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A study of *TLRs* gene polymorphism in infected women with *Trichomonas vaginalis* in Babylon Province

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يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعمَلُونَ خَبِير

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Supervisors Certification

We certify that this thesis, entitled **A study of *TLRs* gene polymorphism in infected women with *Trichomonas vaginalis* in Babylon Province**, was prepared by Zainab Waddah Nasser Kermasha under supervision at the College of Medicine, the University of Babylon as a Partial of the Requirements for the Degree of Ph.D. in Medical Microbiology.

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Dedication

I dedicate my Thesis to ***Imam***

Musa ibn Jafar Al-Kadhim

(Peace be upon him)

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Summary

Trichomoniasis is a sexually transmitted infectious disease caused by a protozoan called *Trichomonas vaginalis*. There is an elevated incidence and prevalence rate, in addition to, an increased resistance profile to the standard treatment. Moreover, serious health complications were also reported which highlighted the importance to this infection.

The pivotal aim of this study was evaluating the association between toll-like receptors (TLRs) polymorphism and between *T. vaginalis* infection among infected women. In order to perform this aim, specific objectives were fulfilled including, molecular determination of the corresponding parasite in women vaginal discharge using specific primers by polymerase chain reaction (PCR) technique, studying the single nucleotide polymorphisms (SNPs) in TLRs (Studied SNPs were rs5743708 in TLR2, rs4986790 in TLR4, rs5743810 in TLR6, rs179008 in TLR7 and rs4988453 in myeloid differentiation primary response 88 (MyD88)), and finally estimating the TLR2, TLR6, TLR7 and (MyD88) levels by using enzyme-linked immunosorbent assay (ELISA) technique in Trichomoniasis-infected women as well as control group.

Vaginal swabs and blood samples were collected from 226 female individuals including 40 healthy women (Controls) and 186 female patients, the later patients were admitted to the gynecology clinics in Al-Imam Al-Sadiq Hospital, Babylon Teaching Hospital for Maternity and Children and Al-Hilla Teaching Hospital, in addition to, several private clinics in Babylon in Iraq between February till December 2022. The collected samples were engaged in molecular identification of the parasite, sequencing of the TLR2, TLR4, TLR6, TLR7 and MyD88 genes as well

as performing the immunological studies using ELISA assays for the corresponding TLRs and MyD88. Deoxyribonucleotides were extracted from the swabs and the conserved β-tubulin (BTUB) gene was amplified using PCR and specific primers to be applied in molecular diagnosis.

Out of the 186 female patients, 40 women (21.5%) showed positive PCR outcomes of *T. vaginalis* BTUB gene versus only seven positive cases (3.76%) detected by wet-mount microscopy. Residency and age-related prevalence of *T. vaginalis* infection were not statistically significant, despite the higher positive cases reported in urban area. Additionally, higher percentages of history of abortion were seen in infected females versus non-infected females. Clinical diagnosis demonstrated that vaginal discharge and itching were the most prevalent symptoms reported by infected females. Molecular study of rs5743708 SNP in TLR2 showed that the frequency of non-mutant G allele was clearly higher in infected women (37/64) than controls (27/64), and the GG genotype has significantly higher prevalence (90%) within infected women versus the GA (5%) and AA (5%) genotypes ($p<0.001$).

Regarding rs4986790 SNP in TLR4, the mutant G allele frequency was greater among infected women (7/11) versus controls (4/11), and importantly, homozygous GG genotype in infected women showed two times higher frequency (2/3) than controls (1/3). Genetic analysis of rs5743810 SNP in TLR6, revealed that the mutant G allele was significantly higher in infected women (17/24) than healthy controls (5/24) ($p=0.021$), in addition, the heterozygous AG and the homozygous GG demonstrated significantly higher frequencies (13/16 and 2/3) in trichomoniasis women versus controls (3/13 and 1/3), respectively, $p=0.013$. For rs179008 SNP in TLR7, the mutant T allele revealed significantly more prevalence in women infected

with *T. vaginalis* in comparison with controls ($p < 0.001$), furthermore, AT and TT genotypes distribution were significantly greater in patients than that in controls ($p = 0.0013$). Moreover, MyD88 SNP (rs4988453) genetic analysis showed that the mutant A allele almost has close frequency between patients and controls, and the heterozygous CA and homozygous AA genotypes were almost normally distributed between controls and patients. Finally, the concentrations of all TLRs included in the immunological studies (TLR2, TLR6, TLR7 and MyD88) were significantly elevated in majority of women patients aged between 16-50 years.

Women infected with trichomoniasis were slightly higher in urban area and they showed higher abortion cases. Importantly, vaginal discharge and itching were the most common clinical symptoms reported by infected women. Genetic analysis showed that mutant A alleles of rs5743708 and rs4988453 SNPs in TLR2 and MyD88, respectively, did not associate with increased risk of trichomonas infection, however, mutant alleles of rs4986790 (G), rs5743810 (G) and rs179008 (T) SNPs in TLR4, TLR6 and TLR7, respectively, can make women more sensitive for infection with *T. vaginalis*. Additionally, immunological studies exhibited significantly higher concentrations of TLR2, TLR6, TLR7 and MyD88 in women infected with *T. vaginalis* than controls particularly in women during sexually active ages.

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List of Abbreviations

Abbreviation	Description
AIC	Akaike information criterion
BIC	Bayesian information criterion
BLT1	Leukotriene B4 receptor 1
BLT2	Leukotriene B4 receptor 2
BTUB	β-tubulin gene
DAMPs	Damage-associated molecular patterns
DNA	Deoxy-ribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
ELIZA	Enzyme-linked immunosorbent assay
H5N1	Avian influenza virus
HCMV	Human cytomegalovirus
HCV	Hepatitis C virus
HeLa cells	Epithelial cell line
HIV	Human immune deficiency virus
HRP	Horseradish Peroxidase
HSV	Human herpes simplex virus
HWE	Hardy-Weinberg Equilibrium
Ig	Immunoglobulin

IL	Interleukin
iNOS	Increase gene expression of nitric oxide
kDa	Killo dalton
LPDCs	Lamina propria dentritic cells
LPG	Lipophosphoglycan
LPS	Lipopolysaccharide
M1	Classically macrophage
M2	Alternative activated macrophage
Mbp	Mega- base pair
MD	Myeloid differentiation factor
MIF	Migration inhibitory factor
MyD88	Myeloid differentiation primary response 88
NCBI	National Center for Biotechnology Information
NF-κB	Nuclear factor kappa B
OD	Optical density
ODNs	CpG oligodeoxynucleotides
PAMPs	Pathogen-associated molecular patterns
PBMCs	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PFO	Pyruvate ferredoxin
PRRs	Pattern recognition receptors

RBC	Red blood cell
RNA	Ribonucleic acid
RNIs	Reactive nitrogen intermediates
ROI	Reactive oxygen intermediates
ROS	Reactive oxygen species
RPM	Revolutions per minute
SARM	Sterile-alpha and Armadillo motif-containing protein
SNPs	Single nucleotide polymorphisms
STD	Sexually transmitted disease
TIR	Toll-interleukin-1 receptor
TIRAP	Toll/Interleukin-1 receptor domain-containing adapter protein
TLCK	Tosyl-L-lysine chloromethyl ketone hydrochloride
TLRs	Toll-like receptors
TNF-α	Tumor necrosis factor-α
TRAM	TRIF-related adaptor molecule

Chapter One

Introduction and Literatures Review

1. Introduction

T. vaginalis is an anaerobic parasitic protozoan having characteristic and flagella. It was firstly isolated from vaginal secretion by Alfred François Donné in 1836. *T. vaginalis* was considered as a mild sexually transmitted disease in humans (Menezes *et al.*, 2016). However, there is an elevated incidence and prevalence rate, in addition to, an increased resistance profile to the standard treatment. Moreover, serious health complications were also reported which highlighted the importance to this infection (Secor *et al.*, 2014). The interaction between the *T. vaginalis* parasite with the host cells was reported as a complex interaction through dependent and/or independent mechanisms. Interestingly, *T. vaginalis* does not have mitochondria, and it includes a large molecular weight genome of 176 Mbp. This genome constitutes six chromosomes, which characterizes this parasite (Carlton *et al*, 2007).

Trichomonas vaginalis is considered the unique etiological factor of an infection termed trichomoniasis. The genitourinary tract tissues of both women and men are the natural sites of infection of this parasite (Maritz *et al*, 2014). *T. vaginalis* parasite has round shape with a well-developed membrane and four anterior flagella which are important and characteristic motility tools and also essential for diagnosis. Epidemiologically, it infects about more than 170 million people globally (Coceres *et al*, 2021). Asymptomatic *T. vaginalis* cases are the major type of this infection which represent a challenge in measuring the rates of infection, and early diagnosis as well as the treatment of *T. vaginalis* (Soper, 2004; Bachmann *et al*., 2011; Alderete and Chan, 2023). Symptomatic cases of *T. vaginalis*, although mild, are mainly seen as itching in the genitourinary tissues.

Unfortunately, *T. vaginalis* may be developed in severe complications demonstrated as ulcerations in the cervix, abortion, premature birth or infertility in

both men and women (Rigo *et al*., 2022). Moreover, studies showed an elevated risk of getting HIV, cervical cancer in women and prostate cancer in men when this *T. vaginalis* infection is confirmed (Soper, 2004; Sutcliffe *et al.*, 2006; Zhang *et al*., 2014). Consequently, newer therapeutic options of drugs and vaccines represent a fundamental requirement to control the infection with *T. vaginalis* and prevent its severe complications.

In humans, trichomoniasis produces specific antibodies in the reproductive tract against the parasite and also antibodies that circulate in the serum (Baxt *et al.*, 2008). In addition, *T. vaginalis* triggers innate immune system and the corresponding TLRs. These receptors are types of transmembrane proteins which constitute an important member of the human innate immune system against different microorganisms (Nie *et al*., 2018). In human, ten TLRs were reported in immune response and combinations of these proteins were also recognized in different types of human cells (Jiménez-Dalmaroni *et al.*, 2016). TLRs involve two domains; extracellular and intracellular domains. The function of the extracellular part is to recognize the ligand of microorganism, while the intracellular part, after activation, is involved in the dimerization or association with other intracellular receptor molecules. Additionally, the intracellular domains of toll-interleukin-1 receptors (TIRs) also interact with other intracellular proteins, like the innate immune signal transduction adaptor (MyD88) and TIR domain containing adaptor protein (TIRAP), which engage in signal transduction (Rajpoot *et al*., 2021). TLRs mainly associate with MyD88 to transduce signals, except TLR3, TLR2 and TLR4, and they are associated with MyD88 after they dimerize with TIRAP (Lannoy *et al.*, 2023).

T. vaginalis infection produces inflammatory response in the genitourinary tract of both sex, male and female, by TLRs stimulation. Specimens taken from

cervical and vaginal regions of the infected women stimulated the TLR4 responsive cells of mice spleens to produce cytokine (Yadav *et al*., 2021). Finally, the macrophage apoptosis resulted from *T. vaginalis* infection was shown to upregulate TLRs expression (Particularly, TLR2, TLR4, and TLR9) which are recognized in HeLa cell line after they infected with *T. vaginalis* (Chang *et al*., 2006).

1.1. Aim of study

The main goal of this research project was to study the association between TLRs polymorphism with *Trichomonas vaginalis* infection among women. To accomplish this goal, the following objectives are set:

1. Detection of *T. vaginalis* in vaginal discharge through Microscopically and molecular diagnosis of the parasite using specific primers by PCR technique.
2. Study the TLR gene polymorphism.
3. Estimation of TLR2, TLR6, TLR7 and MyD88 by using ELISA technique in Trichomoniasis-infected women as well as control group.
4. Study the associations between TLR gene polymorphism and trichomoniasis.

1.2. Literature Review

1.2.1. Historical Veiw

In 1836, a French bacteriologist and doctor called Alfred François Donné was first discovered a motile parasite named *T. vaginalis* in purulent vaginal secretions from infected female patients, this parasite later in 1916 was shown as the causative agent of vaginal infection, trichomoniasis (Donne, 1836; Graves *et al.*, 2019; Manny *et al.*, 2022).

Trichomoniasis is demonstrated as a very widely distributed cosmopolitan parasitic sexually transmitted infection (STD) affecting the genitourinary system of humans. Additionally, this infection was reported decades before other STIs such as *Chlamydia trachomatis* and human papilloma virus (HPV) infections (Harp and Chowdhury, 2011).

T. vaginalis is an early existing anaerobic flagellated protists protozoan which branched before kinetoplastids which are of the earliest protozoa containing mitochondria. *T. vaginalis* unlike most eukaryotic cells in that it lacks mitochondria, and it is considered a fermentative organism because it uses hydrogenosomes to perform carbohydrate metabolism utilizing the hydrogen as an electron acceptor. A common ancestry containing mitochondria was shown to have hydrogenosomes depending on the similarities in activity of protein import. The hydrogenosomes differ mainly from mitochondria in that they do not have cytochromes, enzymes of the respiratory chain and genetic materials (DNA) (Ma *et al*., 2022).

1.2.2. Classification

The classification position of *T. vaginalis* is assigned depending on the classification scheme reported by previous studies (Aquino *et al*., 2020). Classification rank includes:

Kingdom: Animalia

Subkingdom: Protista

Phylum: Sarcomastigophora

Class: Zoomastigophora

Order: Trichomonadida

Family: Trichomonadidae

Genus: *Trichomonas*

Species: *Trichomonas vaginalis* (Donné, 1836).

1.2.3. Parasite Structure and Life Cycle

The external appearance of the *T. vaginalis* is highly affected by the physiochemical properties of the environment. In axenic culture, *T. vaginalis* has a typical pyriform shape in the culture media, however, *T. vaginalis* shape may appear as amoeboid especially when the parasite is adherent to the epithelial tissue of vagina (Malli *et al.*, 2020). *T. vaginalis* has dimensions of about 10 μm length versus 7 μm width with five flagella. Four flagella located in the anterior side of the parasite, while the fifth posterior flagellum is located in a fin-like part of the parasite membrane called undulating membrane, and is strengthened by the costa that is a slender noncontractile structure. The flagella as well as the undulating membrane are responsible for the *T. vaginalis* parasite motility, called quivering motility (Cheon *et al.*, 2013). Some reports showed that the parasite may alter its shape to

round and internalize the flagella when the surrounding environment does not favor normal growth (Collántes-Fernández *et al.*, 2017).

Live visualization of cells shows that *T. vaginalis* amoeboid phases actively move across the epithelial cells of infected human rather than simply attaching to cells, and this movement is characterized by coordinated motion with an average rate of 20 μm/min, and also by using of flagella and apical edge as the leading margins (Kusdian *et al*., 2013).

Internally, the *T. vaginalis* parasite involves a prominent nucleus surrounded by a porous nuclear envelope and a rod-like structure, the axostyle, which is an internal rigid structure supporting the parasite body from the anterior to the posterior ends. Axostyle protrudes from the posterior side, the sharp point, of the parasite and it is thought that this structure helps anchoring the parasite to the epithelial cells of the vagina (Malli *et al*., 2020). Organelles of the *T. vaginalis* parasite are catalase negative, meaning that these organelles are not peroxisomes (Schneider *et al*., 2011). *T. vaginalis* organelles generate molecular hydrogen, consequently called hydrogenosomes which are the organelles responsible for generating energy and they play a significant role in metabolism (Tachezy *et al*., 2022).

T. vaginalis shows hydrolase activity and involves lysosome-like components termed, phagosomes (Zimmann *et al.*, 2022). The cytoskeletal structure of *T. vaginalis* involves two types of fibers, actin and tubulin. Concerning tubulin, dissimilar types of these fibers were recognized in this parasite. Similarly, sequences of peptides in the actin fibers were also reported to be different as shown by their different cleavage properties when used with proteolytic enzymes (Riestra *et al*., 2015).

The life cycle of *T. vaginalis* was described as a simple cycle involving the trophozoite stage that is transmitted sexually from person to person and it lacks cyst formation, the trophozoite stage is dividing by asexual reproduction, binary fission. During infection, this fission develops into larger colonies habitats the lumen as well as the mucous membranes of the diseased subject's genitourinary tract (Khalil *et al.*, 2012).

The parasite *T. vaginalis* is visible in culture as several large round shapes in dividing growth phase including, *T. vaginalis* without flagella, *T. vaginalis* with a dividing nucleus and flagella, and *T. vaginalis* with multiple nuclei and flagella (Beri *et al.*, 2020).

Recent studies mentioned that these forms of the parasite are suggested to be developmental stages of *T. vaginalis* life cycle occurring before the development of mononuclear flagellates (Beri *et al.*, 2020). The previously mentioned large round forms of the parasite differ from the small flagellated pyriform not only in morphology, but also in the mechanism of division. It was shown that amitotic budding is reported to be the mechanism of division of *T. vaginalis* round forms instead of the mitotic division (longitudinal binary fission) of the pyriform of the parasite which does not involve the disappearance of the *T. vaginalis* nuclear membrane (Yusof and Kumar, 2012).

1.2.4. Epidemiology

Trichomoniasis has been reported to be precipitated in any climate and season, and can be distributed in all races and socioeconomic groups with a total estimation of more than 170 million medical conditions globally (Workowski *et al.*, 2021). More than 56% of patients who attending sexual health clinics suffer from

trichomoniasis (Meites *et al.*, 2013). In 2005, a study recorded about 63 infected females out from 1000 tested person and about 82 infected men out from 1000 tested person (World Health Organization, 2011). Some studies reported higher prevalence of this corresponding infection in sexual health clinics of particular in low-income residential areas in or near the center of the cities (Carlton *et al.*, 2007). For example, the prevalence of *T. vaginalis* in African Americans who attend public sexual health clinics was about 38% in the capital city of California (Meites *et al.*, 2015).

Annually, 7.4 million of trichomoniasis of newly diagnosed cases were reported in the USA, making the incidence of infection with this parasite to be more than recorded for other sexually transmitted diseases such as chlamydia and gonorrhea (Gaydos *et al*., 2011). Even with this high incidence, trichomoniasis is not considered a nationally notifiable disease. Studies performed on populations of African Americans announced that the prevalence of trichomoniasis is about 11 to 25% (Mavedzenge *et al.*, 2010). Interestingly, it is shown that the incidence is about 38% within a period of 4-month exposure among women infected with human immunodeficiency virus (HIV) in Zaire (Kawuki *et al.*, 2022).

The infection with *T. vaginalis* was recognized in 21.3% of Syrian women participants using direct microscopy, and also in 36% by using Giemsa staining of samples. Gynecological investigation showed that 56.2% of women were diagnosed as vaginitis by clinical examination (Yentür Doni *et al.*, 2016). In Saudi Arabia, out of 155 women involved in a study, 79 subjects were symptomatic versus 76 subjects were a symptomatic. Nested PCR investigation revealed that *T. vaginalis* infection was prevalent in about 20% of symptomatic cases and 9% of control cases (Hawash *et al.*, 2022).

Locally, the prevalence of trichomoniasis was about 1.66% in Sulaimaina governorate in Iraq and the highest rate was seen in women aged between 26 to 35 years old (Kadir and Fattah, 2010). In Baghdad, another study performed in AL-Liqa'a Hospital showed that trichomoniasis is detected in 18 out of 250 tested women and the highest rate of infection was reported in women aged between 25 to 29 years old (Khalil *et al.*, 2012). This high incidence rate of the *T. vaginalis* infection relays primarily on various parameters including age, activity and number of partner sexual behavior, other sexually transmitted diseases, menstruation, diagnosis and laboratory technique.

Epidemiological data have showed an association between trichomoniasis with other sexually transmitted diseases specially gonorrhea (Javanbakht *et al.*, 2013). Additionally, trichomoniasis was also reported to be present with bacterial vaginosis in most *T. vaginalis* infected women (Margarita *et al.*, 2020). Regarding the age, sexually active women are vulnerable to trichomoniasis at any age groups, while other common sexually transmitted diseases exhibited greater prevalence among women in adolescent and young ages. Consequently, trichomoniasis was manifested as an indicator of risky sexual behavior because of the high association with other sexually transmitted diseases as well as the high incidence at any age groups (Hearn *et al.*, 2015).

1.2.5. Modes of Transmission

It was shown that *T. vaginalis* utilizes one natural host that is human. Additionally, sexual intercourse was reported as the main mode of transmission of the parasite between infected and noninfected host (Maritz *et al.*, 2014). Interestingly, male partners of women infected with trichomoniasis demonstrated

higher prevalence than average *T. vaginalis* infection rates in their genitourinary tract, and cure of the recurrent infection was achieved after effective eradication of the *T. vaginalis* parasite in the male partners and infected women as well. Moreover, women with sexually transmitted disease who attending sexual health clinics regularly are more vulnerable for trichomoniasis than women with normal sexual behavior as well as virgin and postmenopausal women (Schwebke and Burgess, 2004; Arbabi *et al.*, 2018).

Although sexual intercourse was described as the main mode of transmission of the parasite, nonsexual mode of infection was also reported in few cases such as through contaminated toilet seats, specula, vaginal douche nozzles and other tools (Adeoye and Akande, 2007). Furthermore, live trophozoites of the parasite have been detected in biological fluids, particularly urine and seminal fluid of infected people even after being expelled outside the body and exposed for air for several hours or in swimming pools (Menezes *et al.*, 2016). Studies performed on infected pregnant women revealed that 2 – 17% of female newborns may acquire *T. vaginalis* from their infected mothers (Mabaso and Abbai, 2021). Socioeconomic factors besides low education level reflected greater prevalence of *T. vaginalis* infection among women (Barbosa *et al*., 2020).

On the other hand, the use of oral contraceptives containing progestins was shown to decrease the incidence of *T. vaginalis* disease, the increase in thickness of the cervical mucosa which may decrease the capability of mucosal penetration not only for sperms, but also for bacteria and parasite (Saheb *et al.*, 2016). Based on the above modes of transmission and because of the high prevalence of the infection, effective control of the trichomoniasis requires medical screening for the *T. vaginalis*

in both women and their male partners as well as the use of effective pharmacological and non-pharmacological therapeutic options (Van-Der Pol, 2016).

1.2.6. Clinical Manifestations

Wide range of clinical manifestations have been recognized with trichomoniasis. In infected women, the clinical manifestations may range from asymptomatic cases as in carrier women to cases with severe clinical symptoms as in flagrant vaginitis. Studies demonstrated that within six months about one third of asymptomatic women may show clinical symptoms (Margarita *et al.*, 2020). Once the infection is confirmed, the parasite primarily infects the squamous cells of the epithelial tissue of the genital tract. The parasite stays longer time in women in the previously mentioned genital tissue than in men. And because the parasite is mainly transmitted through sex, the symptoms are rarely seen in females before menarche and during menopause. In 50% of infections, 4 - 28 days were reported as the incubation period, and the trichomoniasis has been categorized into three main categories, including acute, chronic and asymptomatic infection based on how much the infection is severe (Dalby and Stoner, 2022).

In acute trichomoniasis infection, there is diffuse vulvitis because of the presence of copious leukorrhea associated with a characteristic yellow to green frothy mucopurulent discharge (Schumann and Plasner, 2022). Additionally, 2% of infected women shows a speckled image called strawberry appearance due to the presence of small hemorrhagic spots on the mucosa of vagina and cervix. The occurrence of these characteristic signs and symptoms follows a cyclic behavior and they are getting worse close to the time of menstruation (Tarney and Han, 2014).

When the infection becomes chronic, the symptoms change significantly from that in acute trichomoniasis. Major symptoms during the chronic course of the disease include pruritis and dyspareunia with scanty vaginal secretion that is combined with mucus. The presence of these symptoms in a woman, on the other hand, indicates that this woman has turned into infectious source of the parasite (Seña *et al*., 2014; Kissinger , 2015).

The third category of infection is termed asymptomatic trichomoniasis. About 25-50% of women infected with *T. vaginalis* parasite are asymptomatic with a normal pH level of vaginal secretion (3.8 - 4.2) in addition to normal vaginal flora, vaginal microbiota. However, infected women in this stage called carrier, and about 50% of these asymptomatic carriers can turn into symptomatic within six months (Kissinger , 2015).

