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Association of TLR7 and MyD88 Gene Polymorphism with *Trichomoniasis vaginalis* Infection

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Abstract

Objectives: Toll-like receptor 7 (TLR7) and MyD88 represent important components of the innate immune response which play a crucial role in recognition of *T. vaginalis*. Single nucleotide polymorphisms (SNPs) in TLRs and MyD88 were manifested as key determinant affecting the susceptibility to trichomoniasis. This study aims to examine the impact of two SNPs, designated rs179008 and rs4988453 in TLR7 and MyD88, respectively, on Iraqi women infected with *T. vaginalis*.

Methods: Women vaginal swabs as well as blood samples were collected from 186 female patients diagnosed clinically by gynecologists. These patients were admitting the gynecology clinics in three public hospitals in Babel governorate in Iraq. Clinical samples were obtained for molecular identification of the parasite, sequencing of the TLR7 and MyD88 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 rs179008 SNP in TLR7 showed that 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 ($P < 0.01$). Moreover, genetic analysis of rs4988453 SNP in MyD88 demonstrated 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 TLR7 and MyD88 were significantly elevated in the majority of women patients aged between 16–40 years.

Conclusion: The mutant allele A of rs4988453 SNP in MyD88 did not show an association with increased risk of trichomonas infection, however, the mutant allele T of rs179008 SNP in TLR7 might make women more sensitive for infection with *T. vaginalis*. However, more studies are needed to confirm these findings and to understand the underlying involved mechanisms.

Keywords: *Trichomonas vaginalis*, trichomonas infections, TLR7, MyD88, SNPs

Introduction

The parasite *Trichomonas vaginalis* (*T. vaginalis*) is thought to be the main cause of the infection known as trichomoniasis. The natural infection sites for this parasite are the tissues of the genitourinary tract in both men and women.[1] Epidemiologically, it infects about more than 170 million people globally.[2] The majority of *T. vaginalis* infections are asymptomatic, which makes it difficult to gauge infection prevalence, make an early diagnosis, and administer *T. vaginalis* treatment.[3] Although minor, *T. vaginalis* symptoms are mostly characterized by genitourinary tissue edema and/or itching. Sadly, *T. vaginalis* can cause serious side effects such cervicitis ulcers, abortion, preterm birth, or infertility in both men and women.[4] Additionally, research indicated that when this *T. vaginalis* infection is verified, there is an increased chance of contracting HIV, cervical cancer in women, and prostate cancer in men.[3,5] Consequently, new therapeutic medications and vaccines represent a fundamental requirement to control the infection with *T. vaginalis* and prevent its severe complications.

Infection with trichomoniasis in humans causes the reproductive tract to create particular antibodies against the parasite, as well as antibodies that circulate in the blood.[6] Infection with trichomoniasis also has an unknown mechanism. *T. vaginalis* specifically activates the innate immune system and the associated toll-like receptors (TLRs). These receptors are several transmembrane protein types that play a crucial role in the immune system's innate defenses against various pathogens. Ten TLRs have been identified in humans, and combinations of these proteins have also been found in many 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.

Ten functioning TLRs-labeled TLR1 to TLR10-out of the total 13 TLRs found in mammals have been found in humans. Out of these 10 receptors, TLR2, TLR3, TLR4, TLR6, TLR7, TLR8, and TLR9 have been shown to be the primary TLR types involved in the recognition of the parasite *T. vaginalis*. A traditional ligand for TLR7 is viral single strand RNA. Particularly, it has been demonstrated that the HIV-1 virus activates human TLR7 and TLR8 via uridine-rich oligoribonucleotides. The risk of contracting this virus rises noticeably when it coexists with other STDs, particularly *T. vaginalis*.[9]

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.[10] Consequently, the main goal of this study is to evaluate the association between TLR7 and MyD88 polymorphism with *T. vaginalis* infection among Iraqi women.

Materials and Methods

Study Subjects

T. vaginalis was isolated from 186 female patients between February 1st, 2022, and January 1st, 2023, these female patients with vaginal discharge and/or itching were admitted to the gynecological clinic at Al-Sadiq Hospital in Hilla, Iraq. Participants completed a questionnaire on their age, place of residence, history of abortion, and symptoms after verbally consenting to participate in the study. In 1 ml of normal saline, vaginal swabs were aggressively mixed before being centrifuged at 2000 g for 10 min. The pellet was resuspended in 1 ml of sterile distilled water after the supernatant was removed, and it was then frozen at −20°C for the PCR experiment.[11,12]

Furthermore, 5 ml of patient blood from female participants was drawn and divided into two halves; these samples (blood and serum) were then stored at −20°C, with one portion being utilized for serum isolation and the other being stored 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-112 bp product. PCR amplification of DNA was performed according to Valadkhani et al., (2011) by thermal cycler in final mixture volume of 25 µl.[11] After being stained with ethidium bromide, PCR products were electrophoretically separated on a 1.5% agarose gel and examined under a UV trans-illuminator. A 12.5 µl master mix, 2.5 µl each of the forward and reverse primers, 3 µl of template DNA, and 4.5 µl of nuclease-free water made up the PCR mixes used in the experiment. The five cycles of the PCR experiment included initialization (94°C for 5 min), denaturation (94°C for 1 min), annealing (56°C for 1 min), extension (72°C for 1 min), and final extension (72°C for 10 min).

