Full Length Research Article
Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 02/08/2023;
Date Revised: 09/09/2023;
Date Published Online: 20/10/2023;

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How to Cite:
Kermasha ZW, Al-Masoudi HK, Al-Shaikh SF (2023). Association of TLR2 and TLR6 gene polymorphism with *Trichomoniasis vaginalis* Infection. Adv. Life Sci. 10S(1): 125-131.

Keywords:
T. vaginalis; Trichomoniasis; TLR2; TLR6; SNPs

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Association of TLR2 and TLR6 gene polymorphism with *Trichomoniasis vaginalis* Infection

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Abstract

Background: Toll-like receptors (TLR2 and TLR6) are a group of receptors that play a crucial role in the innate immune response and recognition of *T. vaginalis*. Single nucleotide polymorphisms (SNPs) in TLRs were manifested as important determinant affecting the susceptibility to trichomoniasis. This study aims to examine the impact of rs5743708 SNP in TLR2and rs5743810 SNP in TLR6 on Iraqi women infected with *T. vaginalis*.

Methods: Vaginal swabs and blood samples were isolated from 186 female patients who were admitting the gynecology clinics in three public hospitals in Babel governorate in Iraq. The collected samples were obtained for molecular identification of the parasite, sequencing of the TLR2 and TLR6 genes as well as performing the corresponding immunological studies.

Results: The PCR assays showed 40 positive women (95% CI, 15.85 to 28.11) of *T. vaginalis* β-tubulin gene. Genetic studies 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 ($p<0.001$).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, $p=0.013$. Moreover, elevated concentrations of these two receptors were detected during *T. vaginalis* infection.

Conclusion: G allele of rs5743708 might play a protective role against trichomonas is for TLR2, and women homozygous for AA were not significantly associated with increased risk of trichomonas infection. While the mutant G allele of rs5743810 SNP may make women more sensitive for infection with *T. vaginalis* and subjects carrying AG heterozygous and GG homozygous genotypes might have higher risk of trichomoniasis compared with AA homozygous genotype. However, more studies are needed to confirm these findings and to understand the underlying involved mechanisms.

Introduction

Trichomonas vaginalis(*T. vaginalis*) parasite is considered the causative factor of an infection called trichomoniasis. The genitourinary tract tissues of both women and men are the natural sites of infection of this parasite [1]. Epidemiologically, it infects about more than 170 million people globally [2]. 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* [3]. Symptomatic cases of *T. vaginalis*, although mild, are mainly seen as itching and /or swelling 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 [4]. 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 [3,5]. 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 infection produces specific antibodies in the reproductive tract against the parasite and also antibodies that circulate in the serum [6]. Trichomoniasis infection, in addition, has unclear mechanism. In details, *T. vaginalis* triggers innate immune system and the corresponding toll-like receptors (TLRs). These receptors are types of transmembrane proteins which constitute an important part of the innate response of the immune system against different microorganisms. In human, ten TLRs were reported in immune response and combinations of these proteins were also recognized in different types of human cells [7]. 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 domain of TIRs also interact with other intracellular proteins, like the innate immune signal transduction adaptor (MyD88) and Toll/Interleukin-1 receptor domain-containing adapter protein (TIRAP), which plays an important role in signal transduction [8]. TLRs mainly associate with MyD88 to transduce signals, except TLR3. TLR2 and TLR4, on the other hand, associate with MyD88 after they dimerize with TIRAP.

T. vaginalis infection produces inflammatory response in the genitourinary tract of both genders, 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 [9]. Furthermore, the macrophage apoptosis resulted from *T. vaginalis* infection was shown to upregulate the expression of TLRs (TLR2, TLR4, and TLR9) which is recognized in HeLa cell line after they infected with *T. vaginalis* [10].

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 [11]. Consequently, the main goal of this study is to evaluate the association between TLR2 and TLR6 polymorphism with *T. vaginalis* infection among Iraqi women.