The most characteristic clinical manifestation of trichomoniasis infection in women is vaginitis, however, other symptoms may appear include adnexitis, endometritis, infertility, pyosalpinx, low-birthweight babies and signs of cervical erosion (Fichorova, 2009). Furthermore, an elevated HIV transmission was also reported by some studies (Alcocer-Bruno *et al*, 2020).

In men, however, the infection has been described mainly as asymptomatic, and infected men were considered as asymptomatic carriers of *T. vaginalis* parasite. Some exceptions have been reported, studies were also shown that some men may show clinical manifestation when they are being infected with *T. vaginalis*. Based on that and depending on the presence of symptoms, trichomoniasis in men have been classified into three classes: asymptomatic infection, the men in this category considered as carriers and they have been detected through medical examination as partners of the women infected with *T. vaginalis*. In acute infection, men are

characterized by the presense of urethritis with profuse, clear or mucopurulent urethral discharge. Other symptoms were also recorded like painful urination and/or burning following sexual activity (Van Gerwen *et al*., 2021). And lastly the mild infection, the men have clinical symptoms similar to that seen with nongonococcal urethritis (Ziaei *et al*., 2022). About 11% of nongonococcal urethritis in men were shown to be caused by *T. vaginalis* parasite, with a duration of about 10 days (Meites , 2013). Serious complications of trichomoniasis in men have also been revealed by some studies such as inflammation of urethra, prostate and epididymis and infertility (Schumann and Plasner, 2022).

1.2.7. Virulence and Pathogenesis of *Trichomonas vaginalis*

Studies showed development in understanding the biochemical mechanisms by which the *T. vaginalis* parasite affecting and damaging the cells and tissues of host. *T. vaginalis* parasite possesses several virulence factors such as adhesion molecules (adhesins and cysteine proteinases) (Figueroa-Angulo *et al.*, 2012), cells detaching factors with trypsin-like activity (Gao *et al.*, 2021), pore-forming proteins (Diaz *et al.*, 2020), phospholipase-A-like proteins (Zimmann *et al*., 2022), saposin-like proteins (Diaz *et al.*, 2020), glyceraldehyde-3-phosphate dehydrogenase (Lama *et al*, 2009), α-enolase (Mirasol-Meléndez *et al*, 2018), legumain-like CPs and triacylglycerol lipase (Rendón-Gandarilla *et al.*, 2013).

First of all, the adhesion capability of the infectious parasitic pathogen to the host cells was shown as an important factor in the corresponding pathogenesis. Several adhesive molecules were recognized on the *T. vaginalis* surface which are produced by the parasite to help attaching to its host target. Interestingly, sub-culture investigations aimed to target these adhesion molecules by antibodies revealed a reduction in the adhesion of the parasite into the cells of the host with a reduction in

the cytopathogenic ability (Ryan *et al*., 2011). Further studies reported that *T. vaginalis* adhesion with mammalian cells or tissues resulted in upregulation of adhesion molecules and the parasite shape turns into flattened structure to laminate with the host cell target. *T. vaginalis* cytoadherence highly relays on cysteine proteinase activity to get efficient adhesion molecules adhering the parasites to the target cells (Hernández *et al*., 2014).

Iron also plays an important role in this corresponding adhesion through iron-responsive elements (such as, AGATAACGA) in DNA of *T. vaginalis*, these iron responsive elements induce the transcription of an adhesion protein called ap65-1 (65,000 MW) supporting the role of iron in the control of expression of adhesion molecules (Tsai *et al*., 2002; Figueroa-Angulo *et al*., 2015). Similar to the cell-cell contact seen with leukocytes, research studies showed the relationship between the host cells and the parasite *T. vaginalis* as a complex interaction which subsequently produces efficient cytopathogenicity against mammalian cells of the host (Ryan *et al*., 2011).

Other protein molecules were reported, hydrolases, which were widely described together with cysteine proteinases to be involved in the adhesion process, these hydrolases have 20 -110 kDa molecular weight and considered of the low molecular mass proteinases released by the host cells (Wang *et al*., 2020).

In addition to proteinic cysteine proteinase, *T. vaginalis* parasite has other molecules having carbohydrate moiety as functional groups utilized by the *T. vaginalis* parasite to adhere to epithelial cells in human vagina (Hernández *et al*., 2014). DNA knockouts or gene silencing techniques, like RNA interfering, offer great facilities to understand the exact role of each molecule or product in the process of adhesion and the subsequent cytopathogenicity of the parasite toward human cells (Chen *et al*., 2018; Schwebke and Burgess, 2004). On the host cells sides, there is

no clear information regarding the receptors on the host cells that bind with the parasite, however, some data demonstrated that laminin might be important for adhesion of *T. vaginalis* with the target host cell (Ryan *et al.*, 2011; Núñez-Troconis, 2021).

Besides the adhesion molecules, other molecules produced and released by *T. vaginalis* parasite termed cell-detaching molecules, these molecules have trypsin-like activity and they show their activity on the target cells of human and hamster casing these cells to detach and round up. Studies indicated that *T. vaginalis* products of proteinases and cell-detaching factors act to degrade extracellular matrix proteins, like laminin and vitronectin, affecting host cell release from tissue.

Furthermore, it was noticed that the levels of proteases inhibitors secreted by leukocytes in patients diagnosed with *T. vaginalis* infection is lower than that in non-infected people (Huppert *et al.*, 2013). These findings support the impact of *T. vaginalis* proteases in enhancing the severity of HIV infection among patients infected with *T. vaginalis*.

Biochemical studies determined some of the properties of these proteases. For example, studies determined the structure and specificity of some of these parasite products. Additionally, studies demonstrated that EDTA, pepstatin and phenylmethyl sulfonyl fluoride do not inhibit the enzymatic activity of these proteins, while antipain, leupeptin, iodoacetic acid and finally Nα-Tosyl-L-lysine chloromethyl ketone hydrochloride (TLCK) do inhibit *T. vaginalis* proteases. Proteinases having low molecular masses of 34 kDa or lower were shown to particularly hydrolyze substrates possessing Arg-Arg residues, while other *T. vaginalis* proteinases demonstrated wide range of substrate activity (Schwebke and Burgess, 2004; Núñez-Troconis , 2021).

Recent research projects suggested the presence of cytotoxic molecules released by the parasite into the target cells which mediate the cytopathogenic effects like damaging the outer plasma membrane of the target cell. Other effects include perforin like cytotoxicity through creating pores in the membranes of red blood cells (RBC) as demonstrated by electron microscopy (Wirth *et al.*, 2014). In 2004, a lytic factor was detected by Lubick and Burgess (2004) which had been shown as a membrane-attacking molecule produced by *T. vaginalis* to destroy the nucleated cells as well as the RBCs of the host. This lytic factor was considered as a virulence factor and suggested to have a phospholipase-A_2 like activity due to its ability to destroy the phosphatidylcholine component of the target cell membrane (Lubick and Burgess, 2004; Zimmann *et al*., 2022).

From the parasite defense system point of view, thioredoxin was described as the main antioxidant mechanisms in trichomonads defense systems. This is due to the fact that the expression of thioredoxin and thioredoxin peroxidases is upregulated in elevated oxidative stress caused by environmental changes. Interestingly, the sequence of amino acid of thioredoxin reductase in *T. vaginalis* is different significantly from that found in human host cells, which highly supports the utilization of this structural difference as selective target for therapeutic purposes (Miyamoto *et al.*, 2021).

1.2.8. *Trichomonas vaginalis* -Viruses

T. vaginalis infection of the urogenital tract in human requires the adherence of the parasite to the mucosa of the host. This adherence enables the parasite to obtain necessary nutrients from the host cells in the urogenital environment, and also release small extracellular vesicles. The later vesicles were shown to be involved in

the *T. vaginalis* adherence as well as the modulation of the host-parasite interaction. The other important thing about the parasite is that 40-70% of *T. vaginalis* strains were demonstrated to possess a virus of double-stranded RNA type called Trichomonasvirus in the *T. vaginalis* isolates. The particles produced by this Trichomonas virus may have the ability to trigger proinflammatory response in the host (Rada *et al.*, 2022). However, higher virus prevalence in parasite isolates of about 82% was recorded in previous studies (Da Luz Becker *et al*., 2015).

Furthermore, Trichomonasvirus may affect the expression of certain antigenic molecules on the surface of *T. vaginalis* such as the P270 antigen (Fichorova *et al.*, 2017). Indeed, loss of expression of these antigens was reported in *T. vaginalis* parasites non-infected with Trichomonasvirus (Barrow *et al.*, 2020). In addition to that, researchers showed another Trichomonasvirus, named *T. vaginlis*V2-1, which has the capability to code two capsid proteins including a capsid protein (85 KDa) and a capsid-polymerase fusion product (160-kDa), in addition to two other proteins (Rada *et al.*, 2022). The exact function of this dsRNA Trichomonasvirus in the mechanism of cytopathogenesis of *T. vaginalis* is still under research and needs to be clearly elucidated.

However, the advanced progress in genome sequencing of *T. vaginalis* offered valuable information about the genes responsible for cytopathogenesis of *T. vaginalis* parasite in human host. Recently, the availability of 5X genome sequencing data at (http://www.tigr.org/tdb/e2k1/*T. vaginalis*g/) offers a great comprehensive data regarding the genomic sequence of *T. vaginalis* parasite (Worthey and Myler, 2005).

1.2.9. Host Immune Response

Clinical studies, animal models and in vitro experiments have been largely served as fundamental sources of knowledge of host immune response against *T. vaginalis* parasite and its related species. Immunological studies showed that after trichomoniasis, partially protective immune defense is generated. Indeed, infection may only reoccur in about 30% of people after regular health check-ups (Núñez-Troconis, 2021).

1.2.9.1. Innate Immunity against *Trichomonas vaginalis*

The female reproductive tract has the ability to perform several activities which are required to adapt with different physiological and pathological events. Of these activities, female reproductive tract is considered a particular immunological site which performs an essential role on mucosa protection from pathogens. Additionally, the mucosal immune response of the female genital tract is under regular adaptation to respond effectively to various physiological events at this tissue (Wira *et al.*, 2011). Several factors also have the capability to modulate both innate and adaptive immune responses at the genital tract mucosa, these factors include hormones, pregnancy, conception, cell to cell interaction, specific microbiome and anatomic components (Fahey *et al.*, 2005; Kaushic *et al.*, 2010). Both, host systemic as well as mucosal immune responses have been reported to be activated at the genital tract of females representing a characteristic feature of this tract. Regardless of the site, the development of multiple immunological mechanisms enables the host to inhibit progression of pathogenic infections (Wira *et al.*, 2011).

The mucosa of the urogenital tract of the human is manifested by immunological studies as the first line of protection from the infectious

microorganisms including *T. vaginalis* parasite. The immunity in this physiological barrier involves innate as well as adaptive immunity (Malla *et al.*, 2014). During trichomoniasis , both types of immune responses, innate and adaptive, were reported, and studies prescribed the humoral immune response as a transient partially protective immune response against *T. vaginalis* (Malla *et al.*, 2014 Menezes and Tasca, 2016).

Regarding the adaptive immunity against *T. vaginalis*, T cell mediated immune response participates significantly in elimination of the parasite as stated by previous studies. Both types of immunity were dependent on each other in two directional communications to control and eliminate the *T. vaginalis* infection. For example, T cell mediated immune response to be developed, an earlier response of innate immunity is needed. Alternatively, cytokines of the adaptive immunity are required to maintain the function of innate response. Indeed, IFN-γ has been required to support macrophage function, while interleukins 17 and 22 (IL-17 and IL-22) were shown to assist the function of neutrophiles and production of cathelicidin by host cells epithelium, respectively (Menezes and Tasca, 2016).

The innate immune response against *T. vaginalis* is a local nonspecific defense mechanism involves complement system (alternative pathway) activation, toll-like receptors (TLRs) stimulation and neutrophils as well as macrophages accumulation (Malla *et al.*, 2014; Menezes and Tasca, 2016). The parasite is sensitive to direct killing by complement system, and the later activation can provide C3 opsonin that enhances phagocytosis of *T. vaginalis* by neutrophils (Kalia *et al.*, 2019). However, the parasite may show resistance to phagocytosis because of its ability to produce cysteine proteases which preserve the *T. vaginalis* from lysis by complement system (Hernández *et al.*, 2014). Ibanez-Escribano A and his research team reported the

sequestration of CD59 from different host cells like RBCs by *T. vaginalis* parasite, the corresponding parasite uses CD59 sequestration process to evade immune response of the host (Ibanez-Escribano *et al.*, 2015).

An important part of the host innate immune strategies is the involvement of membrane-bound proteins called Toll-like, these receptors with other receptors have been shown to participate effectively in the recognition of microorganisms and identification of pathogen-associated molecular patterns (PAMPs) (Behzadi and Behzadi, 2016; Nemati *et al.*, 2017). Trichomoniasis infection has been shown to upregulate the expression of several TLRs including TLR2, TLR4 and TLR9 on the membrane of epithelial host cells in the urogenital tract, this over expression highly suggests their essential role in stimulating the immune defense system of the infected host toward the infectious parasite (Chang *et al.*, 2006). The contact of *T. vaginalis* parasite with vaginal epithelial cells stimulate the expression and release of IL-6 and IL-8 and other inflammatory mediators of cytokines and chemokines like macrophage inflammatory protein-3α (Malla *et al.*, 2014; Mielczarek and Blaszkowska, 2016). IL-8 (Chemokine CXCL8) strongly attracts the neutrophiles which present in high number in secretions of reproductive tract in women and considered a fundamental part of the innate immune response of the host. Neutrophils, in addition, have been involved in the generation of reactive oxygen species (ROS) which further improves the ability of neutrophils to kill the corresponding parasite (Song *et al.*, 2008; Isailovic *et al.*, 2015). Different types of ROS are generated by neutrophils such as hydrogen peroxide, superoxide anions, nitric oxide and defensins (Song *et al.*, 2008; Escario *et al.*, 2010).

In addition to neutrophils, infected women vaginal secretions showed elevated numbers of polymorphonuclear leucocytes and high levels of leukotriene B4

(secreted by *T. vaginalis*) which participates in chemotaxis of neutrophils into infected tissue imposing an inflammatory response (Nam *et al.*, 2012). Leukotriene B4 released by *T. vaginalis* binds to its receptors, leukotriene B4 receptor 1 (BLT1) and leukotriene B4 receptor 2 (BLT2), stimulating the generation of IL-8 by immune cells like neutrophils and mast cells (Nam *et al.*, 2011). In women, leukotriene B4 and subsequent generation of IL-8 act as important inflammatory mediators of mucosal inflammatory response. Leukotriene B4 released by host also play a role in stimulating the immune cells to produce ROS and other peptides which have antimicrobial properties like β-defensin-3 and cathelicidin (Le Bel *et al.*, 2014).

Macrophages are also involved in the immune response against *T. vaginalis*. When stimulated by live *T. vaginalis* parasite, these leukocytes have been reported to support host immunity through several pathways including generation of nitric oxide (NO, that is cytotoxic to *T. vaginalis*), increase gene expression of nitric oxide synthase (iNOS) and generation of inflammatory factors including, IL-1β, IL-6, IL-8 and TNF-α (Han *et al.*, 2009). A significant correlation was noticed between concentrations of interleukins (particularly, IL-1β and IL-6) and TNF-α with the level of expression of iNOS in trichomoniasis (Han *et al.*, 2009). In infected women, vaginal secretions and leukocytes isolated from asymptomatic individuals exhibited greater levels of iNOS and reactive nitrogen intermediates (RNIs) in comparison to that in symptomatic and control groups. *T. vaginalis* infection performed in mice also showed elevated levels of RNI (Yadav *et al.*, 2006). It is suggested that high levels of iNOS and RNIs reflects better control of infection as seen with asymptomatic women (Nemati *et al*., 2018).

Macrophages have been classified generally into two main types; classically (M1) and alternatively activated macrophages (M2) (Wang *et al.*, 2014). The M1

type is highly involved in antigen presentation activity, production of IL-12 and IL-23 as well as generation of ROS and RNI (Wang *et al.*, 2014; Muraille *et al.*, 2014). On the other hand, M2 has been shown to produce ornithine and polyamines through the arginase pathway and generate cytokines, particularly IL-10 and TGF-β (Muraille *et al.*, 2014). To understand the task of both M1 and M2 macrophages, identification of these immune cells in infected women vaginal secretions by immunohistochemistry may partly explain the tasks of these M1 and M2 macrophages. It was indicated that the macrophage response goes toward the M1 responses in asymptomatic women with *T. vaginalis* infection which promotes the elimination or at least minimize the symptoms of trichomoniasis infection by generation of ROI and RNI. However, the progress of infection goes toward the M2 responses in symptomatic women, which may favor the progression of infection by generation of immunosuppressive cytokines (Imam *et al.*, 2007).

1.2.9.2. Adaptive Immunity against *Trichomonas vaginalis*

Antigen stimulation together with the existence of cytokines involving IFN-γ/IL-12, TNF-α/IL-6, IL-4, TGF-β/IL-2 and TGF-β/IL-6 stimulate the differentiation of four T helper cells including Th1, Th2, Th17 and Th22 as well as the Treg cells from the CD4+ T cells, respectively (Rodriguez-Perea *et al*., 2016; Eyerich *et al*., 2017; Schmitt and Ueno, 2015).

Furthermore, various types of immune responses have been reported in patients infected with *T. vaginalis* parasite. Immunoassays showed that *trichomoniasis* infection in human has the ability to trigger antibody formation (Fichorova RN, 2009). Previous studies done using subcutaneous or intraperitoneal injections of the parasite in mice demonstrated the formation of antibodies which might be protective.

Additionally, recent research projects performed using subcutaneous injections of various doses of the *T. vaginalis* parasite followed by vaginal inoculation revealed significantly lower intravaginal trichomoniasis infection accompanied with elevated serum as well as vaginal antibody responses in comparison with control group (Schwebke and Burgess, 2004; Xie *et al*., 2017).

Clinical studies demonstrated that trichomoniasis stimulates antibody formation in the urogenital tract in addition to production of circulating functional antibodies in the host serum (Baxt *et al.*, 2008). Immune cells were also involved in the host immune response to *T. vaginalis* infection as shown with lymphocyte proliferation and hypersensitivity reactions. In details, the incubation of lymphocytes isolated from patients infected with *T. vaginalis* showed significant proliferation when incubated with *T. vaginalis* antigens present in parasite cellular products versus no proliferative response was recorded in lymphocytes from non-infected people, these findings highlighted the potential involvement of hypersensitivity immune reaction in modulating the inflammatory response against trichomoniasis (Fichorova RN, 2009). Consequently, the acquired immune response could be formed through natural *T. vaginalis* infection.

In *in-vitro* studies have shown the presence of antiadhesin antibodies, serum-derived antibodies as well as monoclonal antibodies, which inhibit the adhesion of *T. vaginalis* to different human cell lines like HeLa (Nemati *et al.*, 2018), in addition to adhesion to primary epithelial cells of human vagina (Núñez-Troconis J., 2021). Interestingly, the action of these antibodies resulted in lower damage of the mammalian cells which indicates the potential protective ability of these antibodies against the cell cytotoxic effect mediated through adhesion molecules (Nemati *et al.*, 2018). Additionally, studies showed that antibodies targeting the soluble molecules

of *T. vaginalis* parasite like cytoactive molecules, proteases enzymes and phospholipases similarly may protect host cells (Zimmann *et al*., 2022).

In-vivo studies performed to confirm the protective effect of antibodies against cytotoxicity of *T. vaginalis* parasite were limited because the beneficial animal models applicable experimentally in vaginal infections are also limited. Laboratory animals are not easily used as models for vaginal infections. However, experimentally induced vaginal infections in mice through vaginal inoculation of *T. vaginalis* parasite and lactobacilli have been performed (Smith and Garber, 2015). Additionally, successful infection of mice vagina with *Trichomonas foetus* which is a known parasite infecting the genital tract of cattle. Studies suggest that mice may act as a potential animal model to study the immune responses against trichomonads spices such as *T. vaginalis* parasite. Studies, further, showed that more than one antibody may be required to eliminate the *T. vaginalis* infection. Subsequently, both innate as well as acquired cellular immune responses play significant roles in the host response (Menezes and Tasca, 2016).

1.2.9.3. Selectivity of Adaptive Immunity

The most significant sites targeted by the protective antibodies are the adhesion molecules which perform a crucial impact in attachment of the *T. vaginalis* to host cells besides their subsequent role in the destruction of these cells. The *T. vaginalis* parasite was shown to have four surface elements have adhesive properties and being selectively targeted by the protective antibodies. Furthermore, studies mentioned that the parasite upregulates the expression of these adhesive molecules to improve the attachment (Harjunpää *et al.*, 2019).

1.2.9.4 Specific Antibody Responses Against *Trichomonas vaginalis* Infection

Specific antibodies against *T. vaginalis* were detected in serum and secretions withdrawn from hosts infected with *T. vaginalis*. Of these antibodies, IgA, IgG, IgM and IgE were reported by several techniques. The first three antibodies, IgA, IgG and IgM were detected in vaginal secretion as well as serum of women infected with *T. vaginalis*, while the IgE is present at low levels in genital specimens (Kaur *et al.*, 2008; Menezes and Tasca, 2016). Animal studies performed on Balb/c mice infected with *T. vaginalis* revealed that the tested antibodies, IgG1, IgG and IgM, elevated in serum and genital secretion and maximum concentrations were seen after 14 days after infection. Another study demonstrated significantly elevated concentrations of IgG1 and IgM in tested mice which are infected by *T. vaginalis* using samples isolated from women showing clinical symptoms of trichomoniasis, while isolates from asymptomatic women did not show significant levels of the two antibodies (Yadav *et al.*, 2005). In men, similarly, samples isolated from symptomatic men had significant elevated levels of IgG1, IgG2b and IgM as compared with asymptomatic men. Although the level of IgG2a in symptomatic men was elevated, this elevation was statistically insignificant (Imam *et al.*, 2007).

Based on the above data, differential patterns of immune responses against *T. vaginalis* were detected in symptomatic versus asymptomatic patients. An interesting finding was revealed by a study regarding the specific IgM antibody, which was found in serum of symptomatic women infected with *T. vaginalis*, while this antibody was not detected in serum and vaginal secretion of asymptomatic women (Kaur *et al.*, 2008). Based on the aforementioned findings, an association

was suggested between the level of specific antibody IgG1 and the clinical manifestations of trichomoniasis in infected host (Nemati *et al.*, 2018).

The concentration of parasite (parasite load) in addition to variations in strains of parasite might give rise to different levels of specific antibodies in both symptomatic and asymptomatic infected individuals. In addition to symptoms, some studies correlated the parasite load/strain variation with the duration of protection against the parasite and complications (Yadav *et al.*, 2005).

Furthermore, IgA antibody also involved in *T. vaginalis* infection. Indeed, it was shown that IgA concentrations were significantly elevated in tested mice subjects contaminated by isolates from asymptomatic infected women in comparison with symptomatic patients. Consequently, IgA antibody might be involved in protection from severe *T. vaginalis* infection in asymptomatic patients (Nemati *et al*., 2018). *T. vaginalis* infection in infertile women exhibited high levels of IgA antibody in the genital secretions versus low levels of complement proteins, C3 and C4 (Mielczarek and Blaszkowska, 2016). The level of IgG antibody, on the other hand, decreased or being undetected after 1-3 months in serum of women with acute symptoms of *T. vaginalis* infection after pharmacological treatment with metronidazole (Ton Nu *et al.*, 2015a). Interpretation of these findings was attributed to the lack of *T. vaginalis* antigens after effective treatment. But, with some patients who had ineffective treatment or reinfected by infected partners, the levels of specific antibodies (IgG) against *T. vaginalis* parasite stayed elevated as measured by ELIZA, indicating that the infection of these patients was a chronic type of trichomoniasis (Ton-Nu *et al.*, 2015a). An association between IgG antibody with neutrophiles has been seen through complement activation which results in induction of LTB4 release from these immune cells by IgG, a *T. vaginalis* specific antibody

(Malla *et al.*, 2014; Menezes and Tasca, 2016). Through complement activation-related activities, it is indicated that IgG antibody, which show high specificity toward *T. vaginalis*, enhances LTB4 generation.

