

Assessment of the Activity Parameters on *Trichomonas vaginalis* in Iraqi Women

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DOI: <https://doi.org/10.55145/ajbms.2024.03.01.009>

Received November 2023; Accepted January 2024; Available online January 2024

ABSTRACT: Vaginal infections are predominantly caused by *Trichomonas vaginalis*, bacterial vaginosis, and *Candida* infection. These infections are particularly concerning among pregnant women, potentially leading to adverse outcomes, including miscarriage. This study aimed to investigate the association between *Trichomonas vaginalis* infection and hormonal and immune parameters (Vitamin D and Thyroid Stimulating Hormone (TSH) levels). A cross-sectional study was conducted between September 2022 and October 2023. A total of 92 women participated, including 67 patients with recurrent vaginitis (characterized by burning, itching, and discharge) and 25 healthy controls. High vaginal swabs and 5 ml of venous blood were collected from each participant. Serum levels of Vitamin D, TSH, and inflammatory cytokines were measured using immunofluorescence techniques. Women infected with *Trichomonas vaginalis* had significantly elevated serum Vitamin D levels ($P=0.0144$), while the differences in TSH levels between the two groups were not statistically significant. Although associations between TSH, Vitamin D, and *Trichomonas vaginalis* infection were observed, these correlations did not reach statistical significance. This study suggested that *Trichomonas vaginalis* infection may be associated with increased serum Vitamin D levels in affected women. The lack of a significant change in TSH levels and its weak association with the infection indicate a complex interplay between hormonal balance and vaginal infections. Further research is needed to explore the implications of these findings, particularly in the context of infertility and immune modulation during vaginal infection.

Keywords: *Trichomonas vaginalis*, Vitamin D, Thyroid-Stimulating Hormone



1. INTRODUCTION

Trichomonas vaginalis is an extracellular protozoan parasite that is considered an important cause of sexually transmitted infections (STIs), predominantly affecting the human urogenital tract. *T. vaginalis* colonizes the vaginal mucosa causing an alteration of the local microbiota [1]. The infection caused by *T. vaginalis* termed trichomoniasis is prevalent globally and presents with varying signs and symptoms. Trichomoniasis in women is often manifested as pelvic pain, vaginal itching, dyspareunia, abnormal vaginal discharge, and symptoms suggestive of a urinary tract infection. Conversely, men may be asymptomatic or experience sporadic symptoms, including penile discharge, testicular pain, dysuria, and changes in urine appearance [2]. On the other hand, bacterial vaginosis is a dysbiosis of the vaginal microbiota that has been implicated in the pathogenesis of various gynecological conditions such as endometritis and pelvic inflammatory disease. The imbalance in the vaginal flora has been linked with adverse reproductive outcomes, including premature birth and miscarriages. Current evidence showed the critical role of diverse commensal bacterial species in the female reproductive tract. Alterations in vaginal microbiota colonization patterns may contribute to infertility; thus, deeper understanding of the complex mechanisms underlying the correlation between altered microbial ecology and reproductive health outcomes is needed to improve diagnostic, preventive, and therapeutic approaches in addressing female reproductive health [3]. The management of trichomoniasis involves the use of metronidazole that is considered the cornerstone of therapy. Marketed under various brand names including

Flagyl, metronidazole is widely recognized for its efficacy in eradicating *T. vaginalis*. The treatment regimen is generally well-tolerated, with a low incidence of adverse effects, making it a reliable and safe therapeutic option [4,5]. A key virulence factor in *T. vaginalis* is the lipophosphoglycan (LPG), which plays a fundamental role in the pathogenesis of trichomoniasis. This glycoconjugate is essential for the adherence of *T. vaginalis* to vaginal epithelial cells, facilitating colonization and infection. Additionally, *T. vaginalis* LPG is involved in eliciting a species-specific inflammatory response by active stimulation of the production and upregulation of specific chemokines and macrophage inflammatory proteins in human cervical and vaginal epithelial cells [6,7]. Recent studies showed that pregnant women infected with *T. vaginalis* experience a notable activation of the systemic immune response, coupled with a significant elevation in vitamin D levels. Vitamin D, recognized for its role in activating the innate immune system and modulating adaptive immunity, appears to play a key role in the immune response to *T. vaginalis* infection during pregnancy. This interplay between infection, immune activation, and vitamin D levels in pregnant women not only provides insights into the host response to *T. vaginalis* but also raises important considerations for maternal and fetal health in the context of this parasitic infection [8,9]. Thyroid disorders represent one of the most common endocrine diseases with incidence increasing with advancing age. A key marker in the evaluation of thyroid function is the Thyroid Stimulating Hormone (TSH). Elevated levels of TSH in the bloodstream coupled with normal levels of thyroid hormones suggests the presence of subclinical hypothyroidism. The link between Vitamin D levels and the pathophysiology of thyroid disorders remains tentative [10]. This study aimed to explore the association between Vitamin D levels and TSH levels with *T. vaginalis* infection. This exploration involved the determination of the link *T. vaginalis* infection and the levels of inflammatory markers in both pregnant and non-pregnant women.

