation in WBC levels in the TV positive group may suggest an activated or heightened immune response, which is consistent with the body's natural defense mechanism in response to an infection. This finding aligns with previous studies done by Huppert J.S. (25) that had shown an association between T. vaginalis infection and an increase in WBC count as a response to the infection. The higher eosinophil count in the TV positive group disagrees with studies done by Al Gazali, B.S. et al. (26), and Nouraddin, A.S. et al. (27). Additionally, the significantly lower mean lymphocyte count in the T. vaginalis positive group is noteworthy, as lymphocytes are critical components of the immune system, and their reduction may indicate a dysregulated immune response or immune suppression associated with *T. vaginalis* infection.

Lymphocytes are a type of white blood cell that plays a key role in the body's immune response. When an individual is infected with trichomoniasis, the body may mount an immune response to fight off the parasitic infection. This can lead to an increase in the consumption and utilization of lymphocytes, leading to a temporary decrease in their count (28). The statistically significant differences in all three blood components between the T. vaginalis positive and negative groups highlight the potential impact of T. vaginalis infection on the immune response, and these findings could have implications for the understanding of the pathogenesis and outcomes of the infection.

Conclusion

The *T. vaginalis* positive and negative groups did not significantly differ in their levels of IL-13 or IL-15, on the other hand, they did differ in their immune cell counts. Specifically, the *T. vaginalis* positive group had higher average levels of white blood cells (WBC) and eosinophils but a lower mean lymphocyte count than the *T. vaginalis* negative group.

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