Methods

Study Subjects

All studied samples of *T. vaginalis* were isolated from 186 female patients. These females admitted the gynecology clinic in Al-Sadiq Hospital in Hilla city in Iraq between Feb 1st, 2022 and Jan 1st, 2023 who were suffering from vaginal discharge and/or itching. After taking the verbal consent for participation in the study, a questionnaire was taken from the participants regarding their age, residency, history of abortion, and symptoms. Vaginal swabs were vigorously agitated in 1 ml normal saline and then centrifuged at 2000 × g for 10 min. The supernatant was removed, and the pellet was resuspended in 1 ml of sterile distilled water and then kept frozen at -20˚C for PCR assay [12, 13].

Additionally, five ml of patients' blood was collected from female participants and divided into two portion, then these samples (blood and serum) were kept at -20˚C which were used for one for serum isolation and other part kept in EDTA.

DNA Extraction and Immunology Study

Wizard gDNA purification kit was used to extract 500 µl of thawed swab sample according to manufacturer instruction (Promega, USA).The extracted Chromosomal DNAs were used as DNA templates for all PCR based assays.

PCR Amplification for Diagnosis of *T. vaginalis*

Molecular identification of *T. vaginalis* strains was done by using BTUB9/2 gene-specific primers (5'-CATTGATAACGAAGCTCTTTACGAT-3′, and 5'-GCATGTTGTGCCGGACATAACCAT-3′) which produce-112bp product. PCR amplification of DNA was performed according to Valadkhani and his group [12] by thermal cycler in final mixture volume of 25 µl. PCR Products were analyzed by 1.5% agarose gel electrophoresis and illustrated under UV trans-illuminator after staining with ethidium bromide. PCR mixtures used in the assay included 12.5 µl of master

mix, 2.5 µl of both forward and reverse primers, 3 µl of template DNA, and 4.5 µl of nuclease-free water. In addition, PCR assay conditions involved five cycles of initialization (94 ◦C for 5min), denaturation (94 ◦C for 1min), annealing (56 ◦C for 1min), extension (72 ◦C for 1min) and final extension (72 ◦C for 10min).

SNP Polymorphism

In this study, two SNPs were selected in two genes to study the relationship of trichomoniasis with SNP polymorphism. These SNPs are designated: rs5743708 (G>A) in TLR2-encoded gene and rs5743810 (A>G) in TLR6-encoded gene.

Primer Design

The oligonucleotide primers for the studied SNPs (rs5743708 andrs5743810) were designed in this study according to an existing GenBank sequences for studied genes at National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov). These sequences used to design SNP's forward and reverse by Primer3Plus software [13]. Oligonucleotide primers were synthesized by Macrogen company (Korea). All SNPs used in the present study were summarized in **Table 1**.

SNP / Gene name	Primer Sequence	Product	Study
rs5743708 / TLR2	5'-GCAAGCTGC**A**GAAGATAAT-3' 5'-ATTATCTTC**T**GCAGCTTGC-3'	157bp	New design
rs5743810/ TLR6	5'-GTAAGGTTG**G**ACCTCTGGT-3' 5'-ACCAGAGGT**C**CAACCTTAC-3'	245bp	New design

Table 1.SNPs Primers with PCR product length.

PCR Amplification for SNPs Loci

Uniplex-PCR assay performed for the first SNP (rs5743708)was done with a total volume of 25 µl by using GoTaq® G2 Green Master Mix (Promega, USA), through Prime 5 thermocycler (Techno, UK).PCR mixtures used in the assay included 12.5 µl of master mix, 1.5 µl of both forward and reverse primers, 3 µl of template DNA, and 6.5 µl of nuclease-free water. PCR assay conditions involved five cycles of initialization (94 ◦C for 5min), denaturation (94 ◦C for 1min), annealing (55◦C for 1min), extension (72 ◦C for 1min) and final extension (72 ◦C for 10min). However, PCR mixtures and conditions used for the second SNP (rs5743810 SNP) were similar to that utilized for the first SNP except that the annealing temperatures was 53 ◦C.

