Study of Serum IgM, IgG, IL-37 Levels and IL-37 Gene Polymorphism in Women Infected with Trichomonas Vaginalis in Baqubah City

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Abstract Trichomoniasis is the most prevalent non-viral sexually transmitted infection globally, relying on both innate and adaptive immunity to combat the extracellular pathogen Trichomonas vaginalis. This study included 45 infected women and 45 healthy controls in Baqubah, Iraq, from November 2023 to July 2024. Serum levels of IgM, IgG, and interleukin-37 (IL-37) were measured using ELISA, while genotyping of IL-37 SNP rs3811047 was performed using PCR-SSP. Results revealed the highest infection rate (14%) among women aged ≤25 years. Infected women showed a significant increase in IgM (2.09) and IgG (14.32) compared to controls (IgM: 0.07; IgG: 0.23), with p=0.00. IL-37 levels were also significantly elevated in the infected group (14.29) versus controls (7.35), p=0.00. Sanger sequencing of rs3811047 identified AA, GG, and AG genotypes, suggesting possible associations between A and G alleles and susceptibility to infection. The findings highlight a strong immunological response to T. vaginalis and potential genetic factors influencing host defence.

1 INTRODUCTION

Trichomonas vaginalis is the causative agent of trichomoniasis, a common human urogenital infection. It is the most common, treatable, non-viral sexually transmitted disease (STI) worldwide, with an estimated 156 million cases among men and women aged 15 - 49 years [1].

Trichomonas vaginalis is endemic in Iraq, where several epidemiological studies over the past decade in several Iraqi cities, along with Baghdad, Kufa, Najaf, and Mosul, have confirmed effective diagnosis of infection amongst ladies attending governmental hospitals [2], [3]. Although contamination fees are almost equal in ladies and men, occurrence and signs and symptoms are drastically better in ladies (99 million instances in ladies compared to eleven. Five million instances in guys) [1].

The maximum usually used diagnostic methods encompass microscopic examination of vaginal moist swabs, that is cheaper however insensitive [4]. Genetic investigations can shed slight on drug resistance, pathobiology, aetiology, and different epidemiological capabilities of Trichomonas vaginalis and perceive crucial genes [5]. Various strategies were used to perceive and hit upon Trichomonas vaginalis, which includes monoclonal antibody binding antigenic profiling and real-time polymerase chain reaction (RT-PCR) [6]. The host-parasite interplay in trichomoniasis is complicated. It relies upon at the host immune reaction to the trichomoniasis and the virulence elements of the parasite. This results in pathological consequences via the break out of the parasite from the permanent physical barrier of the lady reproductive tract after powerful mobile adhesion of the parasite to epithelial cells and a success destruction and phagocytosis of target cells, ensuing in continual contamination. The energetic or passive immune reaction of T cells of the inflamed host play essential roles in controlling or exacerbating trichomoniasis [7]. Cytokines, along with interleukins, are a huge organization of proteins, which includes peptides and glycoproteins, secreted by specific cells within the immune gadget. They are produced throughout the frame with the aid of diverse immune cells [8].

IL-37 is a dual characteristic cytokine with both intracellular and extracellular capabilities that has large and complex anti inflammatory and immunomodulatory results, slows the unfold of inflammation, and forestalls tissue harm because of inflammation. These outcomes are caused by suppressing the development of some inflammatory cells, producing cytokines, and activating transcription factors and signalling kinases [9].

This case-control study aimed to explore some immunologic parameters (IgM, IgG, IL-37) and the analysis of the genetic sequence of the single nucleotide polymorphisms (SNP of IL-37).

2 MATERIALS AND METHODS

2.1 Sample Collection and Laboratory Methods

The current study was conducted on women with Trichomoniasis who visited Al-Batoul Teaching Hospital and private gynecology clinics in Baqubah, Diyala Governorate, during the period from November 2023 to July 2024. Pregnant women were excluded from this study.

