



## Relationship between Leukotriene B4 (LTB4) and the pathogenicity of *Trichomonas vaginalis*

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### ABSTRACT

Trichomoniasis, which is brought on by the parasite *Trichomonas vaginalis*, is the most prevalent non-viral, curable worldwide sexually transmitted disease that affects millions of individuals every year. Numerous immunological and biochemical elements were discovered to be involved in the pathogenesis of *T. vaginalis*. In our study, we sought to determine how *T. vaginalis* virulence and leukotriene B4 (LTB4) interact. 350 women (15–65 year) who visited a women's and gynecological hospital in Karbala between November 2021 and June 2022 for this study provided swabs of vagina, samples of urine and samples of blood took place. Routine urine analysis and examination of by direct microscopic to vaginal swabs were used to identify parasites, and its ELISA approach was used to calculate serum (LTB4) levels. Participating women filled out a questionnaire that asked about their age, place of residence, marital status, degree of education, reproductive potential, and use of contraception. As a result, only 100 of the 350 cases tested positive for *T. vaginalis*, with an age group (15–24 year) having the highest frequency and the age group 65+ having the lowest incidence (15–24). 55 years). -65) years. By educational background, 18 women (28.57%) were illiterate, 22 (29.33%) in primary education, 42 (28.18%) in secondary education, and 18 (28.57%) in bachelor level. They did not differ significantly from one another ( $P=0.998$ ). In healthy, non-patient-infected women, the mean and standard deviation of serum (LTB4) were 19.14 and 7.14, respectively, but in patient-infected women, they were (96.41 and 25.9). did. ( $P 0.001$ ) Significant difference. We can infer from the current study that women who had *T. vaginalis* infections had significantly higher serum levels of LTB4.

**Keywords:** *Trichomonas vaginalis*, *Trichomoniasis*, *Leukotriene B4*

### INTRODUCTION

A significant worldwide health issue is sexually transmitted infections (STIs). Sexually

transmitted illness like Chlamydia, Gonorrhea, Syphilis, genital warts and trichomoniasis are thought to affect 500 million people annually.

Additionally, her HSV2 has infected over 530 million people, and her HPV has infected over 290 million women [1].

Through sexual contact, *T. vaginalis* is passed from one person to another. The trophozoite of this parasite clings to the mucosal surface of the urogenital canal during its life cycle and divides via longitudinal binary fission. *T. vaginalis* successfully colonizes the host mucosa by a variety of pathogenic mechanisms, including adhesion. interaction with the vaginal microbiome, evasion and control of the host immune system, production of cytotoxic chemicals and soluble substances, and the initiation of immunological responses [2].

Trichomoniasis, a neglected STI with a prevalence of 110.4 million cases and an incidence of 156 million, is brought on by the flagellated protozoan *Trichomonas vaginalis*. [3,4]. According to the World Health Organization's (WHO) most recent estimates, the African region has the largest incidence of trichomoniasis, which is followed by the Americas, Western Pacific, Eastern Mediterranean, Southeast Asia, and finally the European region. Region [5]. Although the majority of cases are asymptomatic, reports of itching, discharge, irritation, and odor persist. Infertility, pelvic inflammatory illness, cervical and prostate cancer, as well as premature birth and low birth weight in babies are all linked to long-term infection with *T. vaginalis*, which can last for months to year [6,7]. In addition, there is evidence of a reciprocal association between *T. vaginalis* infection and human immunodeficiency virus (HIV) transmission and acquisition, with people of *T. vaginalis* infection 1.5 times more likely than healthy individuals to acquire HIV [8].

Leukotrienes (LTs) are lipid mediators produced by the 5-lipoxygenase (5-LO) passageway during the metabolism of arachidonic acid. Leukotriene B4 (LTB4) is created when 5-LO and LTA4 hydrolase act together on LTA4 [9]. Experimental infections caused by microorganisms in vivo and in vitro interact with phagocytes to produce LTs [10]. LTs play essential roles in both innate and adaptive immune responses [11], [12]. Through the

modulation of both Th1 and Th2 immune reactions, LTB4 are implicated in the defense against protozoan and helminthic illnesses [13]. Nitric oxide (NO) and cytokine generation during infection is the mechanism through which LTB4's antimicrobial action [14], antiparasitic activity [15], and antifungal activity occur. [16]. The phagocytic and antibacterial capabilities against microorganisms, including parasites, will be reduced in the presence of any abnormality in LTB4 biosynthetic pathways.[14] [16] bacteria [17] and fungi [15].