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: rs179008 (A > T) in TLR7-encoded gene and rs4988453 (C > A) in MyD88-encoded gene.

Primer Design

The oligonucleotide primers for the studied SNPs (rs179008 and rs4988453) 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.

PCR Amplification for SNPs Loci

Uniplex-PCR assay performed for the first SNP (rs179008) 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 5 min), denaturation (94°C for 1 min), annealing (55°C for 1 min), extension (72°C for 1 min) and final exten(sion (72°C for 10 min).

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 TLR7 and MyD88 Serum Levels

The assay principle for determining the serum levels of TLR7 and MyD88 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 (Thompson et al., 1994) of Geneious Prime Software

Table 1. **SNPs Primers with PCR product length**

SNP/Gene name	Primer sequence	Product	Study
rs179008/TLR7	5'-TGAAGAGAC**T**AATTCTTAT-3' 5'-ATAAGAATT**A**GTCTCTTCA-3'	157 bp	New design
rs4988453/MyD88	5'-CACTTTTAC**A**AGTTTTACA-3' 5'-TGTAAAACT**T**GTAAAAGTG-3'	245 bp	New design

V2021.1 (Biomatters, Inc., North America) to identify SNPs, allele frequency and genotypes.[14] All other bioinformatic and statistical analysis were done according to Xavier Solé et al., (2006).[15] TLR concentration differences between patients and controls were tested using *t*-test, and differences were considered significant when $P < 0.05$.

Results and Discussion

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%.

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 112 bp product size was amplified in all positive samples.

Trichomoniasis is widespread in 21.5% (40/186) of the tested women group (Women with clinical symptoms), according to PCR identification of *T. vaginalis* based on BTUB9/2 gene-specific primers. Using molecular PCR techniques, the prevalence of *T. vaginalis* worldwide was calculated to be at 22%.[16] Another study that used B-tubulin and 18S rRNA gene-targeted real-time PCR assays to test for *T. vaginalis* in tested female patients found that the prevalence of trichomoniasis was close to 18%.[17] *T. vaginalis* was found in 15.37% of women who had vaginal discharge and 28.3% of patients who complained of gynecological illnesses in a systemic examination of the Turkish population.[18]

Genetic Analysis

In the present study, genotyping frequencies of TLR7 gene SNP (rs179008) and MyD88 SNP (rs4988453) 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

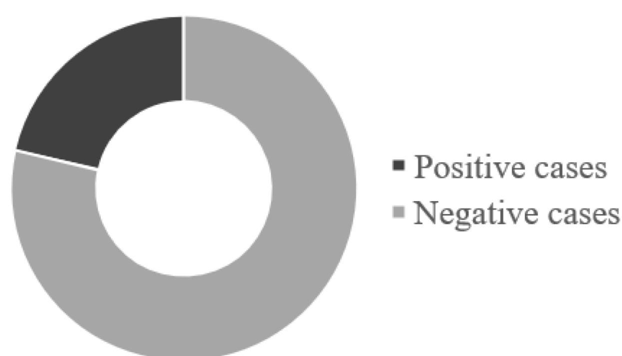

Fig. 1 **Molecular identification of *T. vaginalis* among studied vaginal swabs using PCR-based BTUB9/2 gene-specific primers.**

Table 2. **Exact test for Hardy-Weinberg equilibrium**

Groups	rs179008	rs4988453
All	0.007	0.0014*
Control	0.2	0.1
Patient	0.67	0.01*

*Represent a significant difference at $P \leq 0.05$.

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.

Comparison of rs179008 SNP in T. vaginalis Patients Versus Controls

Data in Table 3 and Figure 2 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).

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.

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.[19,20] Genetic polymorphism in TLR7 elevated the risk of bacterial vaginosis resulting in modulation of women immune responses and susceptibility to sexually transmitted infections.[21,22] 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.[19]

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.[23] TLR7 SNP (rs179008) variants were seen to influence production of immunoglobulin A (IgA) antibody as well as Rotarix vaccine seroconversion in South African individuals.[24]

Moreover, T allele of rs179008 polymorphism has been shown to increase the severity of hepatitis C virus (HCV)

Table 3. **TLR7 (rs179008) SNP distribution frequencies in the screened population**

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
	A/T	11 (0.31)	2 (0.13)	9 (0.45)		13.50 (2.01–90.69)
	T/T	8 (0.23)	1 (0.07)	7 (0.35)		21.00 (1.94–227.21)
	P value	<0.001*	0.001	<0.001*		

*Represent a significant difference at $P \leq 0.05$.