In addition, elevated levels of IgA antibody, which also demonstrate specificity toward *T. vaginalis*, were determined in genital secretions from infected women and compared to control individuals who showed low levels after therapy (Malla *et al.*, 2014). Based on the aforementioned studies, it is demonstrated that the humoral mediated immunity against the *T. vaginalis* parasite is not persistent, and the reports regarding their potent efficacy in eliminating the parasite infection are limited. This is further supported by the challenges in developing effective vaccines against *T. vaginalis* (Menezes and Tasca, 2016).

T. vaginalis lipophosphoglycan (LPG) plays an essential role in parasite adhesion to epithelial cells of women vagina and stimulation of women immune response (Figueroa-Angulo *et al.*, 2012). *T. vaginalis* LPG stimulates leukocytes to release IL-8 and also stimulate the generation of antibodies specific for *T. vaginalis* such as, IgG and IgA. Moreover, these antibodies were shown to initiate synthesis of several factors including cytokines, leukotrienes, MIP-3α and RNI which are related to T helper 1 (Th1) cells (Mielczarek and Blaszkowska, 2016). *T. vaginalis* LPG is thought to be the most common antigen found in *T. vaginalis*-infected women's serum (Bastida-Corcuera *et al.*, 2013).

Alpha (α)-actinin has been reported as the most antigenic protein present in serum of infected women (Xie *et al.*, 2017). The presence of this protein is not limited for women, whereas both men and women may have this immunogen in their serum as evidenced by the production of specific antibodies for α-actinin protein of the *T. vaginalis* parasite (Neace and Alderete, 2013). On α-actinin, about 13 specific

antigenic determinants were detected. To clarify the importance of α-actinin, Balb/c mice were immunized intraperitoneally by two recombinant forms of α-actinins (ACT-F and ACT-T). These recombinant proteins subsequently resulted in enhancing defense against *T. vaginalis* infection, and elevated levels of specific antibodies against *T. vaginalis* (IgG, particularly IgG1) were detected in serum of tested animals. Furthermore, these antigenic determinants resulted in elevated cytokine (IL-6, IL-10, IL-17A and IFN-γ) production and increased proliferation of splenocytes isolated from immunized mice when stimulated in *in-vitro* study (Xie *et al.*, 2017).

It has been revealed that *T. vaginalis* parasite has the ability to produce migration inhibitory factor (MIF) which is a multifunctional cytokine affecting macrophages. MIF of *T. vaginalis* shows 47% identity with human MIF. However, this cytokine is considered an essential triggering factor for different types of autoimmune as well as inflammatory disorders. High level of expression of MIF was seen in cancer and is significantly correlated with progression of cancers, particularly prostate cancer (Twu *et al.*, 2014). Importantly, MIF produced by the *T. vaginalis* was shown to induce antibody production in patients infected with this parasite and also enhance growth and proliferation of prostate cells in men (Mielczarek and Blaszkowska, 2016). Consequently, use of antibodies against MIF of *T. vaginalis* may produce protective effect from development of prostate cancer (Twu *et al.*, 2014).

Another immunogenic antigen, pyruvate ferredoxin oxidoreductase (PFO), showed important attachment characteristics of *T. vaginalis* to host cells. When *T. vaginalis* parasite was incubated specific antibodies against PFO, low level of

proliferation of *T. vaginalis* trophozoites was seen which directly proportional with the concentration of antibodies (Song HO, 2016).

Specific types of antibodies were also found in patients infected with trichomoniasis against *T. vaginalis* legumain-1 (*T. vaginalis*LEGU-1), which is a significant virulence factor involved in *T. vaginalis* lysosomal degradation of nutrients and internal organelles and metabolism as well. Also, this protein was detected on the surface of the parasite, they bind to the surface of HeLa cells with high binding affinity, functioning as another important parasite cytoadherence factor. Particularly, IgG specific for this immunogenic legumain-1 decreased *T. vaginalis* adherence capability for up to 45% (Rendón-Gandarilla *et al.*, 2013).

1.2.9.5. Mechanisms of Humoral Immunity Evasion of *Trichomonas vaginalis*

Planning and development of approaches aiming to prevent and control trichomoniasis infection necessitate the understanding of mechanisms by which *T. vaginalis* parasite evade the immune system of the host, knowing that this parasite has been shown to have different mechanisms being utilized in such evasion. Of these mechanisms, cysteine proteases, which has the ability to degrade different types of immunoglobulins especially the specific antibodies against *T. vaginalis* parasite, including IgG, IgA and IgM in serum as well as genital secretions of infected women (Yadav *et al.*, 2007). The degradation of IgG and IgA antibodies was demonstrated following the incubation of these antibodies with *T. vaginalis* culture supernatants and lysates (Mielczarek and Blaszkowska, 2016). The parasite was shown to have the capability to generate soluble proteins in genital secretion of

women which might deactivate or counteract the activity of immunoglobulins and cytotoxic T cells (Figueroa-Angulo *et al.*, 2012).

An additional mechanism utilized by the *T. vaginalis* parasite to evade the host immune defense system is the presence of different types of immunogens. Some strains of *T. vaginalis* parasite have the ability to perform alternative expression of at least two types of surface antigens to escape host responses (Menezes and Tasca, 2016). *T. vaginalis* parasite expressing certain immunogens was called A+ phenotype, whereas parasite lacking these immunogens was named A- phenotype. These antigens significantly modulate the immune response and subsequently the pathogenicity of the parasite.

In details, A- strains demonstrated more cytotoxicity against HeLa cells through cytadherence-dependent mechanism than that in A+ strains of *T. vaginalis* parasite. Molecular mimicking is another pathway used by the *T. vaginalis* parasite to escape of host immunity, involving expression of molecules resembling the *T. vaginalis* adhesins such as AP33, AP51 and AP65, and also enzymes of the host (Zhang *et al.*, 2020). Hiding under coat of host molecules was also reported as one of the mechanisms by which *T. vaginalis* parasite escapes the host response (Figueroa-Angulo *et al.*, 2012). Moreover, some *T. vaginalis* isolates were shown to produce hemolytic and cytotoxic molecules through contact-dependent and -independent mechanisms (Malla *et al.*, 2008). B cells represent an important component of the humoral immunity of human, and these cells could be affected directly or indirectly by the *T. vaginalis* parasite. Indeed, *T. vaginalis* may kill the B cells either directly through direct contact mechanism, or indirectly through the generation of soluble factors which impose cytotoxic effects on these leukocytes (Mercer *et al.*, 2016).

Consequently, it is concluded that B lymphocytes are killed by *T. vaginalis* parasite more than other types of leukocytes. Other leukocytes, peripheral blood mononuclear cells (PBMCs) were also reported to undergo phagocytosis by *T. vaginalis* (Pereira-Neves and Benchimol, 2007). Understanding these evasion mechanisms would potentially enhance the control and eradication of the parasite.

1.2.10. Toll-like Receptors

Toll-like receptors represent membrane-bound proteins which are a type of pattern recognition receptors (PRRs). They are involved in sensing invading organisms extracellularly and also in lysosomes and endosomes located intracellularly. These receptors are located in innate immune cells of the host, and upon infection, they contribute in recognition of pathogen-associated molecular patterns (PAMPs), they also trigger humoral immunity against pathogen (Miggin and O'Neill, 2006; Sonnex, 2010). Of the total 13 TLRs identified in mammals, 10 functional TLRs were recognized in human which are designated TLR1 to TLR10. Of these ten receptors, TLR2, TLR3, TLR4, TLR6, TLR7, TLR8 and TLR9, were demonstrated as the main types of TLRs involved in the recognition of *T. vaginalis* parasite (Song *et al.*, 2015; Wang *et al*., 2019). When TLRs activated by PAMPs, they induce overexpression of pro-inflammatory mediators including cytokines and chemokines, initiating by that inflammatory response and affecting the degree of host adaptive immunity (Nemati *et al*., 2017).

Furthermore, *T. vaginalis* infection induces upregulation of the expression of specific TLRs in membrane of epithelial cells, this upregulation could significantly initiate the host immunity toward the parasite (Chang *et al*., 2006). Contact of *T. vaginalis* with vaginal epithelial cells stimulate production of different pro-

inflammatory mediators such as, ILs (6 and8) and macrophage inflammatory protein-3α (Malla *et al.*, 2014; Mielczarek and Blaszkowska, 2016).

TLRs are trans-membrane proteins characterized by two structural domains; first domain is an extracellular molecule involving repeated segments (motifs) of leucine rich amino acids, each segment 24 amino acid in length. This domain is located at the N-terminal and it's role is binding with ligands(Chang *et al.*, 2006). The second domain is a conserved intracellular signaling domain of IL-1 receptor that is called Toll/IL-1 receptor (TIR) and located at the C-terminal. Majority of TLRs adaptors were shown to be recognized in the cell membrane while others are expressed in the subcellular endosomal components. TLRs are generated in a wide range of cells including epithelial cells, non-hematopoietic endothelial cells, synovial fibroblasts, parenchymal cells and hematopoietic originated cells of dendritic cells, mast cells, macrophages, neutrophils, T and B cells. Nine members of TLRs including TLR1-TLR9 are considered highly conserved and expressed in humans and mice as well. TLR10, however, is recognized only in humans while TLR11, TLR12 and TLR13 are detected in mice (Takeda *et al.*, 2003; West *et al.*, 2006; Liu *et al*., 2020; Botos *et al.*, 2011).

Based on their location, TLRs could be classified into two subfamilies; cell surface and intracellular TLRs. Cell surface members of TLRs include TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10, while the intracellular TLRs were shown to be located in the endosomes and they involve TLR3, TLR7, TLR8, TLR9, TLR11, TLR12, and TLR13 (Kawai and Akira, 2010; Celhar *et al.*, 2012), As seen in Table (1.1). According to their amino acid sequences, human functional TLRs are classified into five subgroups including TLR2, TLR3, TLR4, TLR5, and TLR9 (Takeda *et al.*, 2003; Liu *et al*., 2020).

The TLR2 subgroup constitutes four members involving TLR1, TLR2, TLR6, and TLR10. These receptors are highly homologous and in the presence of their respective ligands they act in a pair-wise combination. The TLR9 subgroup involves of TLR7, TLR8 and TLR9. Lastly, TLR3, TLR4 and TLR5 subfamilies each involves a unique member which may work alone or in combination with other receptors (Kawai and Akira, 2007). TlR2 were reported to sense different elements, mainly lipoproteins, from viruses, bacteria, Mycoplasma and fungi. To recognize the ligand, TLR2 constitutes heterodimer with TLR1 or TLR6. Each heterodimer TLR1/TLR2 and TLR6/TLR2 has the ability to recognize specific ligands including triacyl and diacyl lipoproteins, respectively. When triacyl or diacyl lipoproteins bind with TLR2, different proinflammatory cytokines were generated in macrophages and dendritic cells.

Table (1.1): Human toll-like receptors involved in recognition of *T. vaginalis*.

Pathogen	Target Receptor	Cytokine	References
Trichomonas vaginalis	TLR 4	IL-8 and TNF-α	Yadav et al, 2021.
	TLR9	IL-8	Chang *et al.*, 2006.
	TLR1,2,3,4,6	IL1A, IL-6 and CCL-2	Soboll *et al.*, 2006.
	TLR7/8, 9	CSF-2, CSF-3 and IL-8	Chang *et al.*, 2006.
	TLR2, 4, 9	IL-8 and TNF-α	Chang *et al.*, 2006.

In addition, TLR2 activation in monocytes induces production of type I interferons (IFNs) in response to viral infection, indicating that TLR2 ligands

binding may result in different cellular responses based on types of cells stimulated (Barbalat *et al*., 2009).

Studies showed that TLR4 recognizes the ligands lipopolysaccharide (LPS) and myeloid differentiation factor type 2 (MD2) on the cell surface. The first ligand, LPS, is considered as a causative factor of septic shock and it constitutes a part of the outer membrane of gram-negative bacteria. Furthermore, TLR4 plays a role in viral cognition through attaching with proteins of viral envelope. TLR4 also controls *T. vaginalis* pathogenicity in addition to avian influenza virus (H5N1) through recognizing damage-associated molecular patterns (DAMPs) rather than recognizing the virus (Imai *et al*., 2008). This opinion is supported by the resistance of mice lacking TLR4 to serious complications of avian influenza virus. Acute lung injury precipitated by the same corresponding virus infection generates oxidized phospholipids, which induce TLR4 that further advocates the role of TLR4 in viral cognition.

TLR5 is largely produced by dendritic cells in small intestine particularly the lamina propria (LPDCs). It has been shown to recognize flagellin protein in bacterial flagellum. When recognition of flagellin occur, LPDCs stimulate differentiation of B lymphocytes into plasma cells which produce IgA and also trigger naive T cells differentiation into Th1 and Th17 antigen-specific cells (Uematsu *et al*., 2008). Studies performed on TLR5 amino acid sequence revealed a close homology between TLR5 and TLR11.

Several studies highlighted the significance of TLRs locations within the immune cell in recognizing ligands (Barton and Kagan, 2009). Importantly, self-nucleotides were considered potential ligands for TLRs which advance autoimmune

responses. For that reason, TLRs recognizing self-nucleotides are usually compartmentalized to escape unwanted immune responses.

The host immunity against pathogens highly depends on the TLR-mediated recognition of pathogen. Additionally, the excessive responses of TLR to their ligands may lead to fatal septic shock. These findings show that proper stimulation of TLRs is fundamental for eliminating invading pathogens from the infected host without precipitating unwanted effects.

1.2.10.1. TLR Signaling Pathways

TLRs recognition of PAMPs upregulates expression of specific genes, depending on which member of TLRs and types of immune cell involved. TIR domain within the adaptor molecules recruited to TLRs may partly explain the variations in TLRs signaling pathways (Akira *et al*., 2006). In general, five TIR domains were reported by previous studies including MyD88, TIRAP/Mal, TIR domain-containing adaptor-inducing interferon-β (TIRF), TRIF-related adaptor molecule (TRAM) and Sterile-alpha and Armadillo motif-containing protein (SARM). Finally, the signaling pathway of TLR is subdivided into two pathways based on which adaptor molecule is utilized (MyD88 and TRIF) (Akira *et al*., 2006).

1.2.10.2. Single Nucleotide Polymorphism (SNP) in TLRs

Single nucleotide polymorphisms (SNPs) have been widely reported in Toll-like receptor (TLR) genes specially those associated with *T. vaginalis* infection, and various studies were performed to understand and correlate the impact of these SNPs on human health after being infected with various infectious pathogens (Medvedev, 2013).

1.2.10.3. SNPs In TLR2

Lin, (2019) found that two SNPs, designated rs4696483/T and rs7656411/T, in TLR2 in women uses condom less than 100% were clearly affected the *T. vaginalis* parasite transmission from women to their partners. The T allele of TLR2 SNP (rs4696483) was shown to decrease the risk of parasite transmission from infected women uses condom less than 100%. The association between SNP in TLR2 and *T. vaginalis* infection was suggested to be corelated indirectly with recognition of the parasite endosymbionts (Lin , 2019). On the other hand, two SNPs in TLR2, designated 597C/T and rs3804100, were observed in Sub-Saharan Africans and they showed lower set-point HIV (Mackelprang *et al.*, 2014). However, two SNPs were detected in HIV patients in Spain including1892A/C and 2258G/A showed no significant role on HIV patients (Soriano-Sarabia *et al.*, 2008).

1.2.10.4. SNPs in TLR4

Several SNPs were found in TLR4 and the impact of these SNPs on human was mentioned by various clinical studies. First of all, rs4986790 SNP designated A1063G with an amino acid change Asp299Gly was widely reported to affect the response of human host against different infectious pathogens (Chen *et al.*, 2013; Chauhan *et al.*, 2019). In details, the corresponding SNP in male patients infected with *T. vaginalis* in the United States showed no association between trichomoniasis and prostate cancer (Chen *et al.*, 2013). Asp299Gly and Thr399Ile SNPs in Iraqi women did enhance the risk of infection by *T. vaginalis* parasite (Abdul-Mohsen and Chaloob, 2014). Other SNP in TLR4 designated rs11536889 CC was detected in Indian women infected with trichomoniasis was shown to elevate the risk of cervical cancer and cervicitis (Chauhan *et al.*, 2019).

1.2.10.5. SNPs in TLR6

Structurally, TLR-6 usually presents in heterodimer with TLR2 (as with TLR1) to facilitate host immune response against the lipopeptides derived from various pathogens. However, few data are available regarding the functional genomic studies concerning TLR6 genetic polymorphisms and their clinical relevance with trichomoniasis. However, SNPs recognized in TLR6 and other genes such as TLR1, TLR2 and TLR4 as well as two adaptor molecules (TIRAP and MyD88) were shown to be associated with sexually transmitted disease among African American women (Taylor *et al.*, 2012). Additionally, an association has been shown between SNPs in TLR1, TLR4 and TLR6 and the development of invasive aspergillosis in 127 patients received allogeneic hematopoietic stem cell transplant (Kesh *et al.*, 2005).

1.2.10.6. SNPs in TLR7

Viral single strand RNA is considered a classical ligand for TLR7. Of particular, HIV-1 virus was shown to stimulate human TLR7 and TLR8 through uridine-rich oligoribonucleotides. The acquisition of this virus increases significantly in association with other sexually transmitted diseases specially *T. vaginalis* (Masha *et al.*, 2019). The binding of these receptors with viral RNA ligands was demonstrated to modulate the susceptibility to infectious pathogens (Schroder and Schumann, 2005). A frequent polymorphism in TLR7 designated Gln11Leu (rs179008) was reported to increase viral load and promote the immune suppression in patients infected with HIV (Oh *et al.*, 2009). Alternatively, a frequent polymorphism 1A>G in TLR8 with an amino acid change, Met1Val (rs3764880), resulted in lower progression of infection in patients infected with HIV (Oh *et al.*,

2008). Additionally, genetic polymorphism in both receptors may reduce the immune response in hosts infected with HCV (Wang *et al*., 2011).

1.2.10.7. SNPs in MyD88

As mentioned previously, MyD88 was found to play a significant role in recognizing *T. vaginalis* through activating signaling pathways of immune responses in association with several TLRs. Similar to TLRs, studies stated that SNPs in MyD88 could affect *T. vaginalis* infection. In this field, significant association was reported between SNP in MyD88 (rs6853/G) and parasite transmission from infected women to their unprotected sex partners (Lin Y, 2019).

In addition to *T. vaginalis*, SNPs in Myd88 were also correlated with other sexually enhanced infections, like bacterial vaginosis. For example, SNPs were determined in MyD88 gene (such as, rs4988457) in association with *Lactobacillus iners* as a vaginal microbiome in Kenyan female individuals (Mehta *et al*., 2020). Two other SNPs were detected in MyD88 human gene, designated 938C>A and 1944C>G, demonstrated an association with elevated susceptibility for *Mycobacterium tuberculosis* infection (Okada and Shirakawa, 2005).

However, SNPs in MyD88 were also studied in animal models. Indeed, in case-control study, DNA analysis and sequencing determinations of SNPs in MyD88 gene showed significant differences among subjects in responses to infection susceptibility to *Salmonella Pullorum*, this study concluded that presence of SNPs in MyD88 might be utilized as a marker for disease resistance in chicken animal model (Liu *et al*., 2015).

Chapter Two

Materials and Methods

2. Materials and Methods

2.1. Materials

2.1.1. Laboratory Equipments and Instruments

The laboratory equipments as well as instruments included in the study were demonstrated all in Table (2.1):

Table (2.1): Laboratory equipment and instruments.

No.	Equipment and instruments	Company	Origin
1	Biological safety cabinet	Thermos scientific	Germany
2	Distillatory	Gallenkamp	England
3	ELISA reader	BioTek	USA
4	Gel electrophoresis	Cleaver	UK
5	Glass EDTA tubes 10 ml	Xinle	China
6	Glass gel tubes 10 ml	Xinle	China
7	Laboratory Centrifuge	Hettich	Germany
8	Medical cotton	Kardelen	Turkey
9	Medical injection syringes	MEDECO	UAE
10	Microcentrifuge tubes 1.5 ml	BIOBASIC	Canada
11	Microcentrifuges	Hettich	Germany
12	Micropipettes + tips	Slamed	Germany
13	Refrigerator	Kiriazi	Egypt
14	Thermocycler	Clever, BIO - RAD	UK, USA
15	Tourniquet for blood	Xinle	China
16	UV - trans-illuminator	Herolab	Germany
17	Vortex, Micro spin Centrifuge	My Fu gene	China
18	Water bath	Memmert	Germany
19	Microscope slides	Sail Brand	China

2.1.2. Kits

Diagnostic kits utilized in the study together with their contents and company names and countries of origins were mentioned in details in Table (2.2).

Table (2.2): Diagnostic kits utilized in the study.

No.	Kits and their materials	Company	Country
1	**Wizard® Genomic DNA Purification Kit** Involves reagents suitable for 500 tests of DNA isolated from 300µl volume of blood samples. Contents: • Technical manual x1 • 500 ml of cell lysis solution • 250 ml of nuclei lysis solution • 125 ml of protein precipitation solution • 10 ml of DNA rehydration solution ➢ Tris-HCl 10 mM (pH 7.4) ➢ EDTA 1 mM (pH 8.0) • 1.25 ml of RNase A solution	Promega Corporation	USA
2	**Human Toll Like Receptor 2 (TLR2) ELISA Kit (SL1722Hu)** • User manual x1 • Closure plate membrane x2 • Sealed bags x1 • Microelisa stripplate x1 • 0.5 ml of Standard (5400 pg/ml) • 1.5 ml of Standard diluent • 6 ml of HRP-Conjugate reagent • 6 ml of Sample diluent • 6 ml of Chromogen Solution A • 6 ml of Chromogen Solution B • 6 ml of Stop Solution • 20ml of Wash solution (30X)	SunLong Biotech Co.,LTD	China

3	**Human Toll Like Receptor 6 (TLR6) ELISA Kit (SL2573Hu)** • User manual x1 • Closure plate membrane x2 • Sealed bags x1 • Microelisa stripplate x1 • 0.5 ml of Standard (5400 pg/ml) • 1.5 ml of Standard diluent • 6 ml of HRP-Conjugate reagent • 6 ml of Sample diluent • 6 ml of Chromogen Solution A • 6 ml of Chromogen Solution B • 6 ml of Stop Solution • 20 ml of Wash solution (30X)	SunLong Biotech Co.,LTD	China
4	**Human Toll Like Receptor 7 (TLR7) ELISA Kit (SL2383Hu)** • User manual x1 • Closure plate membrane x2 • Sealed bags x1 • Microelisa stripplate x1 • 0.5 ml of Standard (13.5 ng/ml) • 1.5 ml of Standard diluent • 6 ml of HRP-Conjugate reagent • 6 ml of Sample diluent • 6 ml of Chromogen Solution A • 6 ml of Chromogen Solution B • 6 ml of Stop Solution • 20 ml of Wash solution (30X)	SunLong Biotech Co.,LTD	China
5	**Human Myeloid Differentiation Factor 88 (MyD88) ELISA Kit (SL3143Hu)** • User manual x1 • Closure plate membrane x2 • Sealed bags x1	SunLong Biotech Co.,LTD	China

	• Microelisa stripplate x1 • 0.5 ml of Standard (2700 pg/ml) • 1.5 ml of Standard diluent • 6 ml of HRP-Conjugate reagent • 6 ml of Sample diluent • 6 ml of Chromogen Solution A • 6 ml of Chromogen Solution B • 6 ml of Stop Solution • 20 ml of Wash solution (30X)		

2.1.3. Chemicals

Chemicals utilized in this study were listed in Table (2.3):

Table (2.3): Chemical substance utilized in the study.