Our earlier research demonstrated that *T. vaginalis* LTB4 release might draw in and stimulate neutrophils. [18]. We also discovered elevated amounts of LTB4 in vaginal discharges from patients with symptomatic trichomoniasis in a preliminary research. [19]. LTB4 also causes a chemotactic response and a number of other reactions, including aggregation, degranulation, and oxidative metabolism, all of which aid in the eradication of invader species [20]. Since neutrophils are known to produce LTB4 and interact with *T. vaginalis* during the inflammatory reaction, [21], A sizable concentration of LTB4 is conceivably expected to liberate and heighten host defenses against *T. vaginalis*. So, in this investigation, we looked into how humoral immunity might control the formation of LTB4 by neutrophils in response of stimulation of *T. vaginalis*.

## MATERIALS AND METHODS

In the current search, swabs of vagina, samples of urine, and samples of blood were collected from 350 women who attended the women's obstetrics and gynecologist hospital in Karbala province between November 2021 and June 2022. The women's ages ranged from 15 to 65. The parasite was found using a comprehensive urine examination and a direct microscopic examination of vaginal swabs, while the serum (LTB4) level was determined using the ELISA approach. The participant women were asked a series of questions on their age, place of residence, marital status, degree of education, fertility, and use of contraceptives.

The samples of venous blood were drawn into anticoagulant-free gel tubes, allowed to clot for

15 minutes at room temperature, and then centrifuged for 10 minutes at 3000 rpm to obtain serum. Serum LTB4 levels were measured using the ELISA method, which uses enzyme-linked immune sorbent assays. The presence of the parasite T. vaginalis was determined using both a general urine test and a direct microscopic inspection of the vaginal swabs.

**The Statistical Tests**

The SPSS version 25 software was used to analyze the statements. For variances, the t-test as well as Chi square were used, P 0.05 was regarded like respectable.

**The Results**

Only 100 (28.57%) of the 350 women in this study tested positive for the parasite T. vaginalis, while 250 (71.42%) tested negative, as shown in the table (1).

**TABLE 1:** shows the number and percentage of positive cases based on microscopic analysis.

Total No. of examined samples	Positive Samples(+)		Negative Samples(-)	
	No.	%	No.	%
	350	100	28.57	250

The results in table (2) showed The relationship between positive samples with the level of fertility, and the table showed that the number of positive samples was 28/12 (35.71%) in infertile

cases, while the table showed that the number of samples in cases without infertility was 90/322 (27.95%). There are no significant differences (P=0.383).

**TABLE 2:** Positive samples distributed according to fertility

P-Value	Positive Cases(+)		Total Number	Fertility
	%	No.		
0.383*	35.71	10	28	Yes
	27.95	90	322	No

As for the relationship between infection with vaginal trichomoniasis and cases of abortion, the table below indicates the incidence of infection, which was (34.09%) for non-abortion women

and (12.35%) for aborted women. The current study found significant differences at the level of likelihood (P > 0.05). There are significant differences at the level of probability 0.05(P=0).

**TABLE 3:** Distribution of parasite infection according to abortions

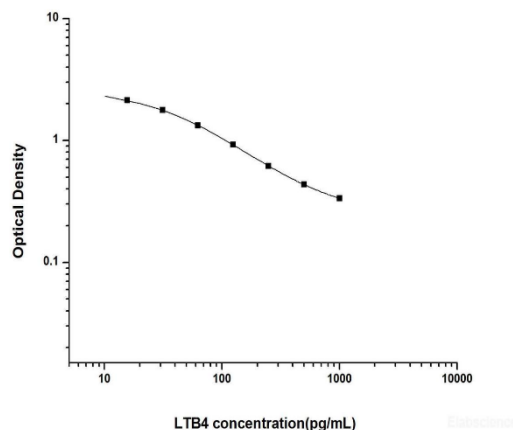
P -Value	Positive Cases(+)		Total Number	Abortions(Abs.)
	%	No.		
0*	12.35	11	89	Yes
	34.09	89	261	No

According to the results in table (4), there was a highly significant difference (P 0.001) between the mean and standard deviation of serum LTB4

levels in the healthy women group (19.147.14) and the infected patient women group (96.4125.9).

**TABLE 4:** The Mean  $\pm$ SD in healthy, LTB4 levels and infected women

The Groups	Mean $\pm$ SD
The Healthy –women	19.14 $\pm$ 7.14
The Patients	96.41 $\pm$ 25.9
Calculated P-Value	<0.001



**FIGURE 1:** Display of the ELISA-generated LTB4 concentration estimate curve

### DISCUSSION

The epidemiology of *T. vaginalis* infection takes into account factors including age, living place, socioeconomic status, pedagogics, marital status, form of contraception used, existence as well as type of vaginal secretions, medicines taken, and history of other STDs. Numerous risk factors may have an impact on. infections [22].