Sequencing of PCR Products

All PCR products obtained were submitted for sequencing as follows. The PCR products were cleaned of amplification primer using the Gel/PCR DNA fragments extraction kit (Geneaid, USA) as per manufacturer's instructions. Purified DNA was sequenced at Macrogen company (Korea) with the sequencing primers for each gene. Bidirectional Sanger sequencing method was carried out on an Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Immunological Examinations

ELISA Assays for Determining TLR2 and TLR6 Serum Levels

The assay principle for determining the serum levels of TLR2 and TLR6 is the same. The ELISA kit used Sandwich-ELISA as the method. After collection of the whole blood, blood allowed to clot by leaving it undisturbed at room temperature for 10-20 minutes. Then clot was removed by centrifugation at 2,000-3,000 rpm for 20 minutes. The test was performed according to the assay procedure of the used kit (SunLong Biotech Co., LTD, China).

Bioinformatic and Statistical Analysis

The raw sequence data was trimmed and aligned to the control sequences. The standard sequences for alignment were taken from GenBank sequences at NCBI (http://www.ncbi.nlm.nih.gov). Multiple alignments were done by using Clustal W v2.0 [14] of Geneious Prime Software V2021.1 (Biomatters, Inc., North America) to identify SNPs, Allele frequency and genotypes. All other Bioinformatic and Statistical analysis were done according to [15]. TLR concentration differences between patients and controls were tested using t-test, and differences were considered significant when $P \leqslant 0.05$.

Results

Demography and Prevalence of *T. vaginalis*

The characteristic data for detection of confirmed positive cases of *T. vaginalis* depend on PCR assay for detection of β-tubulin gene by using specific primers. As shown in Figure 1, the PCR analysis performed on samples taken from 186 subjects, 40 females revealed positive PCR results of *T. vaginalis* β-tubulin gene with a confidence interval of 15.85% to 28.11%, and 146 females revealed negative PCR outcomes with a confidence interval of 71.89% to 84.17%.

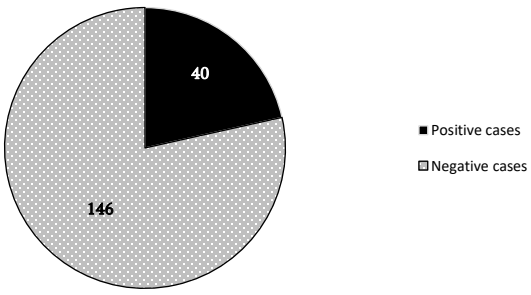

Figure 1: PCR based detection of *T. vaginalis* among studied female samples using BTUB9/2 gene-specific primer.

The female samples of vaginal swaps were assayed by PCR molecular technique using BTUB 9/2 primer. The 40 positive cases were determined by agarose gel electrophoresis. Each set of tests involved both positive and negative controls as well as the DNA marker. The 112bp product size was amplified in all positive samples, as shown in Figure2.

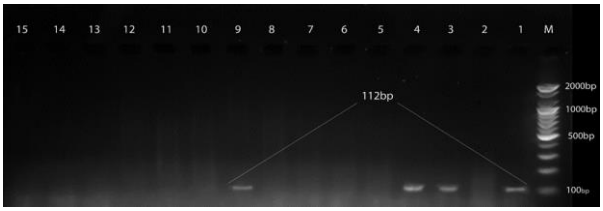

Figure 2: Agarose gel electrophoresis of Uniplex-PCRproducts (112 bp) using *T. vaginalis*-specific primers. Lane M represents universal DNA ladder. Lanes 1,3,4 and 9 represent positive isolates.

Genetic Analysis

In the present study, genotyping frequencies of TLR2 gene SNP (rs5743708) and TLR6 SNP (rs5743810) fitted the Hardy-Weinberg Equilibrium (HWE) among controls (0.0096 and 0.93, respectively). The later model was evaluated by the goodness-of-fit X2 test to compare the observed genotype frequencies with the expected frequencies in controls. The reason for that is to test the assumption that genotype frequencies in a population remains constant from generation to generation, as seen in Table 2.

Groups	rs5743708 / TLR2	rs5743810 / TLR6
All	0.0096*	0.93
Control	0.1	0.33
Patient	0.077	0.35

*Represent a significant difference at p≤0.05.

Table 2: Exact test for Hardy-Weinberg equilibrium for the studied SNPs in TLR2 and TLR6.

