An Investigation of the Variances (rs5744168, rs5744174) of TLR-5 and Susceptibility to Polycystic Ovary Syndrome and Toxoplasmosis in Groups of Iraqi Women

Haider Iskandar Flayh[1], Huda Dhaher Hathal[1], Enas Adnan Abdulrasol[2]

[1] College of Medicine Al-Nahrain University \ Microbiology department
[2] College of Medicine Al-Nahrain University \ Gynecology department

Abstract— **Polycystic Ovary Syndrome (PCOS) is a prevalent endocrine disorder characterized by a range of reproductive, metabolic, and immune irregularities. Emerging evidence suggests that infectious agents can contribute to the pathogenesis of PCOS, particularly through immune dysregulation. Toxoplasma gondii, an intracellular protozoan parasite, has been implicated in various immune-mediated disorders. Toll-like Receptor 5 (TLR-5), a key component of the innate immune system, plays a pivotal role in recognizing pathogen-associated molecular patterns, including those from T. gondii. This article explores interplay between TLR-5 single nucleotide polymorphisms (rs5744168, rs5744174) and susceptibility to T. gondii and the potential implications for PCOS development. A case- control study of 150 sample patient divided into four groups, amplification-refractory mutation system (ARMS PCR) was used to examine these SNPs. rs5744168 shows a significant difference between PCOS+ toxoplasmosis and toxoplasmosis only with control group. Also, rs5744174 revealed a significant difference between toxoplasmosis patients and control group. Therefore, women that have both SNPs (rs5744168, rs5744174) in TLR-5 become more susceptible to infection with toxoplasma gondii.**

Index Terms— **TLR-5, rs5744168, rs5744174, Polycystic Ovary Syndrome.**

I. INTRODUCTION

Toxoplasmosis typically remains asymptomatic in individuals with a fully functional immune system; however, in immunocompromised individuals, it can lead to severe or even fatal complications [1]. Toxoplasmosis is attributed to Toxoplasma gondii (T. gondii), an obligate intracellular parasite that holds the distinction of being recognized as one of the most prevalent global parasites [2]. Toll-like receptors (TLRs) represent transmembrane receptors that exert a pivotal role in orchestrating both innate and adaptive immune responses. They serve as crucial regulators of inflammatory processes and activation of immune cells aimed at eliminating infectious pathogens and malignant cells [3]. To date, the human repertoire comprises ten distinct types of TLRs, each specialized in the recognition of diverse pathogens and/or molecules [4]. Profilin, a pathogen-associated molecular pattern (PAMP) originating from T. gondii, is capable of eliciting recognition by receptors located on dendritic cells and macrophages. This recognition subsequently triggers cellular activation and provokes the release of proinflammatory cytokines such as interleukin IL-6 and IL-12 [5]. Notably, it has been experimentally established that the activation of TLR-11 by profilin results in robust protein production by mouse dendritic cells. However, it is important to note that the human TLR-11 gene contains numerous stop codons, preventing the transcription of a functional protein [6]. Interestingly, within the TLR phylogenetic tree, there exists an ancient cluster encompassing both human TLR-5 and mouse TLR-11. Consequently, it is hypothesized that human TLR-5 may have retained the biological function of mouse TLR-11 and plays a role in mediating the recognition of T. gondii profilin [7,8]. particularly, the ectodomain of human TLR-5 features shared binding sites for both flagellin and profilin [8]. TLR-5 is encoded by gene comprising six exons situated on the extended arm of human chromosome 1 (hCh1q). Notably, this gene exhibits nine documented potential variations in its promoter and coding regions [9]. In T. gondii infection, inflammatory monocytes respond by triggering the production of pro-inflammatory cytokines, including IL-1 and IL-1β, through TLR-5 and MyD88 signaling pathways. This multifaceted cytokine response contributes to inflammation, subsequently impacting processes associated with ovulation, implantation, and fertilization [10,11]. Researchers Zangeneh et al. emphasized the pivotal role of immunity in the fertilization and implantation of eggs within the uterine environment [12]. Additionally, Escobar-Morreale and colleagues identified factors influencing pro-inflammatory responses and linked them to a correlation with the HPA (Hypothalamus Pituitary Adrenal) axis, which regulates adrenal steroid genesis [13]. Polycystic ovary syndrome (PCOS) is characterized by an abnormal elevation in androgens, especially the male sex hormones testosterone that found in women in limited quantities. The term "polycystic ovary syndrome" denotes the presence of multiple small cysts, or fluid-filled sacs, forming within the ovaries [14]. Research in the field of reproductive biology has revealed that pro-inflammatory cytokines, such as TNF-α, IL-6, and IFN-γ, exert an influence on ovarian function as well as the key processes of ovulation, fertilization, and implantation in individuals with PCOS. Conversely, anti-inflammatory cytokines, including IL-10 and IL-1, play a role in modulating the inflammatory state associated with PCOS

DOI: http://doi.org/10.24086/biohs2024/paper.1256

[15].
This research aimed to:
- Study the association between TLR-5 gene polymorphisms and the susceptibility to toxoplasmosis and PCOS in a sample of infected women.

II. MATERIALS AND METHODS

The study was approved by the Institutional Review Board (I.R.B.) in the Al-Nahrain University College of Medicine (in: 22-Nov.-2021). The present Case-control study was conducted on a total of 150 women grouped as the following :
- Polycystic ovary syndrome women with Toxoplasmosis (25 cases)
- women with PCOS without toxoplasmosis , (25 cases)
- women with toxoplasmosis without PCOS, (25 cases)
- control group (apparently healthy) (75 sample)

A. Inclusion criteria

Healthy female 18 years of age or over (as control group), female with PCOS and Toxoplasmosis, female with PCOS only, female with toxoplasmosis only.