In this study, 5 ml venous blood was taken from the participants and divided into two parts: 3 ml was placed in gel-containing tubes for immunologic investigations IgM, IgG, IL-37 by ELISA technique. The other 2 ml was put in EDTA-containing tubes and kept in the refrigerator for genetic testing. DNA was then extracted using a Promega kit to conduct genetic screening and polymorphism screening of IL-37 using the Sequence-specific primer-PCR (PCR-SSP) technique.

Primers of IL-37F genes were: GTAAAACGACGGCCAGTGCACAGACCCAGT TGTTT, and of IL-37R were: CAGGAAACAGCTATGACGCTCATCTTTCCCG AGTTATC. After PCR amplification, agarose gel electrophoresis became used to confirm the presence of amplification. PCR changed into absolutely dependent on the extracted DNA standards.

2.2 Statistical Analysis

In this study, the Statistical Package for Social Sciences (SPSS-25) become used for statistical evaluation. The statistics have been usually dispensed and expressed as (mean ± SD). An unbiased pattern t-check and one-way evaluation of variance (ANOVA) have been used to evaluate between corporations. The genetic assessments had been finished by Hardy-Weinberg equilibrium (HWE), Fisher's exact chance and Sanger sequencing.

3 RESULTS

The cutting-edge study included 90 women divided into two groups. The first group comprised 45 patients infected with *Trichomonas vaginalis*, with ages ranging from 19 to 43 years and an average age of 32 years. The second group included 45 uninfected controls (healthy women), with ages ranging between 34 and 43 years and an average age of 34 years. The results demonstrated that the highest percentage of *Trichomonas vaginalis* infections (14%) occurred among women in the age group ≤25 years, while the lowest percentage (7%) was observed in the 26-30 years age group, as validated in Table 1.

Table 1: Distribution of study groups according to age.

Cases	≤25 years	26-30 years	31-35 years	36-40 years	≥41 years
Infected	14%	7%	10%	10%	9%
Control	10%	6%	8%	11%	15%
Total	24%	13%	18%	21%	24%

The results in Table 2 showed that there was a highly significant increase in the mean and standard deviation of IgM antibody in women with Trichomoniasis (2.09±0.77) compared to the mean and standard deviation of this antibody in healthy women (0.07±0.12) (p=0.00). The results in Table 2 also showed a highly significant increase in the mean and standard deviation of IgG antibody in women with Trichomoniasis (14.32±6.67) compared to the mean and standard deviation of this antibody in the control group (0.23±0.13) (p=0.00).

Table 3 shows observed and expected numbers and percentage frequencies and Hardy-Weinberg equilibrium (HWE) of IL37 SNP rs3811047 genotypes and alleles in trichomoniasis patients and controls.

The statistical analysis of associations between IL37 SNP rs3811047 genotypes or alleles in trichomoniasis and controls is summarized in Table 4.

Sanger sequencing was used to analyze the IL37 gene's rs3811047 SNP as shown in Figure 1.

Table 2: Mean and SD of immunoglobulins (IgM and IgG) among the study groups.

Antibody type	Mean±SD Infected women	Mean±SD Controls	P value
IgM	2.09±0.77	0.07±0.12	0.00**
IgG	14.32±6.67	0.13±0.23	0.00**

**There are significant differences at a significance level of 0.01.

Table 3: Observed and expected numbers and percentage frequencies and Hardy-Weinberg equilibrium (HWE) of IL37 SNP rs3811047 genotypes and alleles in trichomoniasis patients and controls.

Genotyping of IL37 SNP rs3811047	Patients group No. (%)		Control group No. (%)	
	Observed	Expected	Observed	Expected
AA	3(3.2)	15(16%)	0 (0.8)	0(16)
AG	10 (9.6)	50 (48%)	4 (2.4)	80 (48)
GG	7 (7.2)	35 (36%)	1 (1.8)	20 (36)
Total	20 (100.0)	100 (100.0)	5(100.0)	100 (100.0)
A	16 (40%)	Not estimated	4 (40%)	Not estimated
G	24 (60%)	Not estimated	6 (60%)	Not estimated
P-HWE	0.8522		0.136	

Table 4: Statistical analysis of associations between IL37 SNP rs3811047 genotypes or alleles in trichomoniasis and controls.