No	Chemical substance	Company	Origin
1	Agarose	Bio Basic	China
2	Conventional PCR, PCR pre-Mix reaction (master mix) consisting of: • Each: dNTPs (dATP, dGTP, dCTP, dTTP) • Top DNA polymerase (Taq) • Kcl • Tris-Hcl (pH 9.0) • Tracking dye (pH 8.5) and Stabilizer • Mgcl2		
3	DNA sequencing for detection of TLRs SNPs and gene mutations	Macogen	South Korea
4	DNA ladder 100-4000bp (Promega) Bioneer Korea, consisting of:	Bioneer	South Korea

	• Loading dye has the composition: 0.03% bromophenol blue, 15% ficoll, 0.4% orange G, 0.03% xylene cyanol, 50mm EDTA and 10 Mm of Tris-HCL (pH7.5).		
5	Ethidium bromide solution (5μg/ml)	Sigma	UK
6	Gel-casting platform and Gel comb	Cleaver Scientific	UK
7	Loading dye	Promega	USA
8	Tris EDTA buffer (TE)	Bio basic	Canada
9	Tris-Borate-EDTA Buffer (TBE)	Bio basic	Canada

2.2. Methods

2.2.1. Study Design

2.2.1.1. Study Population

This study was performed in collaboration with the gynecology clinics in three public hospitals: AL-Imam AL-Sadiq Hospital, Al-Hilla Teaching Hospital and Babil Teaching Hospital for Maternity and Children, in addition to, several private gynecology clinics in Babil province, between February 1, 2022 to December 1, 2022. The number of women patients included in the current study was 186 female participants aged between 16 to 5 years old, these female participants were suffering from clinical symptoms and diagnosed clinically with trichomoniasis by gynecologists. Additionally, another group of 40 healthy female participants were included in the study as control making the total number of included individuals is 226 women, the study flow diagram was illustrated in Figure (2.1). The laboratory work was performed in the lab of the Department of Microbiology at the University of Babylon/ College of Medicine.

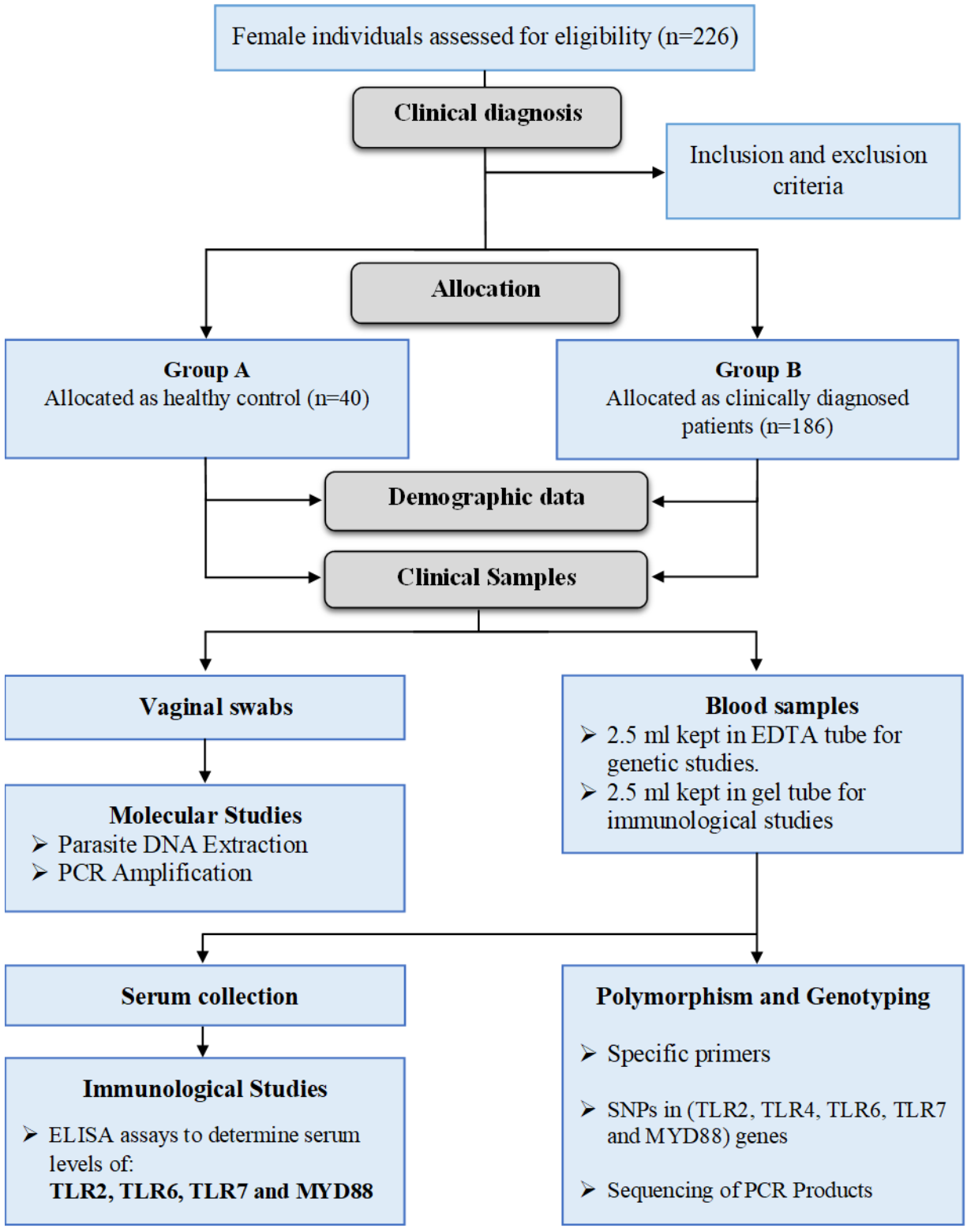

Figure (2.1): Schematic diagram of the study.

2.2.1.2. Demographic Data

The demographic data of trichomoniasis infected women and controls include age, sex, residence, history of abortion and clinical symptoms and severity levels using direct communication questionary.

2.2.1.3. Inclusion and Exclusion Criteria

The potential inclusion criteria were all trichomonas infected women previously diagnosed by the gynecologists. While exclusion criteria involve patients who had other diseases such as inflammatory diseases, autoimmune diseases and other parasitic infections.

2.2.1.4. Ethical Approval

Verbal consent was obtained from all patients before collection of samples. The written ethical approval was first approved by the committee publication ethics at the College of Medicine, Babylon University, as well as the Babylon Health Directorate in Babylon province.

2.2.1.5. Sample Collection

Women blood samples were collected using 5 ml disposable syringes. About five ml blood sample was taken through venipuncture and then slowly kept into two tubes (2.5 ml of the collected blood sample were kept in EDTA tube for the subsequent genetic study, and the remaining 2.5 ml of blood were kept in gel tube at 2˚C). Gel tubes involving blood were centrifuged for 15 minutes at 3000-10000 rpm, and the collected serum was split into three parts which are frozen at -20˚C for further studies.

2.2.2. Molecular Studies

2.2.2.1. Vaginal Swab

All studied samples of *T. vaginalis* were collected from women patients with vaginitis. Vaginal swabs taken from the posterior vaginal fornix were collected in plastic tubes with normal saline (Valadkhani *et al*., 2010). In one ml of normal saline, the swabs of the vagina were vigorously mixed before being centrifuged at 2000 g for 10 min. The pellet was then resuspended in one ml of sterile distilled water, taken out of the supernatant, and frozen at -20 °C.

2.2.2.2. DNA Extraction

Wizard® DNA purification kit was used to extract 500 µl of thawed swab sample according to manufacturer instruction (Promega, USA). Molecular extraction of genetic materials (chromosomal DNAs) helped in providing templates for the corresponding PCR assays.

2.2.2.3. PCR Amplification

Molecular identification of *T. vaginalis* strains was performed using specific primers of BTUB-9 and BTUB-2 genes (Foreword 5'-CATTGATAACGAAGCTCTTTACGAT-3′ and Foreword 5'-GCATGTTGTGCCGGACATAACCAT-3′, respectively). PCR-based amplification procedure of the extracted DNA was done by thermal cycler in a final mixture volume of 25 µl with minor modifications (Valadkhani *et al*., 2010). Amplification outcomes of PCR assay were examined later by agarose gel electrophoresis and also further illustrated by UV trans-illuminator after being stained with the marker, ethidium bromide.

2.2.2.4. The Principle of Polymerase Chain Reaction (PCR).

According to the presence of TLR2, TLR6, TLR7 and MYD88 genes in isolates, PCR technique was utilized to identify genotype of *T. vaginalis* (Alikhani *et al*., 2021).

2.2.2.5. PCR Preparations and Conditions

Master mix of PCR has been prepared using (Maxime PCR PreMix Kit) which was performed based on the company instructions (Promega, USA). The conditions of PCR thermocycler were performed on conventional PCR thermocycler system and the steps as well as the PCR mixtures were demonstrated in Table (2.4).

Table (2.4): Mixtures and conditions of PCR-based identification of *T. vaginalis* strains.

PCR mixtures		PCR conditions		
Contents	**Volume**	**Type of cycle**	**Condition**	**No. of cycles**
Master Mix	12.5 µl	Initialization	94 °C for 5 min	1
Forward Primer	2.5 µl	Denaturation	94 °C for 1 min	35
Reverse Primer	2.5 µl	Annealing	56 °C for 1 min	
Template DNA	3 µl	Extension	72 °C for 1 min	
Nuclase-Free Water	4.5 µl	Final Extension	72 °C for 10 min	1

2.2.2.6. Assay Procedures of Conventional PCR

The PCR procedure was done based on Lorenz, 2012 protocol, as following:

1. 12.5 µl of the Promega master mix added to each reaction tube.
2. 2.5 µl of both forwarding and reverse primers added to the master mix tube.
3. 3 µl of genomic DNA was added to the master mix tube.
4. Variable volume of water (free nuclease) added to the master mix tube to complete the final volume to 25 µl.
5. The components in each tube were mixed by spinning the tubes to avoid the attachment of these components to the wall of PCR tube.
6. Place the master mix tube in a thermal cycler.
7. PCR mixture tube was amplified for 35 cycles.
8. After completing the PCR thermocycler system, 5 µl of PCR product were loaded in electrophoresis system to confirm the presence of amplicons bands. The remaining volume of 20 µl was delivered to Macrogen company in South Korea to examine DNA sequences of amplicons.

2.2.2.7. TLR2, TLR6, TLR7 and MYD88 Genes Amplification for PCR Analysis

The PCR amplification procedure of the tested genes of TLRs were assayed in 25 µl (final volume) reaction mixture using 35 cycles. The resultant copies of DNA were examined using 1.5% electrophoresis on agarose gel, and after being stained with an ethidium bromide marker at 100 V for 30 to 60 minutes, they were seen under a UV-trans illuminator.

2.2.3. Genotyping and SNP Selection

2.2.3.1. Agarose Gel Electrophoresis

Agarose gel (1.5%) is produced by 1X TBE and then dissolved at 100 °C for 15 minutes in a water bath before cooling at 50 °C. Later, it is used to evaluate the PCR products of the TLR genes.

The agarose gel solution was then supplemented with three µl of ethidium bromide dye. Comb carefully removed from the tray and ten µl of PCR product and five µl of (100 bp ladder) were added to each comb's well after the tray had been set in the right position and the agarose gel solution had been put into it. Then, comb solidified at room temperature. In electrophoresis chamber, gel tray is fixed and subsequently 1X TBE buffer was added and electric current was then started.

2.2.3.2. Sequencing of PCR Products

Purified PCR products were obtained and forwarded for sequencing. The Gel/PCR Extraction kit of DNA Fragments (Geneaid, USA) were used to concentrate and extract the DNA fragments from the PCR product. Purified DNA as well as sequencing primers for each gene, which are listed in Table (2.5) were sent to Macrogen Company (South Korea) for sequencing.

3730xl DNA Analyzer (Applied Biosystems, USA) was used to execute the Sanger sequencing procedure. The gold standard for SNPs identification in the TLR gene is DNA sequencing (Teräsjärvi *et al*., 2017).

Table (2.5): Primers upstream and downstream for patients and controls for DNA templates of TLR2, TLR4, TLR6, TLR7 and MYD88 genes.

Gene	SNP ID	Direction	Primer Sequence	PCR product (pb)	Ref.
TLR2	rs5743708	Forward	GCAAGCTGC**A**GAAGATAAT	157 bp	New design
		Reverse	ATTATCTTC**T**GCAGCTTGC		
TLR4	rs4986790	Forward	ACCTCGATG**G**TATTATTGA	574 bp	New design
		Reverse	TCAATAATA**C**CATCGAGGT		
TLR6	rs5743810	Forward	GTAAGGTTG**G**ACCTCTGGT	245bp	New design
		Reverse	ACCAGAGGT**C**CAACCTTAC		
TLR7	rs179008	Forward	TGAAGAGAC**T**AATTCTTAT	232bp	New design
		Reverse	ATAAGAATT**A**GTCTCTTCA		
MYD88	rs4988453	Forward	CACTTTTAC**A**AGTTTTACA	550bp	New design
		Reverse	TGTAAAACT**T**GTAAAAGTG		

2.2.4. Immunological Examination

2.2.4.1. ELISA Assays for Determining TLR2, TLR6 and TLR7 Serum Levels

A. Assay Principle

The assay principle for determining the serum levels of TLR2, TLR6 and TLR7 is the same. The kit of ELISA used the ELISA method called sandwich. In the kit, Microelisa stripplate was pre-coated with antibodies specific for TLRs. Standards and samples solutions added to the appropriate ELISA Microwell Strip Plate well, which were then combined with the indicated matching antibody. After that, a TLR-specific HRP-conjugated antibody was added to each ELISA Microwell Strip Plate well, and this antibody was let to be incubated. After cleaning, the wells were filled with TMB substrate solution. Wells containing TLR and HRP-conjugated TLR antibodies displayed different color (Blue) at the beginning which then altered to yellow following addition of stop solution. Optical density (OD) of this corresponding alteration has been detected by spectrophotometry at 450 nm wavelength. The association of OD value with TLR concentration revealed linear relationship, as shown in Figures (2.2), (2.3) and (2.4) for the corresponding ELISA standard curves of TLR2, TLR6 and TLR7, respectively.

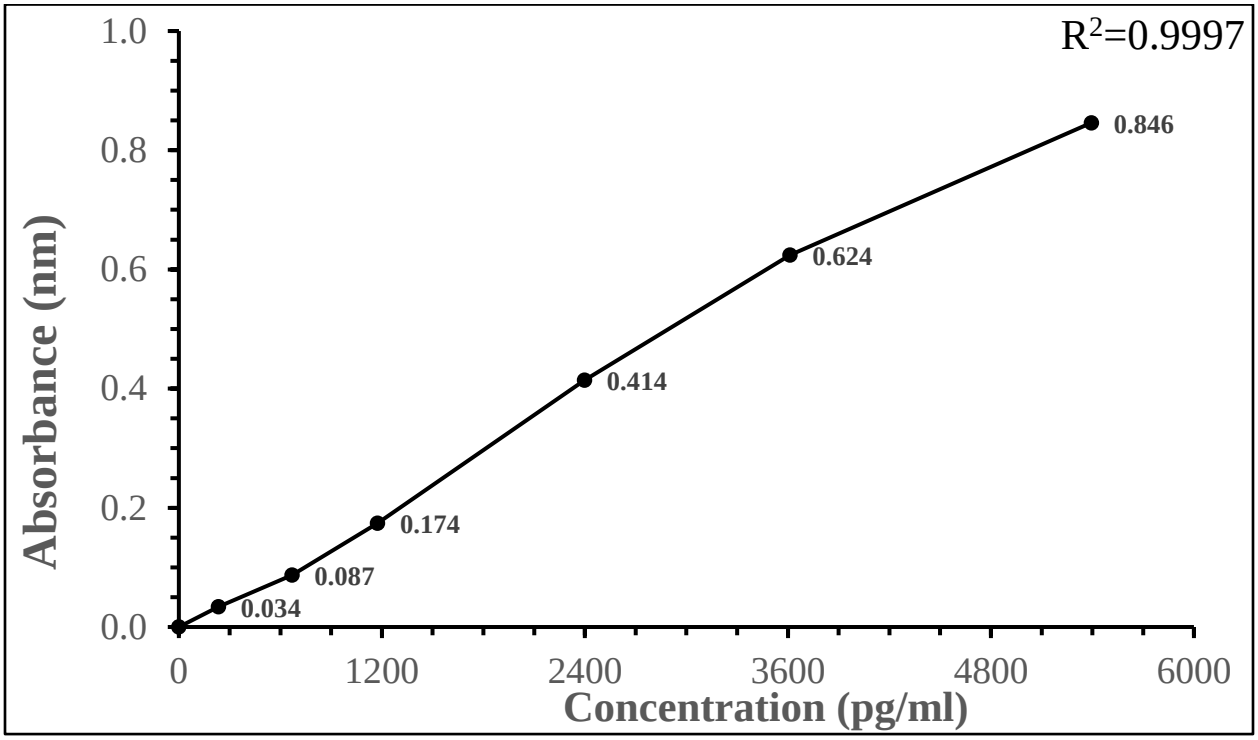

Figure (2.2): ELISA standard curve of TLR2.

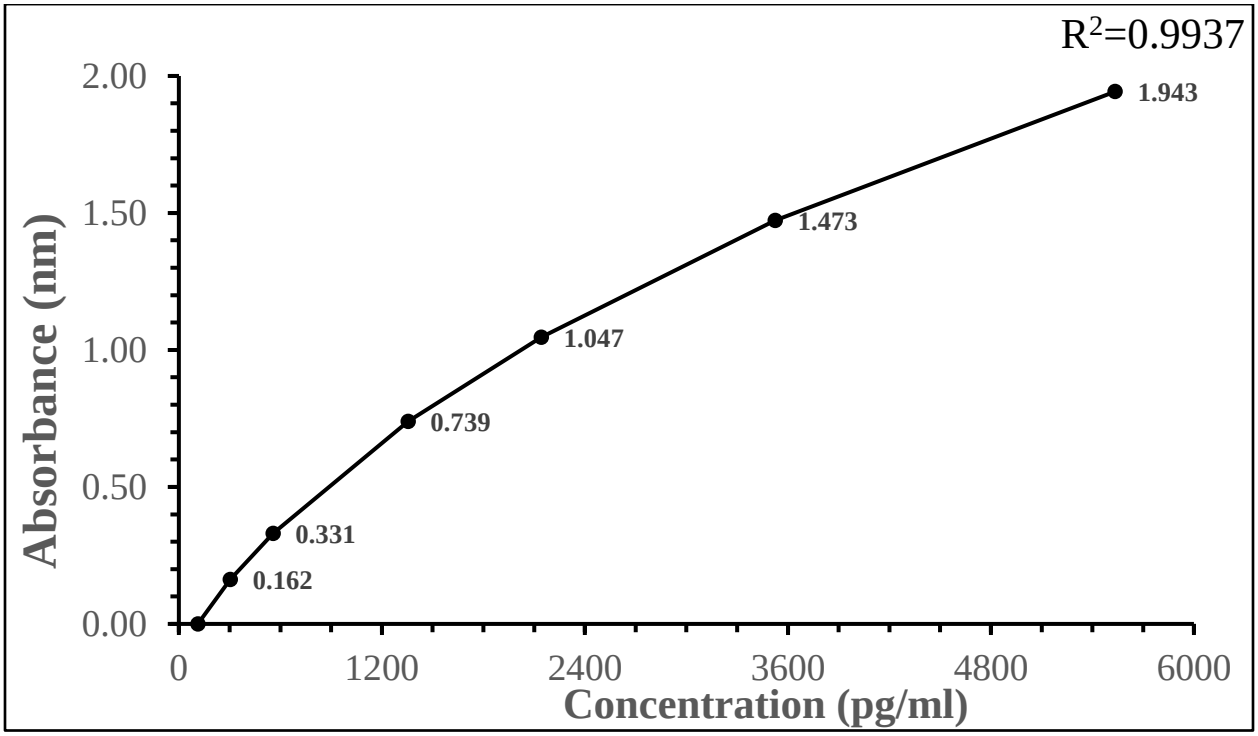

Figure (2.3): ELISA standard curve of TLR6.

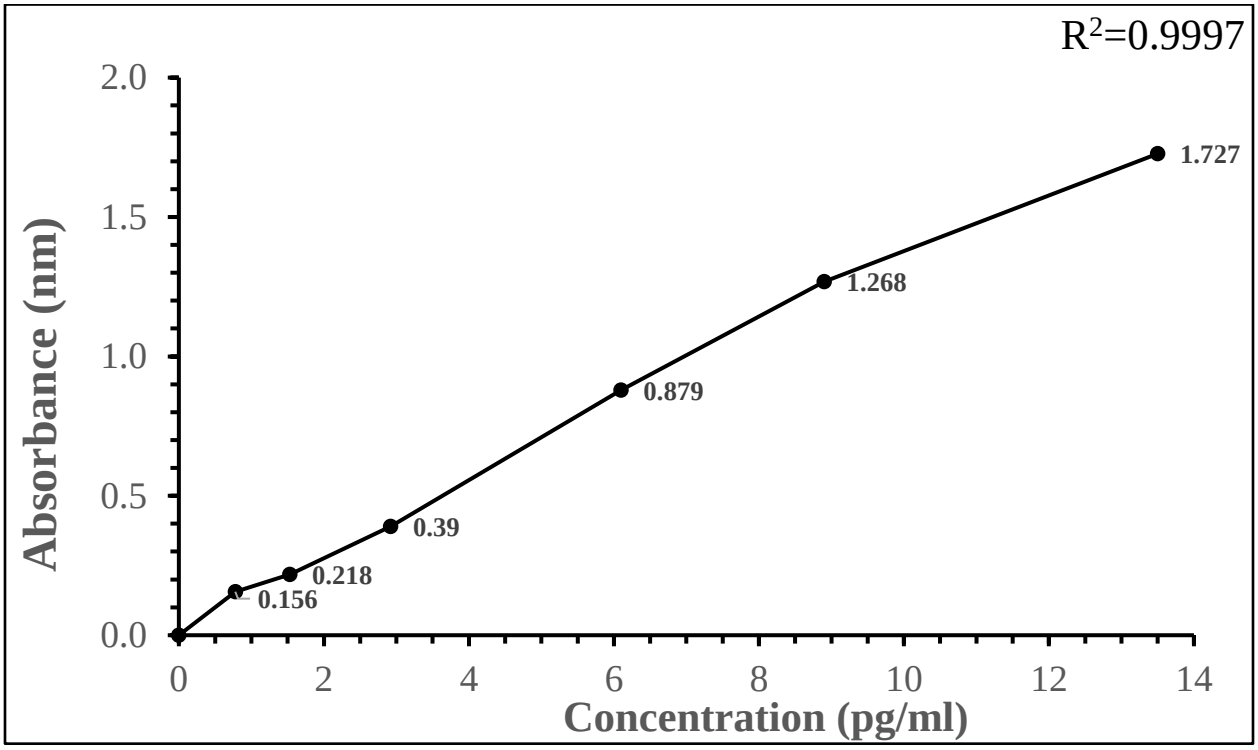

Figure (2.4): ELISA standard curve of TLR7.

B. Serum Preparation

Blood was allowed to clot by being left at room temperature for 10 to 20 minutes following whole blood collection. The blood clot eliminated later by centrifugation for 20 minutes at 3000 rpm.

C. Assay Procedure for Determining TLR2 and TLR7 Serum Levels

1. Standards dilution step:

First, the standards were diluted in micro tubes, two wells were used per tube (total ten wells), and a volume of 50 ul was transferred from each tube to a well.

2. In the Microelisa stripplate, blank control used in an empty well. The wells, involving the sample, received 10 ul and 40 µl of sample in addition to sample

dilution buffer (dilution factor: 5), respectively. Without touching the bottom of the well, samples were placed into it. shaken lightly while completely blending.

3. Incubation step: Closure plate membrane sealed the wells, and then incubated for exactly half an hour at a temperature of 37 °C.

4. Dilution step: Concentration of washing buffer has been reduced 30 and 20 times for 96T and 48T, respectively, by dilution with distilled water.

5. Washing step: following the aspiration and gently eliminating the closure plate membrane, wash solution was used to refill the plates. The wash solution was then disposed out after 0.5 min of relaxation, and the washing procedure was repeated continuously for five times.

6. Later, all wells except control wells (Blank) received 50 µl of HRP.

7. Process of incubation was reperformed as previously described in step 3.

8. Washing steps were carried out as previously demonstrated in step 5.

9. Coloring step: Chromogen Solutions (Designated, A and B) were gently combined and then incubated at 37°C for 15 minutes, light in each well has been avoided.