Comparison of rs5743708 SNP in *T. vaginalis* Patients Versus Controls

The results of genotyping for the 40 infected women with *T. vaginalis* and the 30 controls for the amplicon of 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. 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). 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 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. 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).

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 (0.04-12.64)
	G/A	2 (0.06)	1 (0.07)	1 (0.05)		
	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.

Table 3: TLR2 (rs5743708) SNP distribution frequencies in the screened population.

Comparison of rs5743810SNP in *T. vaginalis* Patients Versus Controls

As shown in Table 4, the A allele of the rs5743810 was less common in patients 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.

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.Limittedinformation were 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 enhance IFN-γ production after vaccination with bacillus Calmette–Guérin (BCG) [23].

Moreover, rs5743810 SNP in TLR6 was associated with an elevated risk for malaria development [24]. On the other hand, polymorphism in TLR6 has been also reported to be associated with non-infectious diseases [25]. 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 [26].

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 (1.85-49.21) 4.40 (0.32-60.62)
	A/G	16 (0.46)	3 (0.2)	13 (0.65)		
	G/G	3 (0.09)	1 (0.07)	2 (0.1)		
	P value	0.008*	0.004*	0.008*	-	-

* Represent a significant difference at p<0.05.
Table 4: rs5743810 SNP distribution frequencies in the screened population (Control and Patients).

Immunological Parameters

To study the association between TLR response and the *T. vaginalis* infection, we examined the concentration of the corresponding TLR genes (TLR2 and TLR6) in patients infected with trichomoniasis compared with the normal controls.

Tol Like Receptor 2 Concentration

The results in Table 5 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 35 years, concentrations of TLR2 were more in infected women than that in controls at high level of statistical significance ($p < 0.01$). In contrast, healthy controls showed only minor levels of TLR2 which involved in the host defense mechanisms against *T. vaginalis* infection and other sexually transmitted diseases.

Tol Like Receptor 6 Concentration

The results in Table 6 demonstrated significantly higher concentrations of TLR6 in patients infected with *T. vaginalis* in all age groups (except 36-40 and 41-45 years) in comparison with the healthy controls. In women agen 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 ($p < 0.01$). However, although the levels of TLR6 were higher in patients aged between 36-45 years than controls, no significant differences were identified. 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.

Parameter	Age (Year)		Concentration (pg/ml) Mean± SD	P value
TLR2	16-20	Patient	114.40±21.30	0.0012 HS
		Control	10.25±4.88	
	21-25	Patient	145.10±28.00	0.0001 HS
		Control	23.95±4.08	
	26-30	Patient	115.30±25.00	0.0001 HS
		Control	44.56±6.87	
	31-35	Patient	174.60±32.00	0.0001 HS
		Control	30.81±14.57	
	36-40	Patient	94.45±17.21	0.0001 HS
		Control	17.10±5.75	
	41-45	Patient	206.40±55.05	0.048 *
		Control	34.26±9.69	
	46-50	Patient	159.70±0.00	0.014 *
		Control	36.50±11.29	

*Significant at p≤ 0.05. HS Higher significant. NS Non-significant.
Table 5: 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
TLR6	16-20	Patient	226.35±29.93	0.0094 HS
		Control	142.39±11.3	
	21-25	Patient	169.79±15.85	0.0053 HS
		Control	143.3±16.2	
	26-30	Patient	171.89±16.94	0.0045 HS
		Control	147.47±20.87	
	31-35	Patient	169.82±13.53	0.0017 HS
		Control	149.6±10.8	
	36-40	Patient	165.62±11.13	0.1171 NS
		Control	147.6±16.23	
	41-45	Patient	163.61±4.89	0.35 NS
		Control	146.8±19.1	
	46-50	Patient	170.04±0.00	0.026 *
		Control	144.69±12.7	

*Significant at p≤ 0.05. HS Higher significant. NS Non-significant.
Table 6: Concentration levels of TLR6 according to the age of patients with *T. vaginalis* versus controls.