2. MATERIALS AND METHODS

2.1 Sample collection

This study was conducted between September 2022 and October 2023 at Baghdad Teaching Hospital, Al-Yarmouk Teaching Hospital. A total of sixty high vaginal swab (HVS) and serum samples were collected from symptomatic non-pregnant and pregnant women of various ages presenting with vaginal itching. Exclusion criteria included women with chlamydial infection or other medical conditions such as diabetes, rubella, autoimmune diseases, blood and circulatory disorders, hormonal disorders, or other infectious diseases. High vaginal swabs were obtained from each participant (both patients and controls) and stored at -20°C for subsequent analysis. The serum samples were analyzed using immunofluorescence techniques to measure TSH and vitamin D levels. TSH detection was performed using a chin-mounted human TSH immunofluorescence kit with a sensitivity of 4.69 pg/ml, as per the manufacturer's instructions. Vitamin D levels were determined using two methods: an immunofluorescence kit (human vitamin D protein.chin) with a sensitivity range of 30-100 ng/ml and an ELISA kit (25-OH vitamin D total (vit D-Direct) Test System, Monobind Inc., USA), following the manufacturer's guidelines. Statistical analysis of the data was conducted using GraphPad Prism software, with P values less than 0.05 considered statistically significant [11].

2.2 Samples examination

For the microscopic examination of vaginal swab samples, two distinct staining techniques were employed to identify *T. vaginalis*. Immediately following collection, each swab sample was placed on a sterile glass slide and mixed with appropriate staining solutions. The preparation was then covered with a slip and examined under a light microscope using a 40 \times objective lens. This process facilitated the identification of motile, pear-shaped protozoans characteristic of *Trichomonas vaginalis*. All samples were analyzed promptly after collection to ensure optimal observation of the parasites [12].

3. RESULTS

The age range of the patients in this study did not differ statistically significantly. The mean values of the pregnant and non-pregnant groups differed, although not to the point of statistical significance. (Table 1A). The quantitative values were allocated and computed based on the type of patient—that is, pregnant or not—that was recorded. The pregnant had the greatest mean for all documented statistically significant differences when compared to the non-pregnant, despite the statistical significance. (table 1B).

Table 1. - Concentration of V.D and TSH in the study patients and controls

Group	N	n(%) <i>T. vaginalis</i> +ve patient	X2 (P-value)
pregnant	5	5	3.6(0.06)
Non-pregnant	20	11	
Pregnant<30 years old	4	4	4(0.2)
Pregnant>30 years old	1	1	

Non-pregnant<30 years old	14	7
Non-pregnant>30 years old	6	4

The current study also looked into the connection between illness and vitamin D levels in the serum. The statistics indicate a substantial difference ($p < 0.5$) between the patient and control groups, with the two groups' mean concentrations being approximately identical (p value 0.0144). (Table 2) There was no significant difference ($p < 0.05$) between the patients' and control groups' connection between vitamin D and TSH, with a p -value of 0.7, as indicated in Table 2. There was no statistically significant difference seen between TSH and vitamin D.

Table 2. - Two inflammatory markers (TSH and V.D.)

factor	T vaginalis +ve (n=16)	T vaginalis -ve (n=9)	P-value
TSH	1.02 ± 1.15	0.87 ± 1.19	The p-value is 0.7 The result is not significant at $p < .05$
Vit.D	24.75 ± 7.64	36.125 ± 13.76	The p-value is 0.0144. The result is significant at $p < .05$.

4. DISCUSSION

Two inflammatory markers (TSH and V.D.) were measured in the blood to see how much of each was present when the female reproductive system was infected. Vitamin D was only considerably raised, despite the fact that the means of concentration for both markers varied between the sera of patients and controls examined in a large body of research, [13]. There is currently no clear explanation for the increased risk of autoimmune thyroid illness in those with *Trichomonas vaginalis*. Infections, psychological stress, and environmental and genetic factors are considered to be contributing contributors to this illness. There has been a suggestion that *Trichomonas vaginalis* and autoimmune are likely related. Theoretically, IR happens when immune cells, including γ -cells, produce cytokines in response to self-antigens. [14,15] The current study looked into the relationship between TSH and vitamin D, although the analysis did not reveal any appreciable variations. Vitamin D has a weak correlation with TSH. however, these were not statistically significant, most likely as a result of the small sample size that was employed [16]. In order to find out how trichomoniasis affects serum cytokine concentration, researchers looked at the levels of the cytokines IFN- γ , IL1 β , TNF- α , IL-6, and IL-8 [17]. Several other research produced varied results. Another study found a negative correlation between acute stroke patients' vitamin D level and CRP and IL-6 [18]. It is widely acknowledged that the level of cytokines may serve as a marker for a number of different medical conditions. To examine their involvement in the production of pelvic adhesions, for example, peritoneal fluid was used to assess IL-1, IL-6, and TNF- α . Laparoscopy included the collection of peritoneal fluid. Women with endometriosis had higher serum IL-6 concentrations, and this was thought to be a good indicator for women who might develop endometriosis. [19]. Furthermore, women with polycystic ovarian syndrome have been shown to have higher CRP levels, and it has been suggested that CRP is a blood marker for cardiovascular disease. [20]. This study was conducted with the aim of investigating the levels of inflammatory markers in sera of patients with vaginitis. It can be concluded that bacteria can affect the levels of inflammatory markers (vitamin D, TSH) and these markers could be risk markers for multiple diseases. [21] It may be inferred from our results and the findings of other research that a high level of vitamin D is a typical result of viral infection. On the other hand, if the infection progresses to create harmful reproductive system problems, like endometriosis, polycystic ovarian syndrome, or pelvic adhesion formation, the TSH level may [22]. As a result, research on the immune system's inflammatory response to *T. vaginalis* in pregnant women has been extensive. The trichomoniasis vaginalis t-infected pregnant women had a significant elevation of pro-inflammatory cytokines.

Funding

None

ACKNOWLEDGEMENT

The acknowledgment is presented to all the staff of the Baghdad Teaching Hospital, Al-Yarmouk Teaching, for their support in blood sampling.

CONFLICTS OF INTEREST

The authors declare no conflict of interest

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