10. Termination step: Reaction has been stopped using 50 µl of stop solution. Consequently, the well's inside color changed clearly from yellow to blue.

11. Last, microtiter plate reader detected the absorbance at wave length of 450 nm. Importantly, the value of OD for the controls are considered zero. At the end, the assay finished when the stop solution was introduced.

D. Assay Procedure for Determining TLR6 Serum Level

1. Standards dilution steps.

Wells number one and two received 100 and 50 µl of standard solution and standard dilution buffer, respectively, which were then well combined by mixing. Each of wells three and four received 100 µl of the previous solutions present in wells one and two, respectively. After that, buffer of standard dilution was then carefully mixed, followed by 50 µl. From Wells 3 and 4, 50 µl of the solution were discharged. Wells number five and six received 50 µl of the solutions present in wells three and four, respectively. The buffer of standard dilution was later carefully mixed, followed by 50 µl. Again, 50 µl of resultant solutions in wells number five and six were put in wells number seven and eight, respectively. Buffer of standard dilution was then carefully combined, followed by 50 µl. Furthermore, wells number seven and eight had given 50 µl of solutions in wells number five and six, respectively. Similarly, buffer of standard dilution was then carefully mixed, followed by addition of 50 µl. The same procedure was done for wells number nine and 10 corresponding their related wells, seven and eight, respectively. From Wells number nine and ten 50 µl of the solution were discharged. Final, a total of 50 µl volume was present in each well after dilution step, and their corresponding concentrations were 3.600, 2.400, 1.200, 600, and 300 pg/ml.

2. A blank well has been left in the ELISA Microwell Strip Plate as control. In wells of samples, 40 µl of dilution buffer of sample (dilution factor: 5) and 10 µl of sample were put and mixed. Samples were then put into the bottom of the well followed by light shaking.

3. The incubation of wells was done at 37°C for half an hour covered with a membrane of closure plate.

4. For 96T and 48T, dilution step was done using distilled water as the diluent to reduce the concentrated washing buffer 20 and 30 times, respectively.

5. Later, washing was done carefully after first removing the membrane from the closure plate, and then aspiration followed by replacing the wash solution. The wash solution was disposed out after 30 seconds and repeated five times.

6. A total of 50 µl HRP-conjugate reagent was added to sample wells (except blank).

7. Process of incubation was reperformed as previously described in step 3.

8. Washing steps were carried out as previously demonstrated in step 5.

9. During coloring step, a total of 50 µl of both solutions of chromogen (A and B) were delivered to wells, after being mixed, they have been kept for at 37 °C for 15 minutes with moderate shaking, and limited light exposure.

10. To terminate the assay: A volume of 50 µl of stop solution has been delivered into wells to halt the reaction that subsequently transformed wells color from blue (initially) to yellow.

11. Last, absorbance of these alterations evaluated at wave length of 450 nm. The record of OD for the control well was considered zero. Finally, 15 minutes after the stop solution was introduced, the assay was finished.

2.2.4.2. ELISA Assays for Determining MyD88 Serum Level

A. Assay Principle

The assay principle for determining the serum levels of MyD88 is similar to that mentioned for TLRs. The kit of ELISA used the ELISA method called sandwich. In the kit, Microelisa stripplate was pre-coated with antibodies specific for MyD88.

Standards and samples solutions added to the appropriate ELISA Microwell Strip Plate well, which were then combined with the indicated matching antibody. After that, a MyD88-specific HRP-conjugated antibody was added to each ELISA Microwell Strip Plate well, and this antibody was let to be incubated.

Wells containing MyD88 and HRP-conjugated MyD88 antibodies displayed different color (Blue) at the beginning which then altered to yellow following addition of stop solution. Optical density (OD) of this corresponding alteration has been detected by spectrophotometry at 450 nm wavelength. The correlation of OD value with MyD88 concentration revealed linear relationship, as illustrated in Figure (2.5).

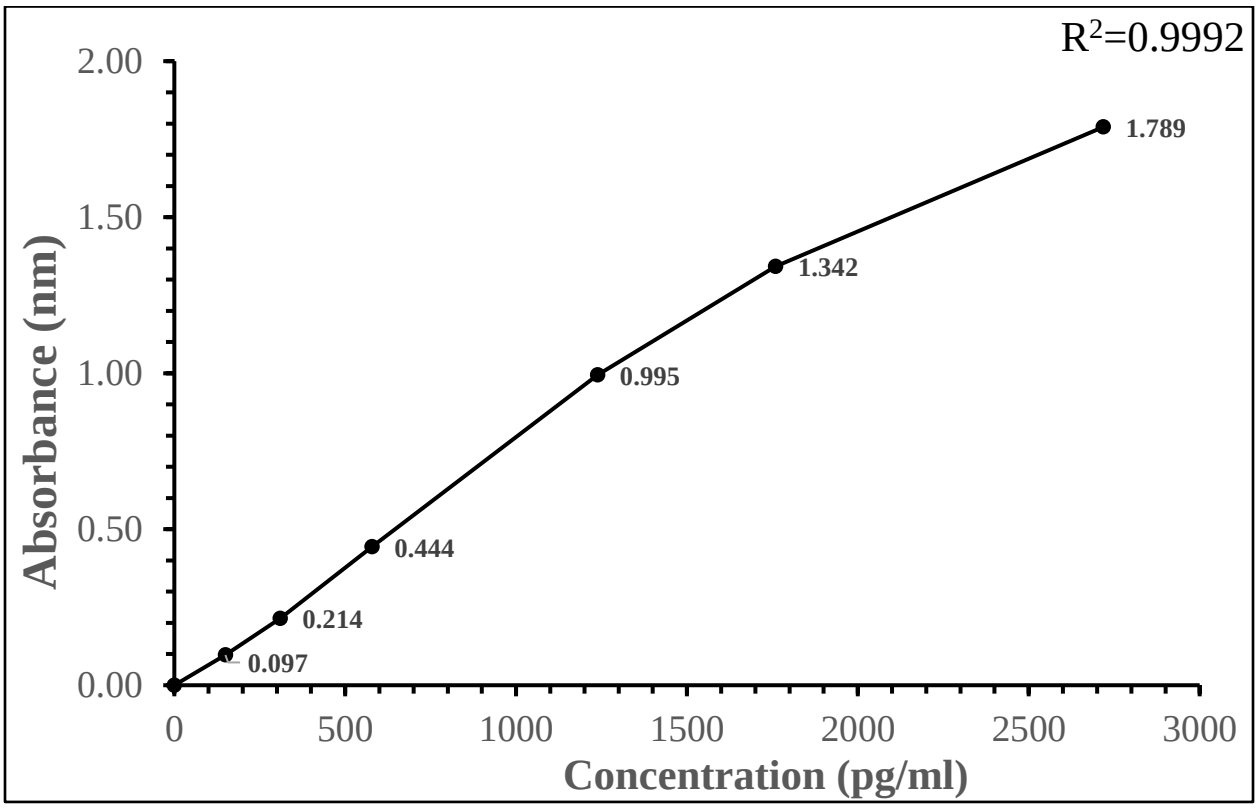

Figure (2.5): ELISA stander curve of MYD88.

B. Serum preparation

Blood was let to be clotted by being left at room temperature for 10 to 20 minutes following whole blood collection. The blood clot eliminated later by centrifugation for 20 minutes at 3000 rpm.

C. Assay Procedure for Determining MYD88 Serum Level

1. Standards' dilution steps

The standard solution has been diluted utilizing micro tubes, 50 ul from solutions were pipetted inside a microplate well (Two wells are used by each tube for a total of 10 wells) in a microplate.

2. A blank well has been left in the ELISA Microwell Strip Plate as control. In wells of samples, 40 µl of dilution buffer of sample (dilution factor: 5) and 10 µl of sample were put and mixed. Samples were then put into the bottom of the well followed by light shaking.

3. The incubation of wells was done at 37°C for exactly half an hour covered with a membrane of closure plate.

4. For 96T and 48T, dilution step was done using distilled water as the diluent to reduce the concentrated washing buffer 20 and 30 times, respectively.

5. Washing step: following the aspiration and gently eliminating the closure plate membrane, wash solution was used to refill the plates. The wash solution was then disposed out after 0.5 min of relaxation, and the washing procedure was conducted out five times.

6. A total of 50 µl HRP-conjugate reagent was delivered to sample wells (excluding blank).

7. Process of incubation was reperformed as previously described in step 3.

8. Washing steps were carried out as previously demonstrated in step 5.

9. During coloring step, a total of 50 µl of both solutions of chromogen (A and B) were delivered to wells, after being mixed, they have been kept at 37 °C for 15 minutes with moderate shaking, and limited light exposure.

10. To terminate the assay: A volume of 50 µl of stop solution has been delivered into wells to halt the reaction that subsequently transformed wells into yellowish color.

11. Last, absorbance of these alterations evaluated at wave length of 450 nm. The record of OD for the control well was considered zero. Finally, 15 minutes after the stop solution was introduced, the assay was finished.

2.2.5. Bioinformatics and Statistical Analysis

The sequencing data were cropped and compared in regard to sequences of controls. These standard sequences of DNA were obtained from GenBank for TLRs genes at National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov). Multiple Sequence alignments were performed utilizing Clustal W version 2.0 (Thompson, *et al*. 1994; Katoh and Standley, 2016) and the software Geneious Prime V2021.1 (Biomatters, Inc., North America) to examine SNPs, corresponding genotypes and related allele frequencies. Other bioinformatic analyses were performed based on Xavier (Solé, *et al*. 2006).

Statistical analysis of serological tests of TLRs were done using Package of Social Sciences (SPSS) version 23 (Inc., Chicago, IL, USA) computer software, and

Microsoft Excel (2019, Microsoft). Mean ± stander error (SD) and correlation were used to express data and measure significant values. A probability value less than 0.05 ($p < 0.05$) level of statistical analysis was considered significant. The probability also examined by using student T-test and ANOVA test. For genotyping and alleles frequencies, the odd ratio was 95% confidence interval, and calculations were done using Online Hardy-Weinberg calculator. Descriptive statistics are performed using Chi-Square test to study the association between the outcome and different factors. MedCalc version 20 software was used for the statistical analysis. $P < 0.05$ is considered statistically significant.

Chapter Three

Results and Discussion

3. Results and Discussion

3.1. Molecular identification of *T. vaginalis*

The characteristic features for detection of positive cases of *T. vaginalis* depend on either microscopic examination (wet-mount) or PCR assay of β-tubulin gene. As shown in Table (3.1), initial assays to set the PCR analysis were done on samples taken from 186 females. Of these females, 40 women showed positive PCR outcomes of *T. vaginalis* β-tubulin gene.

Table (3.1): Direct and molecular identification of *T. vaginalis* among all studied samples using BTUB9/2 genes-specific primers.

Results	Direct detection		Molecular detection		95% Confidence Interval
	No.	Percentage	No.	Percentage	
Positive	7	3.76%	40	21.5%	15.85% to 28.11%
Negative	179	96.24%	146	78.5%	71.89% to 84.17%
Total	186	100%	186	100%	-

The samples of vaginal swaps were identified by molecular technique (PCR) using BTUB 9/2 primer. The 40 positive samples were identified by agarose gel electrophoresis, samples of agarose gel electrophoresis examinations were shown in Figure (4.1). Sets of tests involved both positive as well as negative control in addition to DNA marker. The 112 bp product was amplified in all positive samples.

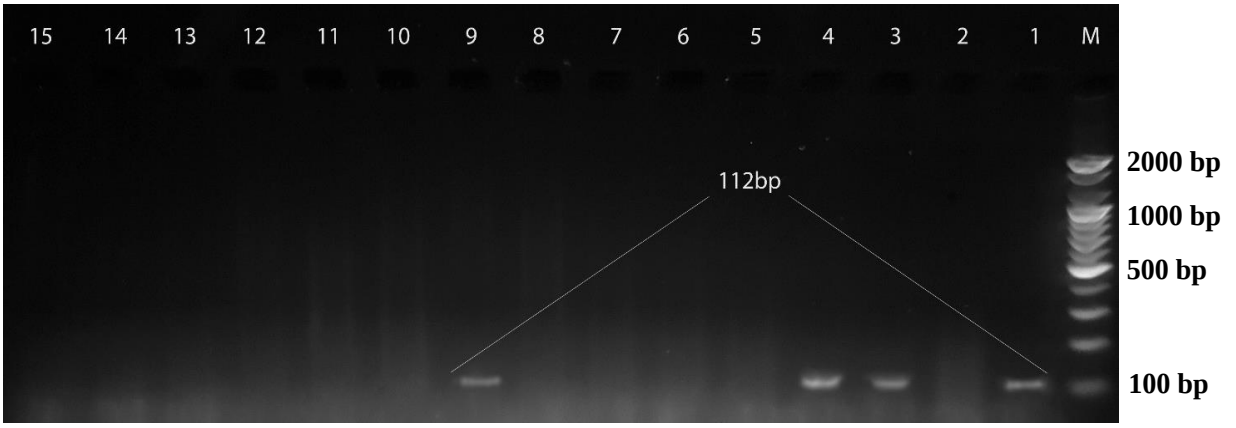

Figure (3.1): Uniplex-PCR products analysis by agarose gel electrophoresis of using *T. vaginalis*-specific primers.
Lane M represents universal DNA ladders.
Lanes 1, 3, 4 and 9 represent positive isolates.

However, of the 40 PCR positive samples, only seven patients demonstrated positive wet-mount microscopy. In this study, PCR analysis helped in offering a fast diagnostic technique to recognize *T. vaginalis*. Similarly, studies showed that PCR analysis reported high and accurate prevalence of 48% positive cases out of 155 total examinations (Li *et al*., 2020). Inversely, only 3.2% of samples taken from women with vaginal discharge reported as positive utilizing wet mount and culture analysis (Yarizadeh *et al*., 2021). The high prevalence of trichomoniasis infection among female individuals recently probably results as a consequence of the elevated sensitivity of detection by PCR in comparison to wet-mount test. PCR assay was a considerably fast diagnostic technique of *T. vaginalis* in addition to that it was highly sensitive and specific. Consequently, this would reduce the transmission of the infection, specially from asymptomatic infected patients. Moreover, additional information has strengthened the significance of *T. vaginalis* infection sequelae in women, including higher risk of cervical cancer, preterm delivery, and other unfavorable pregnancy outcomes (Meites *et al*., 2015; Kissinger *et al*., 2021). The

current study demonstrates that PCR assays represent an advance molecular technique characterized by high sensitivity and specificity because it depends on amplification of specific highly conserved DNA region in the parasite genome including, the β-tubulin gene.

3.2. Characteristic data of *T. vaginalis* infection

3.2.1. Determination of residency-related prevalence of *T. vaginalis*

According to geographical area, the Hilla city center had the greatest number of samples, and it showed the most positive cases, indeed, the percentage of women infected with *T. vaginalis* of the total examinations was higher in the city center (22.3%) when compared with that in rural area (20.3%), see Table (3.2).

Table (3.2): Residence-related prevalence of *T. vaginalis* infection.

Residence	Examinations (%)	Infections (%)	Infections / Examinations	P value
Rural	74 (40%)	15 (37.5%)	15/74 (**20.3%**)	0.740
Urban	112 (60%)	25 (62.5%)	25/112 (**22.3%**)	
Total	186 (100%)	40 (100%)	40/186 (**21.5%**)	

Furthermore, based on the residence-related prevalence of *T. vaginalis* infection, it was found that the higher number of admitted women to the gynecology clinic was from the urban area. Moreover, although statistically non-significant ($p > 0.05$), the percentage of females diagnosed with trichomoniasis using PCR assay out of the total examinations was greater in urban area in comparison to that in rural area. It is well known that urban areas have high residential density than rural areas,

making people in these areas more vulnerable to infectious diseases because of the elevated probability of infection within crowded places. Consequently, high population density may in part increase the chance for more sexual contact as a result of increasing male-male competition for mates and sexual harassment of females, especially within low to middle income societies of low educational status (Dreiss *et al*., 2010 ;Ton Nu *et al*, 2015b). In addition, researchers propose that emphasis be given to the development of standardized molecular detection techniques of *T. vaginalis* and that these become part of regular STD testing since *T. vaginalis* demands a higher profile in urban STD clinic settings (Lusk *et al*., 2010).

Similarly, difference between urban and rural prevalence of trichomoniasis was prominent in a comparable study in Korea, higher prevalence was seen in urban area which had the greatest average population as well as the biggest number of positive cases (Kim *et al*., 2022; Al-Hasnawy and Rabee, 2023; Al-Hamzawi & Al-Awsi, 2023). However, no significant differences were reported between urban and rural areas in some studies (Kadhum *et al*., 2020; Alikhani *et al*., 2022).

3.2.2. Determination of age-related prevalence of *T. vaginalis*

Furthermore, the ages of infected women were between 16 to 50 years, the prevalence of trichomoniasis among females based on their age was listed in Table (3.3). According to results, higher prevalence was seen in women aged between 21-40 years old, and it started o decline after the age of 40 years old. Determination of age-related prevalence of infection by *T. vaginalis* infection exhibited that the majority of women diagnosed with *T. vaginalis* were sexually active women, particularly those aged between 16-50 years old as seen with previous studies (Tine *et al*., 2019; Lindrose *et al*., 2022). Furthermore, women usually become pregnant

during this age period which further elevate the chance for getting *T. vaginalis* infection. Indeed, *T. vaginalis* is very common in pregnant women, and those who are pregnant at an early gestational age are at more risk (Oyeyemi *et al*, 2016; Lazenby *et al.*, 2019). However, data from other studies suggest a high level of medical care for pregnant women including antibiotic administration which may limit or prevent the incidence of trichomoniasis (Li *et al*., 2022).

Table (3.3): Age-related prevalence of *T. vaginalis* infection.

Age (Year)	Examinations (%)	Infected NO.	Percentage % (of 40)	P value
16-20	15 (8.1%)	1	2.5%	0.180
21-25	27 (14.5%)	8	20%	
26-30	49 (26.3%)	11	27.5%	
31-35	43 (23.1%)	8	20%	
36-40	24 (12.9%)	9	22.5%	
41-45	16 (8.6%)	2	5%	
46-50	12 (6.5%)	1	2.5%	
Total	186 (100%)	40	100%	

3.2.3. History of abortion among women infected with *T. vaginalis*

According to the results of current study, out of the 146 negative samples women, 31 had a history of abortion, and 11 out of the 40 infected ones had an abortion history. As demonstrated in Table (3.4), infected women possessed the highest history of abortion than non-infected women.

Table (3.4): History of abortion among non-infected and women infected with *T. vaginalis.*

Results	N	History of abortion (%)	P value
Positive	40	11 (27.5%)	0.40
Negative	146	31 (21.2%)	
Total	186	44 (48.7%)	-

Preconception infection with trichomoniasis elevated slightly the risk of abortion as shown by this study, the history of abortion determination among infected and non-infected women with *T. vaginalis* showed that the infected females revealed slightly higher percentage of previous abortion events in comparison with the negative cases but these results were statistically insignificant. However, it was reported that the impact of trichomoniasis on increasing risk of spontaneous abortion in pregnant women is corelated with co-infection by other reproductive tract infections (Silver *et al.*, 2014; Zeng *et al*., 2022).

3.2.4. Determination of clinical symptoms among women infected with *T. vaginalis*

Regarding the clinical symptoms, vaginal discharge was the most common clinical symptoms among patients, and about 70% of positive patients were complaining of clinical symptoms, see Table (3.5).

Table (3.5): Clinical symptoms in females infected with *T. vaginalis*.

Symptoms	Negative cases	Positive case	p-value
Discharge	112 (76.7%)	38 (95%)	0.001
Itching	65 (44.52%)	25 (62.5%)	
Dysuria	48 (23.8%)	12 (30%)	
Others	27 (18.5%)	0 (0%)	

Based on clinical symptoms in females infected with *T. vaginalis*, clinical diagnosis of trichomoniasis revealed that 38 women of the 40 positive cases were complaining of vaginal discharge, 25 women were suffering from itching, and 12 women were feeling of dysuria in their genital tract, these data were statistically significant at p = 0.001. These clinical symptoms were also reported by other studies as the main clinical symptoms seen in women diagnosed with *T. vaginalis* (Rafiei *et al*., 2021). Some women who were not infected with trichomoniasis demonstrated some symptoms which are not related to *T. vaginalis* infection, these symptoms were included in the table as others.

However, still some females did not complain of clinical symptoms (asymptomatic), despite the fact that these women were infected with the corresponding parasite. Consequently, these findings highlight the important role of PCR assay as an essential and accurate diagnostic tool to *T. vaginalis*.

3.3. Genetic analysis

In the present study, genotyping frequencies of five TLRs SNPs, designated rs5743708 in TLR2, rs4986790 in TLR4, rs5743810 in TLR6, rs179008 in TLR7 and rs4988453 in MyD88, were fitted with the Hardy-Weinberg Equilibrium (HWE) among controls (0.0096, 0.004, 0.93, 0.07 and 0.0014, respectively). The goodness-of-fit X2 test was used to assess the latter model by contrasting the observed genotype frequencies with those anticipated in controls. To test the notion that genotype frequencies in a population stay constant from generation to generation, as seen in Table (3.6).

Table (3.6): Exact test for Hardy-Weinberg equilibrium.

Groups	TLR2 rs5743708	TLR4 rs4986790	TLR6 rs5743810	TLR7 rs179008	MYD88 rs4988453
All	0.0096*	0.004*	0.93	0.07	0.0014*
Control	0.1	0.2	0.33	0.2	0.1
Patient	0.077	0.01*	0.35	0.67	0.01*

* Represent a significant difference at $p < 0.05$.

3.3.1. Comparison of the investigated TLR2 SNP in *T. vaginalis* patients versus controls

The results of genotyping for the 40 infected women with *T. vaginalis* and the 30 women control for the amplification of their PCR products were achieved by sequencing for primers of TLR2. The genotype and allele frequencies of TLR2

(rs5743708) polymorphism were compared between infected patients versus controls and listed in Table (3.7) and further demonstrated in Figure (3.2).

Although it is statistically non-significant, the frequency of the G allele carriers of TLR2 gene polymorphism was higher (37/64) in women diagnosed with trichomoniasis than that in healthy control (27/64), these results may suggest that G allele may not show protection from trichomoniasis for TLR2. Furthermore, the trichomoniasis infected women showed significantly higher frequency of G allele (92%) versus that of mutant A allele (8%) ($p<0.001$). Similarly, the control women demonstrated significantly higher frequency of G allele (90%) versus that of A allele (10%) ($p < 0.001$).

Table (3.7) showed that the homozygous GG genotype has slightly higher frequency (18/31) in trichomoniasis infected women when compared with that in healthy individuals (13/31), whereas the GA and AA genotypes were found to be equal in trichomoniasis women and controls. These findings showed that majority of controls and patients having homozygous GG genotype. The genotype and allele frequencies of the infected women and the control group, however, did not significantly vary from one another depending on the resultant p value ($p= 0.95$). The GG genotype showed significantly higher prevalence (90%) within infected women when compared with GA (5%) and AA (5%) genotypes ($p<0.001$).

According to the corresponding results, individuals with two homologous alleles of A alleles (AA homozygous) were not significantly associated with increased risk of trichomonas infection among women. The analysis of different inheritance models of TLR2 SNP (rs5743708) between controls and patients were listed in Table (3.8). TLRs are considered the first immunological defense against microbes. These receptors link host innate and adaptive immunity, mediating cytokine as well as chemokine responses.

Table (3.7): TLR2 SNP (rs5743708) distribution frequencies.

TLR2 SNP	Allele	Frequency	Controls	Patients	p value	OR (95% CI)
rs5743708	G	64 (0.91)	27 (0.9)	37 (0.92)	0.712	0.730 (0.137-3.898)
	A	6 (0.09)	3 (0.1)	3 (0.08)		
	P value	<0.001*	<0.001*	<0.001*		
	Genotypes					
	G/G	31 (0.89)	13 (0.87)	18 (0.9)	0.95	1.00
	G/A	2 (0.06)	1 (0.07)	1 (0.05)		0.72 (0.04-12.64)
	A/A	2 (0.06)	1 (0.07)	1 (0.05)		0.72 (0.04-12.64)
	P value	<0.001*	<0.001*	<0.001*		

*Represent a significant difference at $p < 0.05$.