According to the study's findings, there are noticeable distinctions between infection and abortion, as the highest infection rate was recorded among women who did not suffer from abortion, amounting to 34.09%, compared to 12.35% for aborted women. 2008. And Al-Kazragee (2013) recorded the highest infection rate among women with normal pregnancies (non-abortions), amounting to 5.82%, compared to aborted women, 4.67%, and the results of this study were inconsistent with some studies, such as the study conducted by Al-Ziyadi (2004) in Najaf, when an infection was recorded High in aborted women, with a rate of 19.76%, compared to women with normal pregnancies, 19.4% also agreed with Al-Khatawy (2012) . The highest rate of infection was recorded among women with normal pregnancies, at a rate of 10%, which is higher than that recorded for aborted women

by 6.67%. It was found that the parasite *Trichomoniasis* is able to pass into the Fallopian tubes, carrying many pathogenic organisms that cause infections and infertility (Lujan et al, 2022).

As for the occurrence of miscarriage, it is attributed to many reasons, including those that are embryonic causes. It is possible that infection of the placenta leads to inflammation of the membranes surrounding the fetus and the release of the prostaglandin hormone, or miscarriage may occur as a result of the early activity of the uterus (Terzic & Aimagambetova et al, 2021)

The current study's findings also showed that there were notable disparities in the distribution of parasite infection between pregnant and non-pregnant women, with the highest infection rate among non-pregnant women being 35.71% and the infection rate among pregnant women being 27.95%. The rate of infection among non-pregnant women was 25.79% higher than that of pregnant women at 13.69%, also in agreement with Al-Saeedi (2016). The rate of parasite infection among non-pregnant women was more than that of pregnant women. The reason for the increase in the infection rate in non-pregnant

women is attributed to the abnormal conditions of the vagina, as well as the person's health level, and that pregnant women may not have the effectiveness for the occurrence of the disease, and the occurrence of infection in those women may be attributed to the change that occurs to the environment of the vagina from the conditions of acidity that change Animal growth from Moniliasis to Trichomoniasis. The appearance of the infection in pregnant women may be due to the hormonal changes that occur during pregnancy and the enlargement that occurs in the vaginal epithelium, as well as the high level of glycogen and estrogen, and this helps in providing a suitable medium for parasite growth and reproduction (Studd, 2008).

According to Dahab et al. (2012) and Al-Ibrahimi (2008), non-pregnant women had the greatest infection rates, 5.6% and 13.3%, respectively. This result supports the need for routine clinical examinations for the early detection and treatment of these diseases in women. Pregnant women are advised to visit health units regularly for a pregnancy check-up (Adeoye & Akende, 2007).

According to the results of the current investigation, there was a highly significant difference in the level of LTB4 between the trichomoniasis infected women and the healthy control women. The percentage of the LTB4 immune criterion in the serum of infected women was (19.14 7.14) compared to control women. This is consistent with a rise in leukotriene as a result of parasite infection, which led to the development of immunity in the lining tissues. The vagina contains a large number of monocytes and thus produces large amounts of LTB4. The findings of this study are consistent with a study by Eida et al. (2015), which found that women with symptoms of *T. vaginalis* infection had higher levels of the leukotriene LTB4. LTB4 is a leukotriene stimulating factor that *T. vaginalis* secretes. Nemati et al. (2018) show that through the interaction of the leukotriene LTB4 with the leukotriene receptors BLT of host cells, *T. vaginalis* has the capacity to communicate directly with immune cells, This modification of the host immune response and activation of neutrophils is the result of this communication. As of Min et al.(2017), These results also

correlate well with previous animal studies where leukotrienes LTB4 has been demonstrated to be increased in animals infected with other nematodes and other parasites such as *Entamoeba histolytica* (Jimenez et al., 2021).