Type of Comparison		Relatives risk (%)	OR	95% CI	Fisher's exact probability
Patients Versus Controls	AA	9.1	2.20	0.12-38.81	1.000
	AG	6.0	0.25	0.03-2.12	0.341
	GG	22.0	2.69	0.31-23.23	0.628
Patients Versus Controls	A	37.8	1.40	0.26-3.87	1.000
	G	10.0	1.00	0.26-3.87	1.000
OR - odds ratio, 95% CI - confidence intervals					

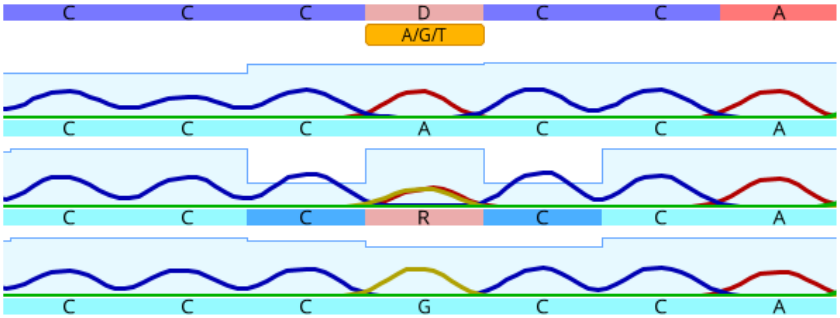

Figure 1: Sanger sequencing become used to analyze the IL37 gene's rs3811047 SNP. A unmarried "A" peak indicates the presence of an A homozygous allele. A unmarried "G" top indicates a G homozygous allele. The presence of the "A" and "G" top indicates the A/G heterozygous allele.

The analysis of the IL-37 gene polymorphism (rs3811047) revealed significant associations between specific genotypes and disease susceptibility. The homozygous AA genotype was notably more prevalent in patients than in controls (63.2% vs. 18%), suggesting a strong risk association (OR = 2.20, $p < 0.0001$). This indicates that individuals carrying the AA genotype may have a higher predisposition to develop the disease=

Similarly, the A allele frequency was higher in patients (40%) compared to the control group (44%), with a moderate risk association (OR = 1.40, p = 0.01), supporting its potential role as a contributing risk factor.

Interestingly, the homozygous GG genotype was also more frequent in patients (18%) than in controls (7%), indicating another risk association (OR = 2.69, p = 0.028). However, the G allele showed equal frequency between patients and controls (60% vs. 60%), with no observed risk effect (OR = 1.00, p = 1.000).

Collectively, these results suggest that both homozygous AA and GG genotypes might act as significant genetic risk factors in the pathogenesis of the disease, whereas the allelic distribution alone (especially for G) may not fully explain the observed associations. Further functional studies are warranted to clarify the biological mechanisms underlying these genetic effects.

4 DISCUSSION

The present day study was performed on 45 women with Trichomoniasis and (45) healthy uninfected women as a control group. Our results showed that the highest infection rate occurred in the age group (≤25) years and the lowest percentage of infections occurred among women in the age group 26-30 years, and this result agreed with Sutton et al [10] who showed a higher rate of infection among young women aged 18-25 years and also agreed with Tine et al.'s study [11] who showed that women under 25 were the most affected. However, our results did not agree with Younis and Elamami who observed that trichomoniasis was higher in the age group more than 40 years [12].

As for the emergence of high rates of infection in young women, it could be attributed to sexual activity and the high concentration of sex hormones, as well as the increase in glycogen, which causes the pH to shift to the alkaline side. Also, the high rates of infection in middle-aged women may have been due to previous infections that were cured and the antibodies to the parasite remained [13].