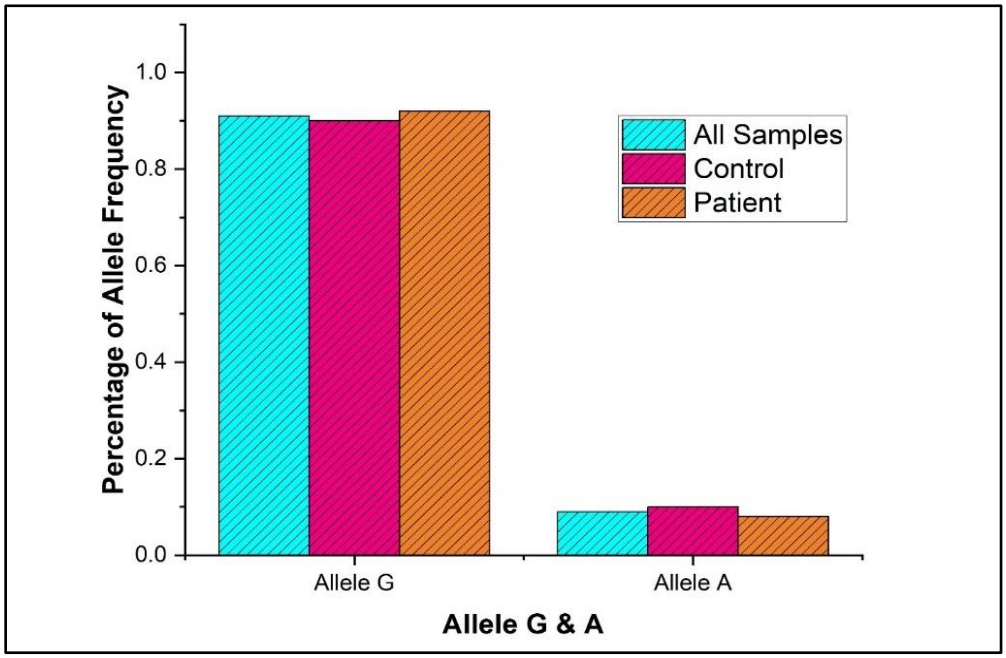

Figure (3.2): Distribution frequency of the TLR2 rs5743708 polymorphism between patients infected with trichomoniasis and controls.

Table (3.8): Analysis of different inheritance models of the TLR2 SNP (rs5743708) between controls and patients.

Model	Genotype	STATUS=0-Control	STATUS=1-Case	OR (95% CI)	P value	AIC	BIC
Codominant	G/G	13 (86.7%)	18 (90%)	1.00	0.95	53.7	58.4
	G/A	1 (6.7%)	1 (5%)	0.72 (0.04-12.64)			
	A/A	1 (6.7%)	1 (5%)	0.72 (0.04-12.64)			
Dominant	G/G	13 (86.7%)	18 (90%)	1.00	0.76	51.7	54.8
	G/A-A/A	2 (13.3%)	2 (10%)	0.72 (0.09-5.81)			
Recessive	G/G-G/A	14 (93.3%)	19 (95%)	1.00	0.83	51.8	54.9
	A/A	1 (6.7%)	1 (5%)	0.74 (0.04-12.82)			
Over-dominant	G/G-A/A	14 (93.3%)	19 (95%)	1.00	0.83	51.8	54.9
	G/A	1 (6.7%)	1 (5%)	0.74 (0.04-12.82)			
Log-additive	---	---	---	0.82 (0.22-3.06)	0.77	51.7	54.8

Akaike information criterion (AIC), Bayesian information criterion (BIC)

TLR2 G/A polymorphism (rs5743708) involves guanine substitution at nucleotide 2258 with adenine in the TLR2 gene, as indicated on the chromatogram in Figure (3.3), which results in substitution of Gln amino acid at position 753 by Arg. This amino acid substitution locates in the c-terminal region of TLR2 at a highly conserved region. It was shown that women characterized by symptomatic trichomoniasis demonstrated high TLR2 expression during early time of infection (Yadav *et al*., 2021). rs5743708 SNP in TLR2 (Arg753Gln) was correlated with increase susceptibility to septic shock (caused by staphylococcal infection), tuberculosis in Turkish population, infective endocarditis (Ogus *et al*., 2004;

Bustamante *et al*., 2011;). TLR2 is either associated with TLR1 or TLR6 recognizing ligands from fungi, protozoa, gram-positive bacteria, mycobacteria and viruses.

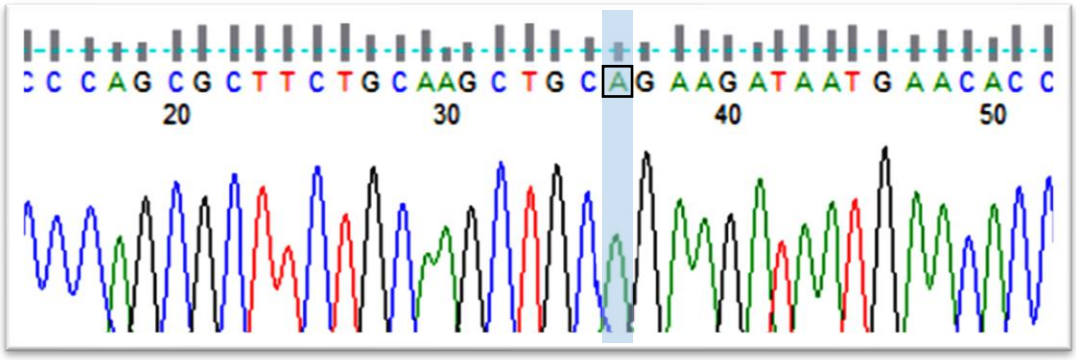

Figure (3.3): DNA sequencing chromatogram of rs5743708 SNP in TLR2 gene.

3.3.2. Comparison of the investigated TLR4 SNP in *T. vaginalis* patients versus controls

The outcomes of genotyping of amplicon of PCR products for the 40 women infected with *T. vaginalis* as well as the 30 healthy controls were sequenced using primers of TLR4, the genotype and allele frequencies of TLR4 (rs4986790) polymorphism were compared between *T. vaginalis* infected patients versus controls were shown in Table (3.9) as well as in Figure (3.4). Although it is statistically non-significant (p = 0.56), the frequency of A allele carriers was higher [33/59 (55.9%)] in women infected with trichomoniasis in comparison with that in healthy controls [26/59 (44.1%)]. In addition, the frequency of G allele showed even greater distribution [7/11 (63.6%)] in infected women in comparison with that in healthy controls [4/11 (36.3%)]. Subsequently, the presence of A allele may in part enhance the defensive mechanism against trichomoniasis for TLR4 SNP (rs4986790). Also, because the distribution of G allele in infected women was greater than that recorded

by A allele, G allele carriers of this SNP might have more sensitivity for trichomoniasis.

Additionally, Table (3.9) showed that the prevalence of the three genotypes, AA, AG, and GG, among infected women was 75%, 15% and 10% respectively, $p<0.001$, while the prevalence of the same genotypes was 80%, 13% and 7% respectively, in control group $p<0.001$. Genotype comparison between patients and controls revealed clearly that the homozygous AA genotype frequency was only slightly higher [15/27 (55.5%)] in infected women when compared with healthy individuals [12/27 (44.4%)] which may indicate the protective role of AA genotype against trichomoniasis. Similarly, the heterozygous AG genotype frequency was found to be higher in infected women [3/5 (60%)] versus controls [(2/5 (40%)]. Importantly, the homozygous GG genotype in infected women showed not just higher but even two times higher frequency [2/3 (66.6%)] than that recorded in healthy controls [1/2 (33.3%)]. These results although statistically non-significant, they may in part show that the women with GG genotype, as well as the heterozygous AG genotype, might be more sensitive for infection with *T. vaginalis* than the homozygous AA genotype.

Table (4.10) demonstrates the frequencies of alleles of the corresponding SNP (rs4986790) in the TLR4 gene between infected patients versus controls in the current study and previous studies. It is clearly seen that the minor allele frequency of G allele among infected individuals was higher than that reported by previous studies performed locally in Iraq, as well as other countries (Yarizadeh *et al*., 2021). In addition, analysis of different inheritance models of TLR4 SNP (rs4986790) between controls and patients were shown in Table (3.11).

Table (3.9): TLR4 SNP (rs4986790) distribution frequencies.

TLR4 SNP	Allele type	Frequency	Control population	Patients population	p value	OR (95% CI)
rs4986790	**A**	59 (0.84)	26 (0.87)	33 (0.82)	p = 0. 56	1.379 (0.364-5.22)
	G	11 (0.16)	4 (0.13)	7 (0.18)	p = 0. 56	
	p value	< 0.001*	< 0.001*	< 0.001*	-	-
	Genotypes					
	A/A	27 (0.77)	12 (0.80)	15 (0.75)	p = 0.72	1.00 1.20 (0.17-8.38) 1.60 (0.13-19.84)
	A/G	5 (0.14)	2 (0.13)	3 (0.15)	p = 0.86	
	G/G	3 (0.09)	1 (0.07)	2 (0.10)	p = 0.75	
	p value	<0.001*	0.001*	<0.001*	-	-

*Represent a significant difference at $p < 0.05$.

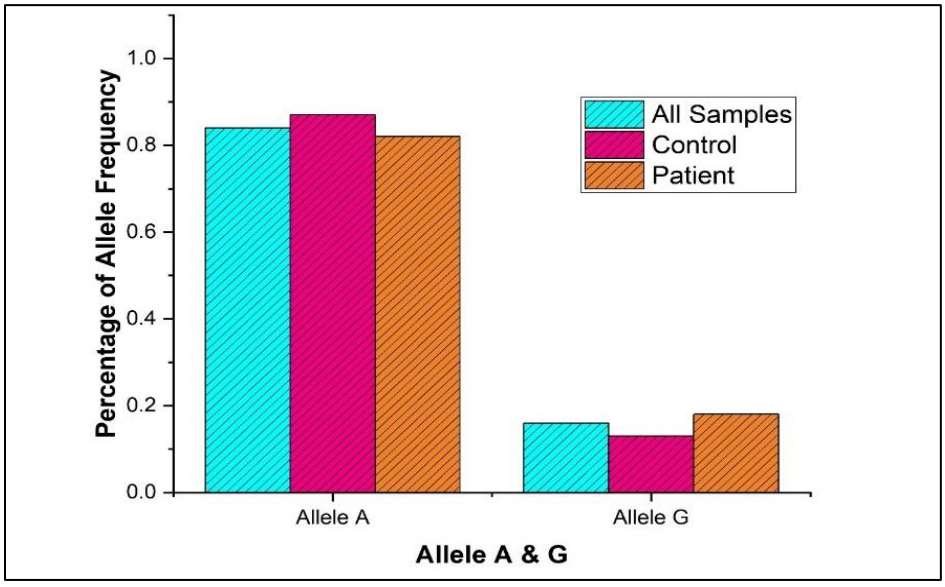

Figure (3.4): Distribution frequency of the TLR4 rs4986790 polymorphism between patients infected with trichomoniasis and controls.

Table (3.10): Allele frequencies of the TLR4 single nucleotide polymorphism rs4986790 (A>G) between patients infected with trichomoniasis and controls.

Population	Country	Year of Publication	Patients	Controls	Reference
			(A/G) Allele Frequencies	(A/G) Allele Frequencies	
Caucasians	USA	2009	93.03 / 6.97	95.85/ 4.15	1000 Genomes Project (Kazemi *et al*., 2004)
Asians	India	2019	86.56/ 13.44	92.96/ 7.04	(Yarizadeh *et al*., 2021)
Middle East	Iraq	2019	96.00/ 4.00	98.00/ 2.00	(Kim *et al*., 2022)
Middle East	Iraq	2023	82.00/18.00	87.00/ 13.00	Current study

Table (3.11): Analysis of different inheritance models of TLR4 SNP (rs4986790) between controls and patients.

Model	Genotype	STATUS=0-Control	STATUS=1-Case	OR (95% CI)	P-value	AIC	BIC
Codominant	A/A	12 (80%)	17 (85%)	1.00	0.66	53	57.6
	A/G	2 (13.3%)	1 (5%)	0.35 (0.03-4.35)			
	G/G	1 (6.7%)	2 (10%)	1.41 (0.11-17.40)			
Dominant	A/A	12 (80%)	17 (85%)	1.00	0.7	51.7	54.8
	A/G-G/G	3 (20%)	3 (15%)	0.71 (0.12-4.11)			
Recessive	A/A-A/G	14 (93.3%)	18 (90%)	1.00	0.72	51.7	54.8
	G/G	1 (6.7%)	2 (10%)	1.56 (0.13-18.95)			
Over-dominant	A/A-G/G	13 (86.7%)	19 (95%)	1.00	0.38	51	54.2
	A/G	2 (13.3%)	1 (5%)	0.34 (0.03-4.18)			
Log-additive	---	---	---	0.96 (0.32-2.89)	0.94	51.8	54.9

Akaike information criterion (AIC), Bayesian information criterion (BIC)

In parallel, this SNP was also reported in previous studies and it revealed an association with sensitivity to trichomoniasis infection among individuals. rs4986790 SNP was found in two genotypes, AA and AG, in both patients and controls. The reported AG genotype frequency was significantly greater in infected patients (20%) than in healthy controls (6%) (OR=3.92, 95%CI=1.01-15.22, p=0.037). In addition, the same study found that the frequency of the mutant G allele was also significantly greater (10%) in infected patients when compared with the controls (3%) (Kim *et al*., 2022). rs4986790 was further genetical studied in patients with leishmaniasis, Rasouli and his research team investigated patients diagnosed with kala-azar (visceral leishmaniasis) and healthy controls to determine the association of rs4986790 SNP with the leishmaniasis among Iranians population. However, no statistically significant association was reported of rs4986790 SNP with the corresponding infection. Furthermore, the genetic haplotypes obtained from this SNP did not differ significantly between infected patients and controls (Tine *et al*., 2019). It is still unclear how the rs4986790 SNP may modify the structure and/or activity of TLR4. Importantly, rs4986790 SNP, designated as 896A/G as shown in Figure (3.5), is a non-synonymous mutation changes the conserved Asp acid residue to a Gln at position 299 at the extracellular protein structure. It is located at exon number three of the TLR4 gene (Ohto *et al*., 2012).

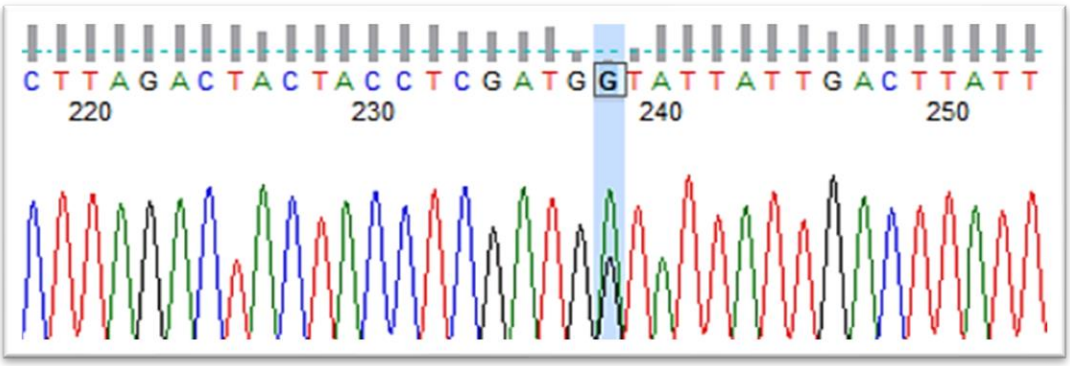

Figure (3.5): DNA sequencing chromatogram of rs4986790 SNP in TLR4 gene.

However, G allele (mutant) may alter TLR4 gene expression, signaling, or ligand binding. Of these three effects, expression of TLR4 gene was reported by several researchers not to be affected by this SNPs (Zeng *et al*., 2022; Rafiei *et al*., 2021). In epithelial cell line (HeLa cells) in human, *T. vaginalis* infection elevates TLR4 expression, inducing the production of two cytokines, including interleukin-8 (IL-8) and tumor necrosis factor-α (TNF-α). These two cytokines have the ability to trigger human immune response through attracting two white blood cells, macrophages and neutrophils, at the infected sites, and enhance the T-cell activity (Özel *et al*., 2022). Interestingly, ligands of TLR4 were demonstrated to control/protect cellular damage resulted from protozoan infection in the HeLa cell line (Chang *et al*., 2006). Recently, it was shown that CpG oligodeoxynucleotides (ODNs) enhanced survival of *T. vaginalis* parasite in HeLa cell line by downregulating the caspase-3 pathway avoiding by that the apoptosis mechanism. Additionally, a previous study performed in 2013 reported no statistically significance association between risk of prostate cancer and *T. vaginalis* infection in the presence of TLR4 SNP (rs4986790) (Kim *et al*., 2005).

Another local study was performed to determine the association between TLR4 SNPs (rs4986790) with susceptibility to *T. vaginalis* infection in Iraqi women. This study concluded that no statistically significant association was imposed on susceptibility to trichomoniasis by the TLR4 SNPs (rs4986790) (Chang , 2005; Banerjee *et al*., 2006). TLR4 SNP (rs4986790) was also studied in Indian and revealed that this corresponding SNP has increased the viral load in patients infected with HIV (Kim and Jeong, 2020). The mechanism behind this elevation of viral load was correlated to the role of TLR4 on alteration of innate immune response in the mucosal surfaces, as well as changing the ability of host to counteract the invasive pathogens rather than interaction of TLR4 with the pathogen. Based on these

findings, Ding and his research team concluded that TLR4 rs4986790 SNP affects the severity of cervicitis infection among patients (Ding *et al*., 2017). To conclude, although the studied TLR4 SNP (rs4986790) demonstrated higher frequency in women patients infected with *T. vaginalis* in comparison with healthy controls, there was no statistically significant association between TLR4 SNP and susceptibility to *T. vaginalis* infection in studied women population.

3.3.3. Comparison of the investigated TLR6 SNP in *T. vaginalis* patients versus controls

As shown in Table (3.12) and demonstrated in Figure (3.6), the A allele of the rs5743810 was less common in patient with *T. vaginalis* (23/48) than healthy individuals (25/48) (P=0.021), add ratio 3.696 and confidence interval (1.174-11.633) respectively. Interestingly, the mutant G allele was more common (17/24) in infected women than healthy controls (5/24). These results significantly highlight that women with G allele are at greater risk to get trichomoniasis compared with those bearing the A allele. In addition, the homozygous AA genotype showed lower frequency (5/16) in trichomoniasis infected women when compared with that in healthy individuals (11/16), whereas the heterozygous AG and homozygous GG demonstrated significantly higher frequencies (13/16 and 2/3) in trichomoniasis women versus controls (3/13 and 1/3), respectively, at p value equal to 0.013 OR (1.00) Cl (1.85-49.21). Based on that, all subjects carrying heterozygous AG and homozygous GG genotypes might have higher risk of trichomoniasis compared with AA homozygous genotype. In the current study, the studied TLR6 SNP has the possibility of determining the pathophysiology of trichomoniasis. Furthermore, different inheritance models of TLR6 SNP (rs5743810) between controls and patients were analyzed and shown in Table (3.13).

Table (3.12): TLR6 SNP (rs5743810) distribution frequencies.

SNP	Allele	Frequency	Controls	Patients	P Value	OR (95% CI)
rs5743810	A	48 (0.69)	25 (0.83)	23 (0.57)	0.021*	3.696 (1.174-11.633)
	G	24 (0.31)	5 (0.17)	17 (0.42)		
	P value	0.005*	<0.001*	0.343		
	Genotypes					
	A/A	16 (0.46)	11 (0.73)	5 (0.25)	0.013*	1.00
	A/G	16 (0.46)	3 (0.2)	13 (0.65)		9.53 (1.85-49.21)
	G/G	3 (0.09)	1 (0.07)	2 (0.1)		4.40 (0.32-60.62)
	P value	0.008*	0.004*	0.008*		

*Represent a significant difference at $p < 0.05$.

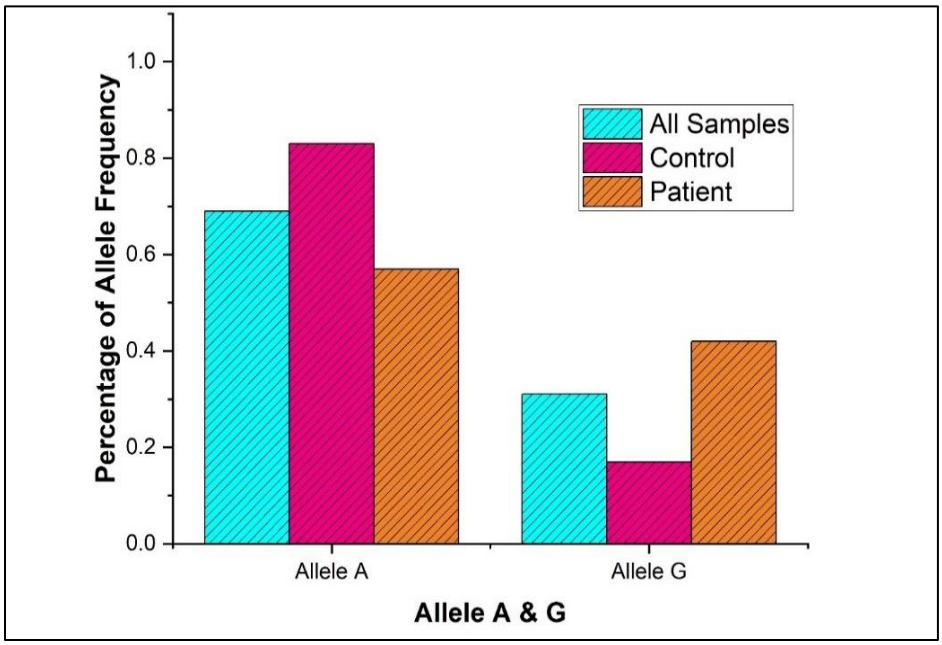

Figure (3.6): Distribution frequency of the TLR6 rs5743810 polymorphism between patients infected with trichomoniasis and controls.

Table (3.13): Analysis of different inheritance models of TLR6 SNP (rs5743810) between the controls and patients.

Model	Genotype	STATUS=0-Control	STATUS=1-Case	OR (95% CI)	P-value	AIC	BIC
Codominant	A/A	11 (73.3%)	5 (25%)	1.00	0.013	45.1	49.8
	A/G	3 (20%)	13 (65%)	9.53 (1.85-49.21)			
	G/G	1 (6.7%)	2 (10%)	4.40 (0.32-60.62)			
Dominant	A/A	11 (73.3%)	5 (25%)	1.00	0.0038	43.4	46.5
	A/G-G/G	4 (26.7%)	15 (75%)	8.25 (1.79-38.01)			
Recessive	A/A-A/G	14 (93.3%)	18 (90%)	1.00	0.72	51.7	54.8
	G/G	1 (6.7%)	2 (10%)	1.56 (0.13-18.95)			
Over-dominant	A/A-G/G	12 (80%)	7 (35%)	1.00	0.0067	44.5	47.6
	A/G	3 (20%)	13 (65%)	7.43 (1.56-35.48)			
Log-additive	---	---	---	4.52 (1.22-16.79)	0.013	45.6	48.7

Akaike information criterion (AIC), Bayesian information criterion (BIC)

As shown in Figure (3.7), the studied SNP, rs5743810, involves adenine to guanine nucleotide change at position 745 in the TLR6 gene which is associated with an amino acid substitution Ser249Pro.