## REFERENCES

1. W.H.O. (2012). Global incidence and prevalence of selected curable sexually transmitted infections-2008. In: World Health Organization. Geneva, Switzerland: 1-20.
2. Secor, W.E.; Meites, E.; Starr, M.C.; Workowski, K.A. (2014). Neglected parasitic infections in the United States: Trichomoniasis. *Am. J. Trop. Med. Hyg.*, 90, 800–804.
3. Rowley, J.; Vander Hoorn, S.; Korenromp, E.; Low, N.; Unemo, M.; Abu-Raddad, L.J.; Chico, R.M.; Smolak, A.; Newman, L.; Gottlieb, S. (2019). Chlamydia, gonorrhoea, trichomoniasis and syphilis: Global prevalence and incidence estimates. *Bull. World Health Organ.*, 97: 548–562.
4. Menezes, C.B.; Frasson, A.P.; Tasca, T. (2016) Trichomoniasis—Are we giving the deserved attention to the most common non-viral sexually transmitted disease worldwide? *Microb. Cell*, 3: 404–419.
5. Ghosh, I.; Mandal, R.; Kundu, P.; Biswas, J. (2016). Association of genital infections other than human papillomavirus with pre-invasive and invasive cervical neoplasia. *J. Clin. Diagn. Res.*, 10, XE01–XE06.
6. Masha, S.C.; Cools, P.; Sanders, E.J.; Vanechoutte, M.; Crucitti, T. (2019). *Trichomonas vaginalis* and HIV infection acquisition: A systematic review and meta-analysis. *Sex. Transm. Infect.*, 95: 36–42.
7. Harp, D.F. and Chowdhury. I. (2011). Trichomoniasis: evaluation to execution. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 157(1): 3-9.
8. Golden, M.P. and Henderson, W.R. (2007) Mechanisms of disease: Leukotrienes. *The New England Journal of Medicine*, 357: 1798-1854.
9. Golden, M.P., Canetti, C., Mancuso, P. and Coffey, M.J. (2005). Leukotrienes: underappreciated mediators of innate immune responses. *The Journal of Immunology*, 174: 589-594.
10. Rogerio, A.P. and Anibal, F.F. (2012). Role of Leukotrienes on protozoan and helminthes infections. *Mediators of Inflammation*, 595-694.
11. Mancuso, P., Nana-Sinkam, P. and Peters-Golden, M. (2001). Leukotriene B4 augments neutrophil phagocytosis of *Klebsiella pneumoniae*. *Infection and Immunity*, 69: 2011-2016.
12. Machado, E.R., Ueta, M.T., Lourenço, E.V., Anibal, F.F., Sorgi, C.A., Soares, E.G. and



- Faccioli, L.H. (2005). Leukotrienes play a role in the control of parasite burden in murine Strongyloidiasis. *The Journal of Immunology*, 175: 3892-3899.
13. Medeiros, I.A., Sa-Nunes, A., Soares, E.G., Peres, C.M., Silva, C.L. and Faccioli, L.H. (2004). Blockade of endogenous Leukotrienes exacerbates pulmonary histoplasmosis. *Infection and Immunity*, 72: 1637-1644.
  14. Anibal, F.F., Rogerio, A.P., Malheiro, A., Machado, E.R., Martins-Filho, O.A., Andrade, M.C. and Faccioli, L.H. (2007). Impact of MK886 on eosinophil counts and phenotypic features in toxocarosis. *Scandinavian Journal of Immunology*, 65: 344-352.
  15. Peres-Buzalaf, C., de Paula, L., Frantz, F.G., Soares, E.M., Medeiros, A.I., Peters-Golden, M., Silva, C.L. and Faccioli, L.H. (2011). Control of experimental pulmonary tuberculosis depends more on immune stimulatory Leukotrienes than on the absence of immunosuppressive Prostaglandins. *Prostaglandins Leukotrienes and Essential Fatty Acids*, 85: 75- 81.
  16. Gillin, F.D. & Sher, A. (1981). Activation of the alternative complement pathway by *Trichomonas vaginalis*. *Infection and Immunity* 34: 268.
  17. Holbrook, T.W., Boackle, R.J., Vesely, J. & Parker, B.W. (1982). *Trichomonas vaginalis*: alternative pathway activation of complement. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 16:473.
  18. Rein M.F., Sullivan J.A. & Mandell G.L. (1980). Trichomonacidal activity of human polymorphonuclear neutrophils: killing by disruption and fragmentation. *Journal of Infectious Disease*. 142:575.
  19. Shaio, M.F., Chang F.Y., Hou S.C., Lee C.S. & Lin P.R. (1991). The role of immunoglobulin and complement in enhancing the respiratory burst of neutrophils against *Trichomonas vaginalis*. *Parasite Immunology*, 13: 241-50.
  20. Shaio M.F., Lin P.R., Lee C.S., Hou S.C., Tang P. & Yang K.D. (1992). A novel neutrophil-activating factor releasing by *Trichomonas vaginalis*. *Infection and Immunity*, 60:4475-82.
  21. Shaio M.F. & Lin P.R. (1995). Leukotriene B4 levels in vaginal discharges from patients with trichomoniasis. *Annals of Tropical Medicine and Parasitology*. 89(1):85-8 .
  22. Samuelsson B. (1983). Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. *Science*. 220(4597):568-75.
  23. Ford-Hutchinson A.Q., Bray M.A., Doig M.V., Shipley M.E. & Smith M.J.H. (1980). Leukotriene B4, a potent chemotactic and aggregating substance released from polymorphonuclear leukocytes. *Nature*. 286:264.
  24. Liarte S, Bernabé-García Á, Nicolás FJ. (2020). Role of TGF- $\beta$  in Skin Chronic Wounds: A Keratinocyte Perspective. *Cells*, 9(2):306.