Discussion

PCR diagnosis of *T. vaginalis* based on BTUB9/2 gene-specific primers demonstrated that trichomoniasis is prevalent in 21.5% (40/186) in tested women population (Women with clinical symptoms). In parallel, the worldwide prevalence of *T. vaginalis*was estimated to about 22% using molecular PCR techniques [16]. Other study which utilized real-time PCR assays to test for *T. vaginalis*, targeting the B-tubulin and 18S rRNA genes showed close prevalence of trichomoniasis of 18% in tested female patients [17]. In a systemic review of Turkish population, *T. vaginalis*

was reported in 28.3% of patients who complain of gynecological illness, and 15.37% in women having vaginal discharge [18].

Genetic Analysis of rs5743708 SNP

These results may suggest that G allele may not show protection from trichomoniasis for TLR2, These findings showed that majority of controls and patients having homozygous GG genotype. 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. TLRs in general are considered the first immunological defense against microbes. These receptors link host innate and adaptive immunity, mediating cytokine as well as chemokine responses. In regard to TLR2, the rs5743708 SNP involvesguanine substitution at nucleotide 2258 with adenine in the TLR2 gene, which results in substitution of Gln amino acid at position 753 by Arg.This amino acid substitution is located in the c-terminal region of TLR2 at a highly consereved region.

It was shown that women characterized by symptomatic trichomoniasis demonstrated high TLR2 expression during early time of infection [19]. rs5743708 SNP in TLR2 (Arg753Gln) was correlated with increase susceptibility to septic shock (caused by staphylococcal infection), tuberculosis in Turkish population, infective endocarditis [20 - 22]. TLR2 is either associated with TLR1 or TLR6 recognizing by that ligands from fungi, protozoa, gram-positive bacteria, mycobacteria and viruses.

Genetic Analysis of rs5743810 SNP

Results of rs5743810 SNP significantly highlight that women with G allele are at greater risk to get trichomoniasis compared with those bearing the A allele. In addition, 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.

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.Limittedinformation were available regarding the association of TLR6 polymorphisms (rs5743810) with parasitic infections. However, TLR6 play a role in mediating the host immune response for various microorgnisms 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 haematopoietic stem cell transplantation therapy, this polymorphism was reported enhace IFN-γ production after vaccination with bacillus Calmette–Guérin (BCG) [23]. Moreover, rs5743810 SNP in TLR6 was associated with an elevated risk for malaria development [24]. On the other hand, polymorphism in TLR6 has been also reported to be associated with non-infectious diseases [25]. 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 [26].

Tol Like Receptor 2 Level

Human TLR2 is considered a cell surface receptors [7]. 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 [10]. 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 as well was reported to be elevated in resonse to *T. vaginalis,* and higher levels of proinflammatory cytokines including TNF-α, IL-6 and IFN-γ were detected from macrophages isolated from mice infected with trichomoniasis in comparison with macrophages islated from TLR2 knockout mice [27].

Tol Like Receptor 6 Level

However, although the levels of TLR6 were higher in patients aged between 36-45 years than that in controls, no significant differences were identified. The elevated concentrations of these receptors during the sexually active ages of women is expected, because during these ages the infection with *T. vaginalis*and other sexually transmitted diseasesis common. Very limited data were available regarding the expression of TLR6 in patients immune cells during the infection with *T. vaginalis*. Studies manifested that *T. vaginalis* infection elevates the expression of TLRs (TLR2, TLR4, TLR6 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 [10, 28]. Importantly, macrophages lacking TLR6 expression showed failure

in respose 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 [29]. Epithelial cell lines from women vagina and cervices were shown to express TLR1-TLR6, similarily, primary epithelial cells of uterine were also reported to express TLR1-TLR9 [30]. TLR6 is expressd by neutrophils, macrophages and B lymphocytes [31].

Author Contributions

Zainab Waddah Kermasha: Drafted the manuscript, collected the samples and worked on it for her PhD thesis.
Hayam Khalis Al-Masoudi: Supervisor of Zainab Kermasha, reviewed the document.
Suhaila Fadhil Mohammed: Co supervisor of Zainab Kermasha, reviewed and took responsibility of the document.

Competing Interests

The authors declare that they have no conflicts of interest.

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