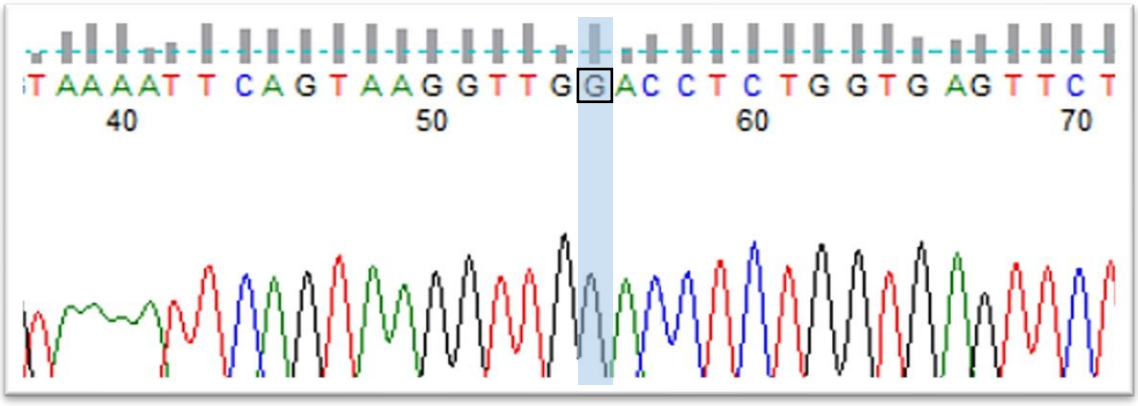

Figure (3.7): DNA sequencing chromatogram of rs5743810 SNP in TLR6 gene.

Limited information is available regarding the association of TLR6 polymorphisms (rs5743810) with parasitic infections. However, TLR6 play a role in mediating the host immune response for various microorganisms lipopeptides usually through dimerization with TLR2 or TLR1. An association was detected between SNP in TLR6 (Ser249Pro) and increasing the risk of invasive aspergillosis infection in patients receiving hematopoietic stem cell transplantation therapy, this polymorphism was reported to enhance IFN-γ production after vaccination with bacillus Calmette–Guérin (BCG) (Randhawa *et al*., 2022).

Moreover, rs5743810 SNP in TLR6 was associated with an elevated risk for malaria development (Leoratti *et al*., 2008). On the other hand, polymorphism in TLR6 has been also reported to be associated with non-infectious diseases (Skevaki *et al*., 2015). Indeed, studies showed that rs5743810 SNP in TLR6 has strong association with breast cancer protection in women. Interestingly, the G allele of rs5743810 SNP has been suggested to be a diagnostic biomarker of breast cancer in the tested women population (Semlali *et al*., 2018).

3.3.4. Comparison of the investigated TLR7 SNP in *T. vaginalis* patients versus controls

Data in in Table (3.14) and Figure (3.8) showed that allele A had significantly more prevalence in control individuals than patients, while allele T revealed significantly more prevalence in infected patients versus controls ($p < 0.001$). Within infected women, allele T demonstrated more frequency (57%) than allele A (42%), however, these findings were statistically non-significant ($p = 0.343$). The genotyping data showed that homozygous AA has significantly greater frequency in healthy controls (12/16) than infected women (4/16), whereas heterozygous AT genotype distribution was significantly higher in patients (9/11) than controls (2/11).

Table (3.14): TLR7 SNP (rs179008) distribution frequencies.

TLR7 SNP	Allele	Frequency	Controls	Patients	P Value	OR (95% CI)
rs179008	A	43 (0.61)	26 (0.87)	17 (0.42)	<0.001*	8.794 (2.583-29.942)
	T	27 (0.39)	4 (0.13)	23 (0.57)		
	P value	0.056	<0.001*	0.343		
	Genotypes					
	A/A	16 (0.46)	12 (0.8)	4 (0.2)	0.0013*	1.00 13.50 (2.01-90.69) 21.00 (1.94-227.21)
	A/T	11 (0.31)	2 (0.13)	9 (0.45)		
	T/T	8 (0.23)	1 (0.07)	7 (0.35)		
	P value	<0.001*	0.001	<0.001*		

*Represent a significant difference at $p < 0.05$.

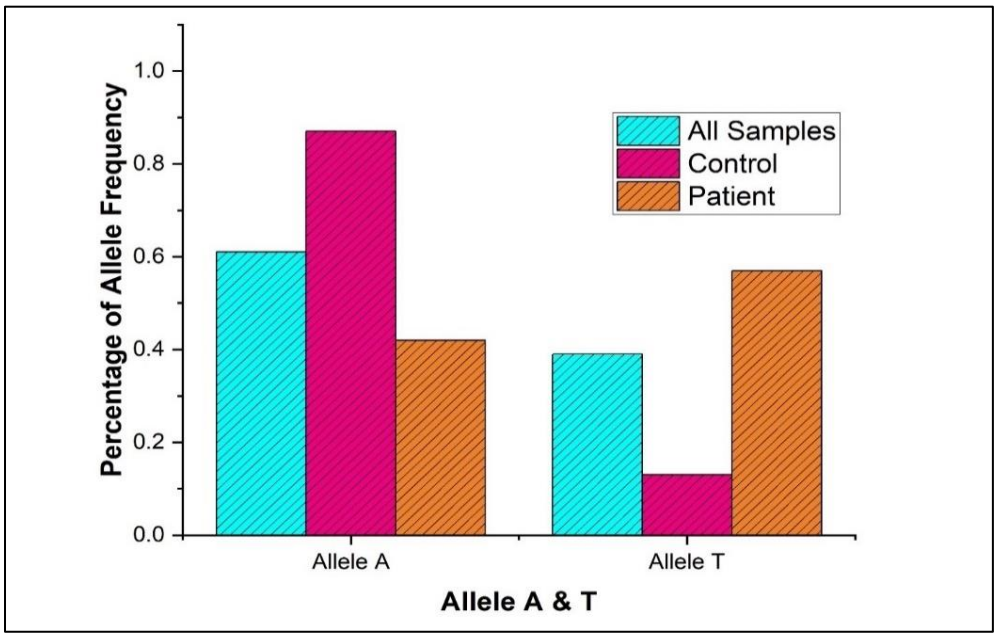

Figure (3.8): Distribution frequency of the TLR7 rs179008 polymorphism between patients infected with trichomoniasis and controls.

Similarly, homozygous TT genotype showed significantly higher distribution in patients (7/8) than that determined in controls (1/8) (p = 0.0013). Within infected patients, the AT bearing women showed significantly the higher distribution among other genotypes followed by TT bearing individuals (p = 0.001).

The interpretation of these findings highlights that women with T allele may be at greater risk for infection with *T. vaginalis*, and A allele might have a protective role against this corresponding parasite. Consequently, women with genotypes AT and TT could be more vulnerable for trichomoniasis infection than women having AA genotype. As seen in Table (3.15), different inheritance models of TLR7 SNP (rs179008) between controls and patients were analyzed.

Table (3.15): Analysis of different inheritance models of TLR7 SNP (rs179008) between controls and patients.

Model	Genotype	STATUS=0-Control	STATUS=1-Case	OR (95% CI)	P-value	AIC	BIC
Codominant	A/A	12 (80%)	4 (20%)	1.00	0.0013	40.5	45.1
	A/T	2 (13.3%)	9 (45%)	13.50 (2.01-90.69)			
	T/T	1 (6.7%)	7 (35%)	21.00 (1.94-227.21)			
Dominant	A/A	12 (80%)	4 (20%)	1.00	3e-04	38.6	41.7
	A/T-T/T	3 (20%)	16 (80%)	16.00 (3.00-85.31)			
Recessive	A/A-A/T	14 (93.3%)	13 (65%)	1.00	0.036	47.4	50.5
	T/T	1 (6.7%)	7 (35%)	7.54 (0.81-69.91)			
Over-dominant	A/A-T/T	13 (86.7%)	11 (55%)	1.00	0.039	47.5	50.6
	A/T	2 (13.3%)	9 (45%)	5.32 (0.94-29.99)			
Log-additive	---	---	---	6.46 (1.79-23.30)	5e-04	39.7	42.8

Akaike information criterion (AIC), Bayesian information criterion (BIC)

As displayed in Figure (3.9), the rs179008 SNP in human TLR7 gene involves adenine substitution at nucleotide number 32 with thymine, which in turn results in amino acid change of Gln to Leu at position 11 in the protein structure. T allele has a global allele frequency of 19.9% versus 80.1% for the reference A allele. The association between TLR7 polymorphism, particularly rs179008, and susceptibility to microorganisms has been mentioned by different studies (Alseoudy *et al*., 2022; Mackelprang *et al*., 2015). Genetic polymorphism in TLR7 elevated the risk of bacterial vaginosis resulting in modulation of women immune responses and susceptibility to sexually transmitted infections (Rosentul *et al*., 2014; Royse *et al*., 2012). The genotype TT as well as the allele T of TLR7 SNP (rs179008) were genotyped through TaqMan real-time PCR and demonstrated significant elevation in risk of COVID 19 pneumonia infection (Alseoudy *et al*., 2022).

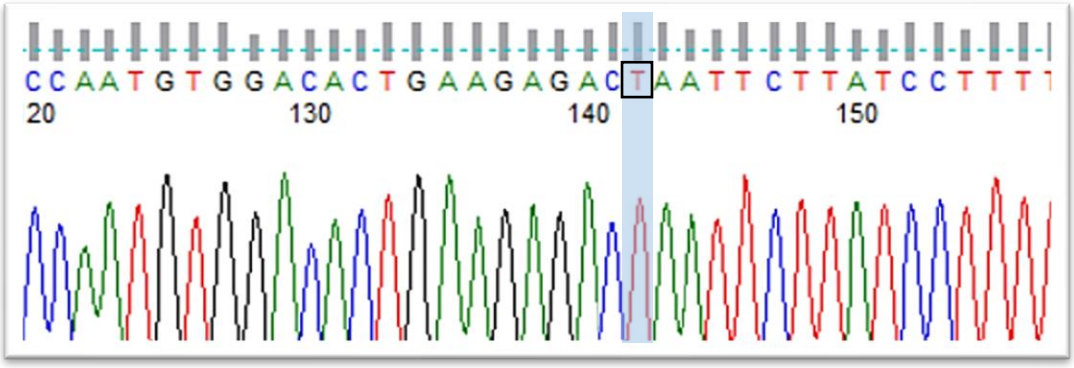

Figure (3.9): DNA sequencing chromatogram of rs179008 SNP in TLR7 gene.

This SNP (rs179008) was also studied in pregnant Brazilian females and revealed increase risk of postpartum placental infection and pregnancy health complications due to human herpes simplex virus types 1 and 2 (HSV 1 and 2) and human cytomegalovirus (HCMV) infection (Sánchez-Luquez *et al*., 2021). TLR7 SNP (rs179008) variants were seen to influence production of immunoglobulin A

(IgA) antibody as well as Rotarix vaccine seroconversion in South African individuals (Miya *et al*., 2021).

Moreover, T allele of rs179008 polymorphism has been shown to increase the severity of hepatitis C virus (HCV) infection in Moroccan subjects after genotyping by TaqMan PCR assays (Fakhir *et al*., 2018). Patients with HIV bearing TLR7 polymorphism (Gln11Leu) exhibited greater viral load in addition to faster progression to advance levels of immune suppression (Oh *et al*., 2009).

3.3.5. Comparison of the investigated MyD88 SNP in *T. vaginalis* patients versus controls

Results in Table (3.16) and Figure (3.10), although statistically non-significant, demonstrated that allele C of rs4988453 SNP in MyD88 had higher frequency in infected women (35/59) versus healthy controls (24/59), while mutant allele A of the same SNP showed almost close frequency between patients and controls, 5/11 and 6/11, respectively. Furthermore, 88% of the infected women had the C allele, whereas only 12% of these patients had the A allele.

Genotyping examination of rs4988453 polymorphism revealed that patients bearing CC genotype were more (17/28) than healthy controls who bearing the same genotype (11/28), however, these findings were statistically non-significant. While the heterozygous CA and homozygous AA genotypes were almost normally distributed between controls and patients. The distribution of genotypes CC, CA and AA among infected women were 85%, 5% and 10%, respectively. Also, different inheritance models of MyD88 SNP (rs4988453) between healthy controls and patients infected with trichomoniasis were analyzed and demonstrated in Table (3.17).

Table (3.16): MyD88 SNP (rs4988453) distribution frequencies.

MYD88 SNP	Allele	Frequency	Controls	Patients	P value	OR (95% CI)
rs4988453	**C**	59 (0.84)	24 (0.8)	35 (0.88)	0.394	0.571 (0.156-2.08)
	A	11 (0.16)	6 (0.2)	5 (0.12)		
	P value	<0.001*	<0.001*	<0.001*		
	Genotypes					
	C/C	28 (0.80)	11 (0.73)	17 (0.85)	0.63	1.00 0.32 (0.03-4.01) 0.65 (0.08-5.29)
	C/A	3 (0.09)	2 (0.13)	1 (0.05)		
	A/A	4 (0.11)	2 (0.13)	2 (0.1)		
	P value	<0.001*	0.005*	<0.001*		

*Represent a significant difference at $p < 0.05$.

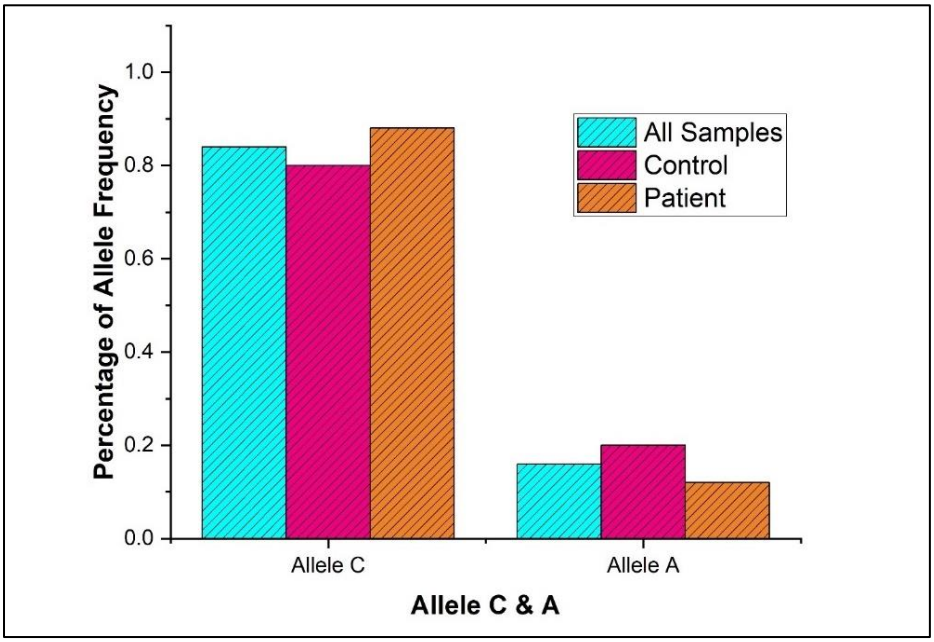

Figure (3.10): Distribution frequency of the MYD88 rs4988453 polymorphism between patients infected with trichomoniasis and controls.

Table (3.17): Analysis of different inheritance models of MyD88 SNP (rs4988453) between controls and patients.

Model	Genotype	STATUS=0-Control	STATUS=1-Case	OR (95% CI)	P-value	AIC	BIC
Codominant	C/C	13 (86.7%)	17 (85%)	1.00	0.92	53.6	58.3
	C/A	1 (6.7%)	1 (5%)	0.76 (0.04-13.41)			
	A/A	1 (6.7%)	2 (10%)	1.53 (0.12-18.76)			
Dominant	C/C	13 (86.7%)	17 (85%)	1.00	0.89	51.8	54.9
	C/A-A/A	2 (13.3%)	3 (15%)	1.15 (0.17-7.90)			
Recessive	C/C-C/A	14 (93.3%)	18 (90%)	1.00	0.72	51.7	54.8
	A/A	1 (6.7%)	2 (10%)	1.56 (0.13-18.95)			
Over-dominant	C/C-A/A	14 (93.3%)	19 (95%)	1.00	0.83	51.8	54.9
	C/A	1 (6.7%)	1 (5%)	0.74 (0.04-12.82)			
Log-additive	---	---	---	1.16 (0.36-3.69)	0.8	51.7	54.9

Akaike information criterion (AIC), Bayesian information criterion (BIC)

These outcomes may indicate that C allele of the rs4988453 SNP in MyD88 did not show a protective role against *T. vaginalis* infection. Indeed, individuals with C allele have high risk for infection with trichomoniasis. In parallel, individuals bearing CC genotypes are more susceptible for trichomoniasis than the other genotypes. In the current study, the frequency of alleles C and A were 0.84 and 0.16, respectively. However, allele frequencies of rs4988453 polymorphism reported by previous studies were 0.96 and 0.037 for C and A alleles, respectively (Tryka *et al*., 2014).

rs4988453 SNP in MyD88 involves the nucleotide cytosine substitution at location number 938 into adenine as demonstrated in Figure (3.11), sharing the same promotor region with the protein acetyl coenzyme-A acyl-transferase-1 (Klimosch *et al*., 2013).

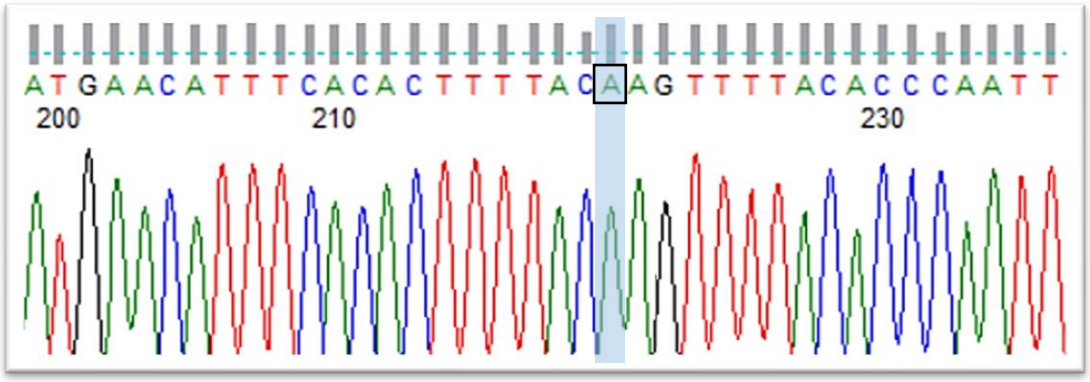

Figure (3.11): DNA sequencing chromatogram of rs4988453 SNP in MYD88 gene.

Nonetheless, studies examined the association of MYD88 genetic polymorphisms with parasitic infection are limited. A study was performed to assess the association of TLRs SNPs, including MyD88, with *T. vaginalis* transmission from infected women to their partners, this study revealed statistically significant association between SNPs in TLR2, TLR3, TLR4, TLR8 and MyD88 with *T. vaginalis* transmission (Lin, 2019). SNPs detected in MyD88, TIRAP and TLRs have been shown to be associated with sexually transmitted disease among African American women (Taylor *et al*., 2012).

Other studies were performed to examine the association of this SNP in MyD88 with susceptibility to Tuberculosis infection, this corresponding polymorphism showed an increased risk for Tuberculosis (more than 5 folds) which was suggested to be a diagnostic marker for people who are at increased susceptibility to Tuberculosis. Indeed, rs4988453 polymorphism has been shown to modify the

interaction between MyD88 with other TLRs, inhibiting by that nuclear factor kappa B activation together with modifying protein function and folding (Aggelou *et al*., 2016).

Furthermore, rs4988453 polymorphism showed an association with progression of sarcoidosis (Daniil *et al*., 2013). MyD88 represent an important component of signaling pathways of human immune response together with other TLRs. Importantly, the mechanism by which rs4988453 SNP can affect the function of MyD88 is could be through inhibiting the promotor region of the MyD88 gene. In parallel with that, reducing the expression of MyD88 can decrease nuclear factor kappa B (NF-κB) activation which potentially attenuate immune response or susceptibility to pathogenic microorganisms (Klimosch *et al*., 2013).

3.4. Immunological Assays

To understand the association between TLR response and the *T. vaginalis* infection, the concentrations of the corresponding TLR genes (TLR2, TLR6 and TLR7) as well as MyD88 in patients infected with trichomoniasis were examined and compared with the normal controls.

3.4.1. Toll-like receptor 2 Concentration

The results in Table (3.18) showed significantly higher levels of TLR2 in patients infected with trichomoniasis in all age groups when compared with the healthy controls. In women aged between 16 to 40 years of particular, concentrations of TLR2 were more in infected women than that in healthy controls at high level of statistical significance ($p < 0.001$ and $p < 0.01$). In contrast, healthy controls (as

shown in Figure (3.12) showed only minor levels of these receptors which involved in defense mechanisms against *T. vaginalis* and other sexually transmitted diseases.

Human TLR2 is considered a cell surface receptor (Jiménez-Dalmaroni *et al*., 2016). Trichomoniasis was reported to upregulate the expression of several TLRs including TLR2, TLR4 and TLR9 on the membrane of epithelial host cells in the urogenital tract, which highlight the important impact of these receptors in stimulating the immune defense system of the infected host toward *T. vaginalis* (Chang *et al.*, 2006). In addition, TLR2 and other TLRs play a significant role in the recognition of *T. vaginalis* (Song *et al*., 2015; Wang *et al*., 2019)

Trichomoniasis infection was shown to induce macrophage apoptosis. Furthermore, *T. vaginalis* has been shown to upregulate the expression of TLR2 in HeLa cells treated with this corresponding parasite as revealed by a study (Chang *et al*., 2006). Similarly, higher levels of these receptors (TLR2) were detected *in vitro* as well as *in vivo* studies in response to infection by *T. vaginalis*. However, it is not demonstrated whether asymptomatic or symptomatic trichomoniasis may affect the expression level of TLR2.

Interestingly, the expression level of TLR2 and two other types including TLR4 and TLR9 were reported to be elevated in response to *T. vaginalis*. Moreover, higher concentrations of cytokines (TNF-α, IFN-γ and IL-6) were produced from macrophages isolated from mice animal model infected with trichomoniasis in comparison with macrophages isolated from TLR2 knockout mice (Li *et al*., 2018).

Table (3.18): Concentration levels of TLR2 according to the age of patients with *T. vaginalis* versus controls.

Parameter	Age (Year)		Concentration (pg/ml) Mean± SD	p value
TLR2	16-20	Patient	114.40±21.30	0.0012 **
		Control	10.25±4.88	
	21-25	Patient	145.10±28.00	0.0001 ***
		Control	23.95±4.08	
	26-30	Patient	115.30±25.00	0.0001 ***
		Control	44.56±6.87	
	31-35	Patient	174.60±32.00	0.0001 ***
		Control	30.81±14.57	
	36-40	Patient	94.45±17.21	0.0001 ***
		Control	17.10±5.75	
	41-45	Patient	206.40±55.05	0.048 *
		Control	34.26±9.69	
	46-50	Patient	159.70 ± 18.03	0.014 *
		Control	36.50 ± 11.29	

* p < 0.05, ** p < 0.01, *** p < 0.001

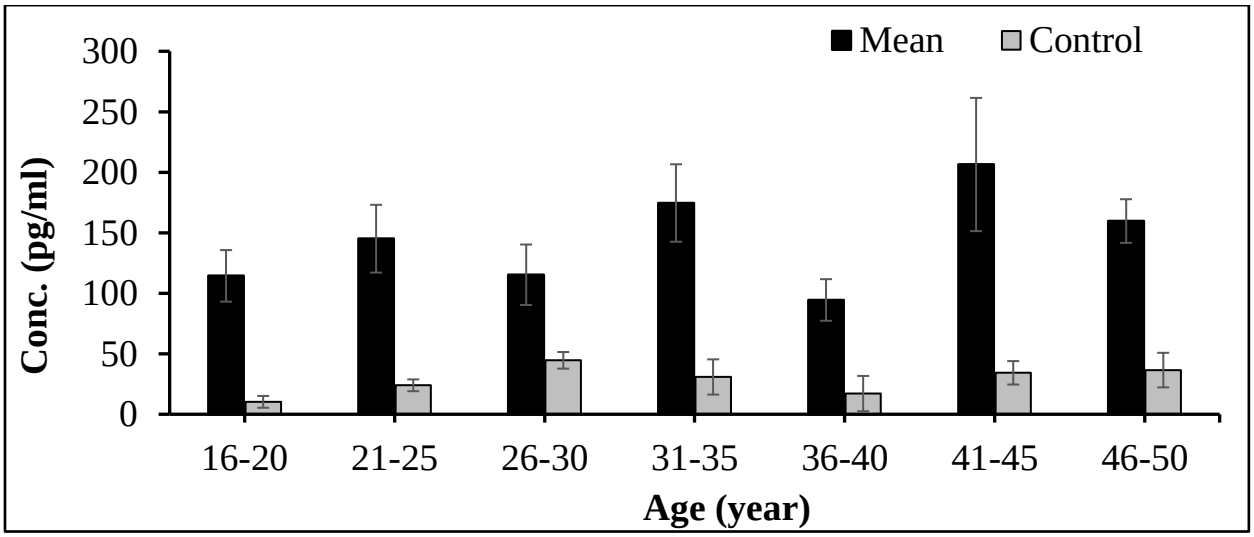

Figure (3.12): Concentration of TLR2 according to the age of patients infected with *T. vaginalis* versus controls.