With regard to immunoglobulins (IgM and IgG), our results pointed out a relatively large increase in their levels in women infected with Trichomonas vaginalis. These results were in agreement with the study of [13], which showed the increased levels of IgM and IgG in women infected with trichomoniasis. Moreover, in the last forty years the presence of anti-T. Vaginalis antibodies (IgA, IgM, and IgG, and its subclasses) in serum and cervicovaginal secretions has been established by radioimmunoassay, ELISA, and immunofluorescence techniques [14]. Immune variables play an important role in maintaining human health. Infection with some parasites and changes in those variables can result in a variety of symptoms and signs, leading to increased production of immune cells such as B and T lymphocytes. This furthermore results in increased levels of antibodies such as IgG and IgM [15].

IgM is the first type of antibody secreted during the primary immune response, and therefore its presence in plasma is considered a diagnostic tool. It indicates the presence of a recent infection, and due to the multiple binding sites for antigens, it has a high capacity to agglutinate antigens [16]. IgG is the most abundant form of antibody in plasma, as it represents 70-80% of all antibodies. It is characterized by its ease and speed of movement in cells because it is smaller in size and more abundant. It protects against various types of disease causes such as bacteria, viruses, fungi, and toxins [17].

The understanding of the mechanisms involved in the interaction between T. Vaginalis and the host immune response may contribute to the development of new goals to combat the parasite [18].

The IL-37 gene is located on chromosome 2q12-13, close to the regulatory regions of the IL-1a and IL-1b genes. This specific location may be critical for the positioning of IL-37 as an inflammatory response inhibitor. The IL-37 gene is situated on chromosome 2q12-13, which is extremely close to the regulatory regions of the IL-1a and IL-1b genes. This particular position may be critical for the function of IL-37 as an inflammatory response inhibitor [19].

Among the cytokines is IL-37, and its immunological role in viral, bacterial, and fungal infections is receiving increasing attention [20]. Furthermore, accumulating data revealed that SNPs of the IL37 gene are conserved across humans through selection force, and their potential participation in the control of immune responses and susceptibility to human disorders has been hypothesized [21].

IL-37 functions as an anti-inflammatory cytokine, inhibiting inflammatory reactions by influencing the production of pro-inflammatory cytokines [22]. Because of this immunological

characteristic, IL-37 has been shown to play an important role in the pathogenesis of a variety of inflammatory and autoimmune illnesses, and dysregulated IL-37 expression has been reported under these conditions. Furthermore, the antiviral, antibacterial, and antifungal properties of IL-37 have also been identified [20].

To the best of our knowledge, the SNPs of IL-37 have not been examined in any human disease, therefore, limited information on their effects in human infectious diseases is available.

Genetic association studies have related several single nucleotide polymorphisms (SNPs) within the IL-37 gene to vulnerability to a number of autoimmune, inflammatory, and infectious diseases, including Behçet disease, systemic lupus erythematosus, periodontal infection, hepatitis viral infection, and tuberculosis [23].

A number of viral, inflammatory, and autoimmune diseases, including ankylosing spondylitis, rheumatoid arthritis, autoimmune thyroid disease, Behcet's disease, and Vogt-Koyanagi-Harada disease, have been linked to the polymorphism rs3811047 [24].

5 CONCLUSIONS

The present study identified a distinct epidemiological pattern, with the highest rate of T. vaginalis infection observed among women aged ≤25 years. From an immunological perspective, the infection triggered a pronounced immune activation, as evidenced by significantly elevated levels of IgM and IgG (indicating a robust humoral response) and IL-37 (suggesting a modulation of the inflammatory response) compared to healthy controls. Additionally, our investigation into the genetic variant rs3811047 of the *IL-37* gene revealed a distribution of AA, GG, and AG genotypes that implies a potential genetic predisposition to infection, although this requires validation in a larger cohort. In summary, these findings underscore the complex interaction between T. vaginalis and the host's immune system, involving both humoral and cellular mechanisms. This work contributes to the growing body of evidence on the immunology of trichomoniasis and opens avenues for further research into genetic factors influencing disease susceptibility.

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