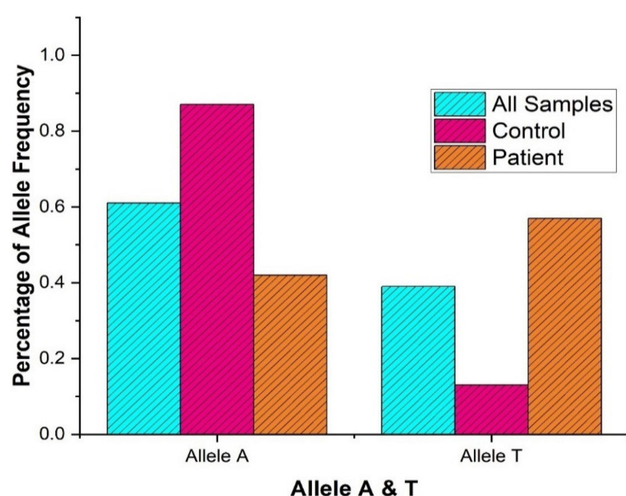

Fig. 2 **Distribution frequency of the TLR7 rs179008 polymorphism between patients infected with trichomoniasis and controls.**

infection in Moroccan subjects after genotyping by TaqMan PCR assays.[25] Patients with HIV bearing TLR7 polymorphism (Gln11Leu) exhibited greater viral load in addition to faster progression to advance levels of immune suppression.[26]

Comparison of rs4988453 SNP in T. vaginalis Patients Versus Controls

Results in Table 4 and Figure 3 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.

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.[27]

rs4988453 SNP in MyD88 involves the nucleotide cytosine substitution at location number 938 into adenine, sharing the same promotor region with the protein acetyl coenzyme-A acyl-transferase-1.[28] 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.[29] SNPs detected in MyD88, TIRAP and TLRs have been shown to be associated with sexually transmitted disease among African American women.[30]

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.[31]

Furthermore, rs4988453 polymorphism showed an association with progression of sarcoidosis.[32] 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.[28]

Immunological Parameters

To understand the association between TLR response and the *T. vaginalis* infection, we examined the concentration of the

Table 4. **rs4988453 SNP distribution frequencies in the screened population (control and patients)**

MYD88 SNP	Allele	Frequency	Controls	Patients	*P* value	OR (95% CI)
	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*		
rs4988453	Genotypes					
	C/C	28 (0.80)	11 (0.73)	17 (0.85)	0.63	1.00
	C/A	3 (0.09)	2 (0.13)	1 (0.05)		0.32 (0.03–4.01)
	A/A	4 (0.11)	2 (0.13)	2 (0.1)		0.65 (0.08–5.29)
	P value	<0.001*	0.005*	<0.001*		

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

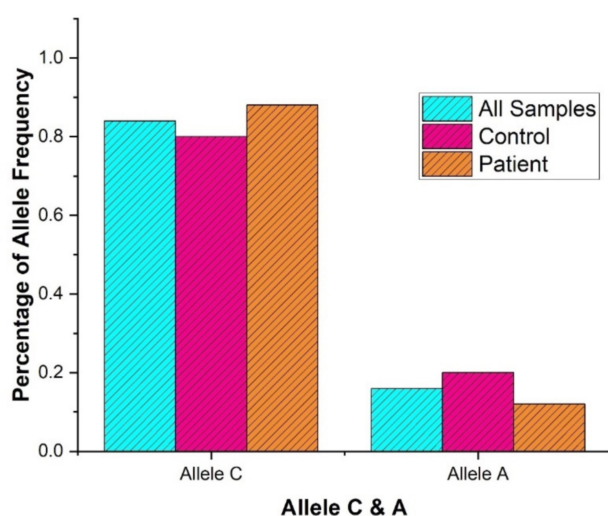

Fig. 3 **Distribution frequency of the MYD88 rs4988453 polymorphism between patients infected with trichomoniasis and controls.**

Table 5. **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	

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

corresponding TLR7 gene as well as MyD88 in patients infected with trichomoniasis compared with the normal controls. The levels of TLR7 in Table 5 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. 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*.[33]

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.[34] 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.[35] 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.[36] In addition to *T. vaginalis*, TLR7 plays an important role in recognition of genetic materials (DNA and RNA) derived from pathogenic viruses and bacteria.[37,38]

MyD88 Concentration

Determinations of MyD88 concentrations were listed in Table 6. 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 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

Table 6. **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	

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

others, implying that they are an important component of human immune response against different types of infections.[39] Nishiya et al. (2007) suggested that activation of TLRs signaling pathway by infections results in high expression of MyD88.[40] 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.[41] 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.[42] TLRs-MyD88 signaling has been considered as a main pathway of protozoan pathogen recognition by host innate immune defense system.[43]

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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