3.4.2. Toll-like receptor 6 concentration

The results in Table (3.19) demonstrated significantly higher concentrations of TLR6 in patients infected with *T. vaginalis* in all age groups in comparison with the healthy controls. Interestingly, in women aged between 16 to 35 years, concentrations of these corresponding receptors in infected women were greater than that detected in controls at high level of statistical significance ($p < 0.01$). The elevated concentrations of these receptors during the sexually active ages of women are expected, because during these ages the infection with *T. vaginalis* and other sexually transmitted diseases is common, as demonstrated in Figure (3.13).

Very limited data were available regarding the expression of TLR6 in patients' immune cells during the infection with *T. vaginalis*. TLR6 is one of the TLRs shown to be particularly involved in the early recognition of *T. vaginalis* (Song *et al*., 2015 ;Wang *et al*., 2019). Studies manifested that *T. vaginalis* infection elevates the expression of TLR6 (and others such as TLR2, TLR4 and TLR9) in epithelial cell line of human (HeLa cells), which eventually leads to release of inflammatory cytokines (IL-8 and TNFα). These inflammatory factors trigger the human immune response through attracting macrophages and neutrophils to the infection site as well as increasing the T-lymphocyte response (Kim *et al*., 2005; Chang *et al*., 2006).

Importantly, macrophages lacking TLR6 expression showed failure in response to *T. cruzi*, these findings indicating that interaction between TLR2-TLR6 complex with CD14 plays an important role in the recognition process of antigenic molecules of parasite (Ropert and Gazzinelli, 2004). Epithelial cell lines from women vagina and cervices were shown to express TLR1-TLR6, similarly, primary epithelial cells of uterine were also reported to express TLR1-TLR9 (Schaefer *et al*., 2004; McClure and Massari, 2014). TLR6 is expressed by neutrophils, macrophages and B lymphocytes (Chandrani *et al.*, 2019).

Table (3.19): Concentration levels of TLR6 according to the age of patients with *T. vaginalis* versus controls.

Parameter	Age (Year)		Concentration (pg/ml) Mean± SD	p value
TLR6	16-20	Patient	226.35± 29.93	0.0094 **
		Control	142.39± 11.3	
	21-25	Patient	169.79± 15.85	0.0053 **
		Control	143.3± 16.2	
	26-30	Patient	171.89± 16.94	0.0045 **
		Control	147.47± 20.87	
	31-35	Patient	169.82± 13.53	0.0017 **
		Control	149.6± 10.8	
	36-40	Patient	165.62± 11.13	0.01*
		Control	147.6± 16.23	
	41-45	Patient	163.61± 4.89	0.04 *
		Control	146.8± 9.1	
	46-50	Patient	170.04 ± 9.0	0.024 *
		Control	144.69 ± 12.7	

* p < 0.05, ** p < 0.01, *** p < 0.001

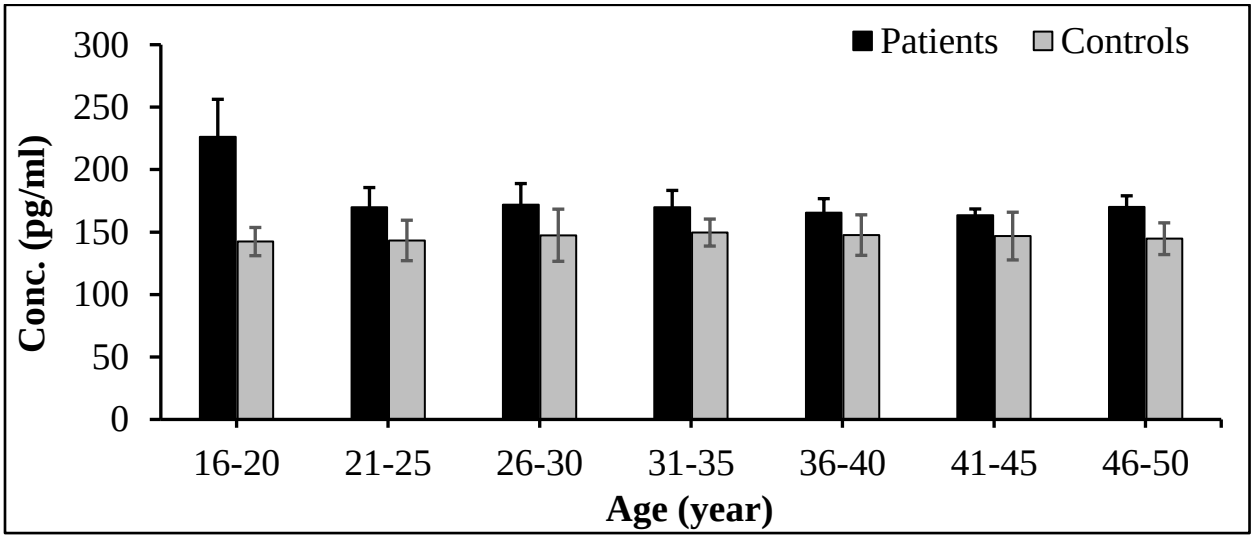

Figure (3.13): Concentration of TLR6 according to the age of patients infected with *T. vaginalis* versus controls.

3.4.3. Toll-like receptor 7 concentration

The levels of TLR7 in Table (3.20) demonstrated significantly higher concentrations of this type of receptors in patients infected with *T. vaginalis* in all tested age groups when compared with that in healthy controls, as further demonstrated in Figure (3.14). Importantly, in women aged between 16 to 40 years (sexually active ages), levels of TLR7 in infected individuals were greater than that in controls at high level of statistical significance ($p < 0.01$, $p < 0.001$). TLR7 as well as other receptors (including TLR2, 3, 4, 6, 8 and 9) are considered the main types of TLRs triggering the immune response by recognizing the *T. vaginalis* (Song *et al.*, 2015).

Table (3.20): Concentration levels of TLR7 according to the age of patients with *T. vaginalis* versus controls.

Parameter	Age (Year)		Concentration (ng/ml) Mean ± SD	p value
TLR7	16-20	Patient	0.136 ± 0.028	0.0015 **
		Control	0.024 ± 0.001	
	21-25	Patient	0.134 ± 0.022	0.0001 ***
		Control	0.029 ± 0.003	
	26-30	Patient	0.130 ± 0.030	0.0001 ***
		Control	0.019 ± 0.009	
	31-35	Patient	0.126 ± 0.030	0.0001 ***
		Control	0.024 ± 0.002	
	36-40	Patient	0.115 ± 0.040	0.0001 ***
		Control	0.014 ± 0.003	
	41-45	Patient	0.164 ± 0.020	0.01 *
		Control	0.024 ± 0.001	
	46-50	Patient	0.139 ± 0.03	0.03 *
		Control	0.019 ± 0.003	

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

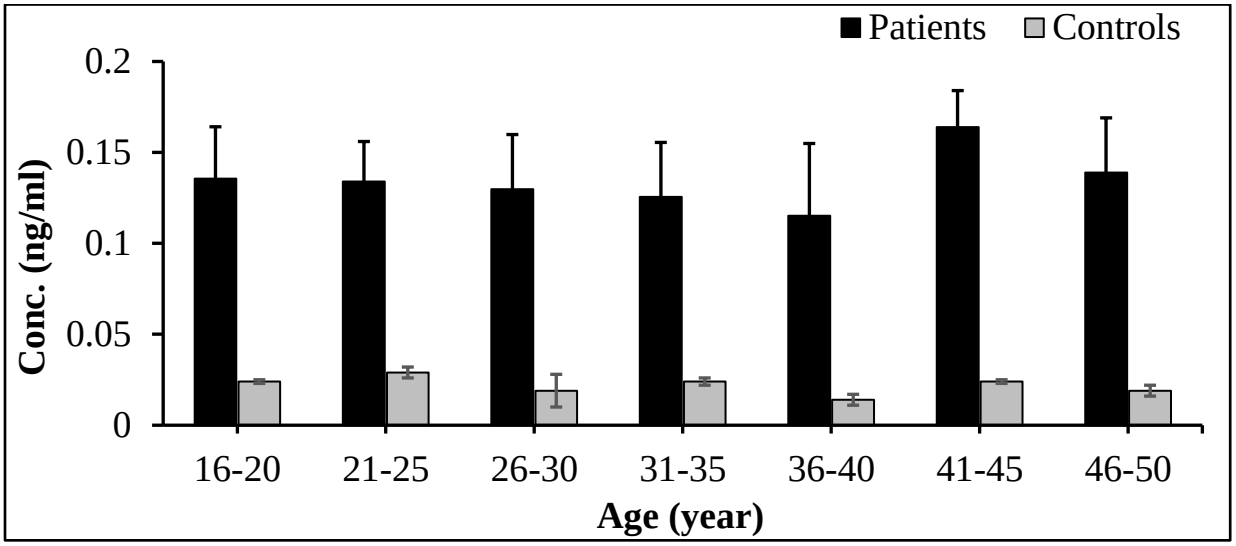

Figure (3.14): Concentration of TLR7 according to the age of patients infected with *T. vaginalis* versus controls.

When these receptors are activated by PAMPs, immune cells overexpress pro-inflammatory cytokines and chemokines initiate inflammatory responses and affecting the intensity of adaptive immunity (Nemati *et al*., 2017).

TLR7 elevated expression has been also reported by previous studies. In details, infection with *T. vaginalis* up-regulated significantly the expression of TLR7 and aided in the suppression of this parasitic infection and other infections as well like, HPV and HIV infections (Yadav *et al*., 2021). Consequently, TLR7 possesses anti-parasitic, anti-viral and anti-bacterial activity as demonstrated by previous studies. Additionally, enhancing or inhibiting signaling pathway of TLR7 was shown to play a crucial role in immune response as well as protection from pathogen-associated molecular pattern and endogenous damage-associated molecular pattern (Patinote *et al*., 2020). In addition to *T. vaginalis*, TLR7 plays an important role in recognition of genetic materials (DNA and RNA) derived from pathogenic viruses and bacteria (Akira *et al.*, 2006; Mancuso *et al*., 2009).

3.4.4. MyD88 concentration

Determinations of MyD88 concentrations were listed in Table (3.21). It is found that there are significantly higher concentrations of MyD88 in patients infected with *T. vaginalis* in all women infected by trichomoniasis when compared with that in healthy controls at all included ages. In women aged between 16 to 40 years, levels of MyD88 in infected individuals were greater than that in controls at high level of statistical significance ($p < 0.001$ and $p < 0.01$). Consequently, these findings indicate that overexpression of these adapters occurs during the sexually active years of women life (as seen in Figure (3.15)) because of the important role of these receptors in human immune response against pathogenic microorganisms.

MyD88 are important adapter proteins of the immune system. These adapters are expressed in various kinds of immune cells including, B and T cells, natural killer cells and others, implying that they are an important component of human immune response against different types of infections (Yadav, S *et al*., 2021). Nishiya *et al*. (2007) suggested that activation of TLRs signaling pathway by infections results in high expression of MyD88 (Nishiya *et al*. 2007). Parasitic infection in mice when their T-cells lacking MyD88 showed more susceptibility to infection, indicating that expression of MyD88 by immune cells is important against parasitic infection (LaRosa *et al*., 2008). Other studies further demonstrated the significant correlation between TLR signaling and MyD88, activation of TLRs on antigen-presenting cells enhanced expression of MyD88 and other costimulatory factors as well as stimulating antigen presentation (Nouri *et al*., 2021). TLRs-MyD88 signaling has been considered as a main pathway of protozoan pathogen recognition by host innate immune defense system (Egan *et al*., 2009).

Table (3.21): Concentration levels of MyD88 according to the age of patients with *T. vaginalis* versus controls.

Parameter	Age (Year)		Concentration (pg/ml) Mean ± SD	p value
MyD88	16-20	Patient	66.9 ±13.6	0.0084 **
		Control	27.3 ± 4.0	
	21-25	Patient	44.8 ± 4.9	0.0001 ***
		Control	32.8 ± 2.6	
	26-30	Patient	56.9 ± 6.8	0.0001 ***
		Control	24.8 ± 1.9	
	31-35	Patient	51.5 ± 11.3	0.0004 ***
		Control	30.8 ± 5.5	
	36-40	Patient	53.8 ± 13.7	0.0001 ***
		Control	19.5 ± 2.7	
	41-45	Patient	63.9 ± 11.0	0.023 *
		Control	37.9 ± 6.3	
	46-50	Patient	44.6 ± 3.0	0.017 *
		Control	30.9± 5.3	

* p < 0.05, ** p < 0.01, *** p < 0.001

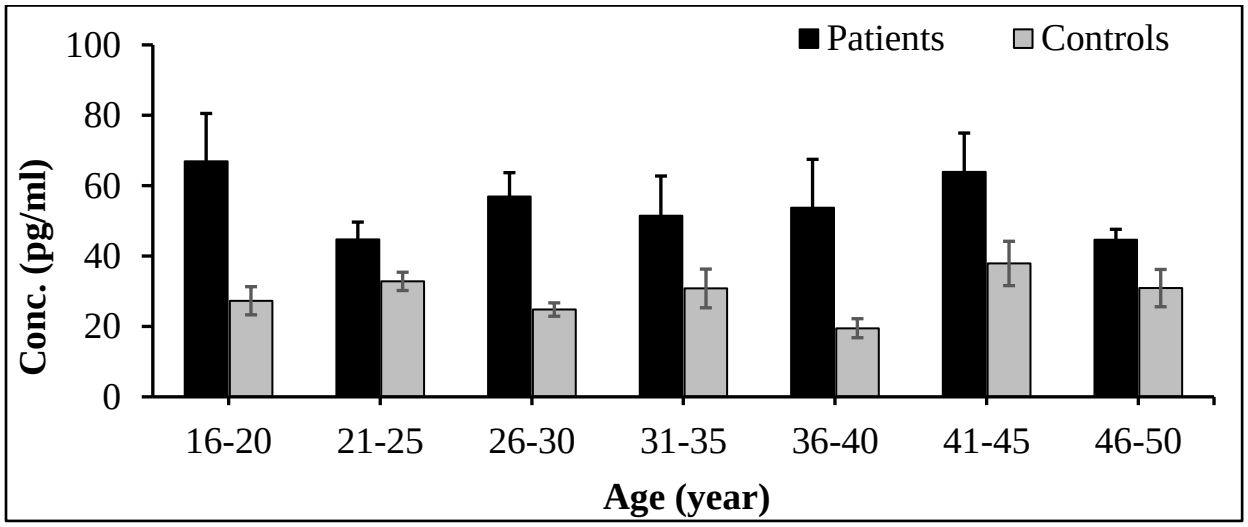

Figure (3.15): Concentration of MyD88 according to the age of patients infected with *T. vaginalis* versus controls.

Conclusions and Recommendations

4.1. Conclusions

1. The percentage of women diagnosed with trichomoniasis using PCR assay out of the total examinations was greater in urban area in comparison to that in rural area. Also, the majority of females infected with trichomoniasis were sexually active women, particularly those aged between 21-40 years old.
2. Genetic studies performed on rs5743708 SNP of TLR2 showed that non-mutant G allele might not play a protective role against trichomoniasis. In addition, women homozygous for AA were not significantly associated with increased risk of trichomonas infection.
3. Regarding rs4986790 SNP of TLR4, Allele A may in part enhance the defensive mechanism against trichomoniasis rather than the mutant G allele. Additionally, the individuals with GG and AG genotypes could be more sensitive for infection with *T. vaginalis* than the homozygous AA genotype.
4. Results of TLR6 polymorphism (rs5743810) significantly highlight that women with mutant G allele are at greater risk to get trichomoniasis compared with those bearing the A allele. Moreover, all women carrying AG heterozygous as well as GG homozygous genotypes might have higher risk of trichomoniasis compared with AA homozygous genotype.
5. Concerning TLR7 SNP (rs179008), women with mutant T allele may be at greater risk for infection with *T. vaginalis*, and A allele might have a protective role against this corresponding parasite. Consequently, women with genotypes AT and TT could be more vulnerable for trichomoniasis infection than women having AA genotype.

6. Finally, MyD88 SNP (rs4988453) genetic analysis showed that women with C allele have high risk for infection with trichomoniasis than the mutant A allele, and individuals bearing CC genotypes are more susceptible for trichomoniasis than the other genotypes.
7. Immunological studies exhibited significantly higher concentrations of TLR2, TLR6, TLR7 and MyD88 in women infected with *T. vaginalis* than controls.

4.2. Recommendations

1. Women with clinical symptoms are recommended to be screened for *T. vaginalis* periodically by PCR based assays to confirm the diagnosis of *T. vaginalis* because of their high sensitivity and specificity.
2. Additional studies are needed to examine more SNPs in TLR2, TLR4, TLR6 and TLR7 and determine their association with *T. vaginalis* susceptibility.
3. In addition, further studies are needed to detect and study more SNPs in MyD88 adapter protein and investigate their association with *T. vaginalis* susceptibility.
4. It is important to study the role of other TLRs in *T. vaginalis* infection as well as studying the association of their SNPs with severity and susceptibility to trichomoniasis.
5. Studying other immunological parameters to fully understand and evaluate the human immune response to *T. vaginalis*.

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الخلاصة

داء المشعرات المهبلية هو مرض معدي ينتقل عن طريق الاتصال الجنسي، وقد ينشأ عن طفيليات تسمى المشعرات المهبلية (*T. vaginalis*). هناك ارتفاع في معدل الإصابة والانتشار، بالإضافة إلى زيادة مقاومة العلاجات الاساسية. علاوة على ذلك ، تم تسجيل مضاعفات صحية خطيرة أبرزت أهمية هذه العدوى.

الأهداف الرئيسية لهذه الدراسة هي تقييم العلاقة بين تعدد الأشكال ل *TLRs* مع عدوى المشعرات المهبلية بين النساء، والتشخيص الجزيئي للطفيلي المذكور باستخدام بادئات محددة بواسطة تقنية تفاعل البلمرة المتسلسل (PCR) من الإفرازات المهبلية للنساء، ودراسة تعدد الأشكال الجينية ل *TLRs* بما في ذلك (rs5743708 في TLR2, rs4986790 في TLR4, rs5743810 في TLR6, rs179008 في TLR7 , rs4988453 في MyD88)، وأخيرًا تقدير التراكيز TLR2، TLR6، TLR7 والاستجابة الأولية للتمايز النخاعي 88 (MyD88) باستخدام تقنية الفحص المناعي المرتبط بالإنزيم في المرضى المصابين بداء المشعرات وكذلك مجاميع السيطرة.

تم جمع عينات من المهبل وعينات الدم من 186 مريضة كن يراجعن عيادات أمراض النساء في مستشفى الامام الصادق ومستشفى بابل التعليمي للولادة والأطفال ومستشفى الحلة التعليمي في محافظة بابل في العراق للمدة من شباط لغاية كانون الأول 2022 . تم الحصول على العينات التي تم جمعها من أجل التحديد الجزيئي للطفيلي ، وتسلسل جينات TLR2 و TLR4 و TLR6 و TLR7 و MyD88 وكذلك إجراء الدراسات المناعية باستخدام ELISA لـ TLR2 و TLR6 و TLR7 و MyD88. تم استخلاص الحمض النووي من المسحات المهبلية وتم تضخيم جين β-tubulin المحفوظ (BTUB) باستخدام بادئات محددة. بالإضافة إلى ذلك، تم جمع معلومات من المشاركات بخصوص العمر ، ومحل السكن، وتاريخ الإجهاض ، والأعراض.

من بين 186 مشاركة ، أظهرت 40 امرأة (21.5%) نتائج PCR إيجابية لجين (BTUB) في المشعرات المهبلية مقابل سبع حالات إيجابية فقط (3.76%) تم اكتشافها بواسطة الفحص المجهري الرطب. لم يكن معدل انتشار الاصابة بالمشعرات المهبلية مرتبطًا بالعمر والمكان ذا دلالة إحصائية، على الرغم من الحالات الإيجابية الأعلى المبلغ عنها في مركز المدينه. بالإضافة إلى ذلك، لوحظت نسب أعلى من الإجهاض في النساء المصابات مقابل النساءغير المصابات. أظهر التشخيص السريري أن الإفرازات المهبلية والحكة كانت أكثر الأعراض انتشارًا النساء المصابات. الدراسات الجينية لـ rs5743708 SNP في TLR2 ، كان تكرار أليل G غير

المتحور أعلى (64/37) في النساء المصابات مقارنة بمجاميع السيطرة (64/27) ، والنمط الجيني GG له انتشار أعلى بشكل ملحوظ (90٪) بين النساء المصابات مقابل النساء المصابات بالنمط الوراثي GA (5٪) و AA 5)٪((P<0.001). فيما يتعلق بـ rs4986790 SNP في TLR4، كان G أليل أكبر في النساء المصابات مقابل مجاميع السيطرة، وكان كل من الأنماط الجينية AG و GG أعلى في النساء المصابات من تلك المسجلة في مجاميع السيطرة الاصحاء. أظهر التحليل الجيني لـ rs5743810 SNP في TLR6 أن أليل G المطفر كان أعلى بكثير (24/17) في النساء المصابات من السيطرة الاصحاء (24/5) (P = 0.021) ، بالإضافة إلى ذلك، أظهر AG و GG نسب أعلى بشكل ملحوظ (16/13 و 3/2) في النساء المصابات بداء المشعرات مقابل مجاميع السيطرة (13/3 و 3/1) ، على التوالي ، p = 0.013. بالنسبة لـ rs179008 SNP في TLR7 ، كشف الأليل المتحور الوراثي T عن انتشار أكبر بشكل ملحوظ في النساء المصابات بـ p =0.0013). علاوة على ذلك، أظهر التحليل الجيني في MyD88 (SNP rs4988453) أن الأليل المتحور A له تردد قريب تقريبًا بين المرضى و مجاميع السيطرة، توزيع CA والأنماط الجينية AA تقريبًا طبيعية بين مجاميع السيطرة والمرضى. أخيرًا، تم اكتشاف تراكيز مرتفعة بشكل ملحوظ لجميع ل *TLRs* المدرجة في الدراسات المناعية (TLR2 و TLR6 و TLR7 و MyD88) في غالبية النساء اللاتي تتراوح أعمارهن بين 16 و 50 عامًا.

كانت النساء المصابات بداء المشعرات أعلى بقليل في مراكز المدينة وأظهرن حالات إجهاض أعلى. الأهم من ذلك ، كانت الإفرازات المهبلية والحكة أكثر الأعراض السريرية شيوعًا التي تعاني منها النساء المصابات. أظهر التحليل الجيني أن rs5743708 و rs4988453 SNPs في TLR2 و MyD88، على التوالي، لا يرتبطان بزيادة خطر الإصابة بعدوى المشعرات المهبلية، بينما rs4986790 و rs5743810 و rs179008 في TLR4 و TLR6 و TLR7, على التوالي، جعلت النساء أكثر حساسية للإصابة بـالمشعرات المهبلية. بالإضافة إلى ذلك، أظهرت الدراسات المناعية تراكيز أعلى بكثير من TLR2 و TLR6 و TLR7 و MyD88 في النساء المصابات بـالمشعرات المهبلية من مجاميع السيطرة خاصة عند النساء خلال الأعمار النشطة جنسياً.

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دراســـة تعدد الأشكــال الجينيـة ل (*TLRs*) عند النساء المصابات بطفيلي المشــعَّرات المــهبليَّة في محافظة بـــابل

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