B. Exclusion criteria

Female with ovarian cancer or other chronic diseases (ex. Diabetes mellitus and Hypertension), pregnant and Breast-feeding female, female with other Gynecological diseases (ex: Endometriosis, Uterine fibroids).

C. Sample collection

Five ml of whole venous blood was taken from each patient, and patients information was taken from the data recorded. Two ml was collected in an ethylene diamine tetra acetic acid (EDTA) tube for extraction of DNA. The other three ml of blood collected in plain gel tube for separation of serum and the Samples collected in the period from January 2022 to June 2022 from Obstetrics and Gynecology Department of Kadhimiya Teaching Hospital, in Baghdad province.
Women participating in the study were already diagnosed with PCOS and investigated for the presence of anti-T. gondii IgG by rapid chromatographic immune technique and confirmed by ELISA technique. The selected SNPs TLR-5 (rs5744174, rs5744168) were detected by using amplification-refractory mutation system (ARMS PCR) with specific primers.

D. Statistical Analysis

SPSS software version 25.0 was used for statistical analysis (SPSS, Chicago). The normality test (Shapiro Wilk test) was performed on continuous data. Information with normally distributions were provided as mean and standard deviation and evaluated using the Student t-test. Non-normally distributed data were presented as median and range and analyzed using the Mann Whitney U test (for two-group comparison) or the Kruskal Wallis test (for three groups comparison). Categorical variables had been recorded as numbers and percentages were examined using the Chi-square test, statistically significant difference was defined as a p-value less than 0.05.

III. RESULTS

A. Age Distribution of the Study Population

The mean age of women with PCOS+Toxoplasmosis was 27.12±6.47 years which did not differ significantly from that of women with PCOS (28.44±5.45 years), women with toxoplasmosis (28.68±6.41 years) of controls (27.0±5.56 years) as shown in figure -1.

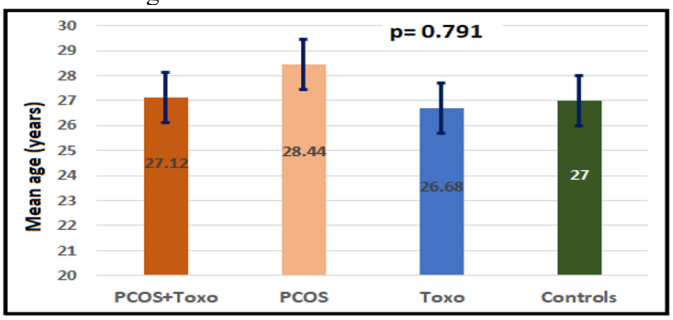

Figure -1: Age distribution of the study population.

B. TLR-5 rs5744168 polymorphism

This polymorphism appeared in two genotypes only: GG and AG in all study groups as shown in Figure -2.

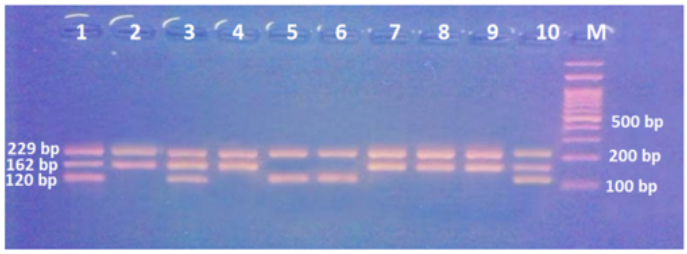

Figure -2: Gel electrophoresis of TLR-5 rs5744168 gene amplified ARMS method. The PCR products were stained with ethidium bromide. M: molecular marker.

The frequency of different genotypes of this SNP was identical between women with PCOS+toxoplasmosis and those with toxoplasmosis only. Thus, the two groups were merged with each other in all tables.
A comparison between women with PCOS+toxoplasmosis/or toxoplasmosis only with those with PCOS only revealed no significant difference in the frequency of genotypes and alleles (Table -1).

Table -1: Genotypes and alleles TLR-5 rs5744168 in women with PCOS/or toxoplasmosis/or toxoplasmosis and those with PCOS.

rs5744168	PCOS+Tox/or toxo (n=25)	PCOS (n=25)	P-values	OR(95%CI)
Genotypes				
GG	19(76%)	22(**88**%)	0.278	1.0
AG	6(24%)	3(**12**%)		2.32(0.51-10.54)
HWE	0.820	0.438		
Alleles				
G	44(88%)	47(94%)	0.303	1.0
A	6(12%)	3(6%)		2.14(0.51-9.07)

DOI: http://doi.org/10.24086/biohs2024/paper.1256

However, women with PCOS+toxoplasmosis/or toxoplasmosis only displayed higher frequency of AG genotype than controls (24% vs. 5.33%) with a significant difference (OR= 5.61, 95%CI= 1.43-21.9, p =0.013). Moreover, allele A was more common in patients with PCOS+toxoplasmosis/or toxoplasmosis only (12%) than controls (2.67%) with a significant difference (OR= 5.0, 95%CI= 1.34-18.43, p =0.016) as shown in table -2.

TABLE -2: GENOTYPES AND ALLELES TLR-5 RS5744168 IN WOMEN WITH PCOS/OR TOXOPLASMOSIS/OR TOXOPLASMOSIS AND CONTROLS.

rs5744168	PCOS+Tox/or toxo (n=25)	Controls (n=75)	P-values	OR(95%CI)
Genotypes				
GG	19(76%)	71(94.67%)	**0.013**	**1.0**
AG	6(24%)	4(5.33%)		**5.61(1.43-21.9)**
HWE	0.820	0.804		
Alleles				
G	44(88%)	146(97.33%)	**0.016**	**1.0**
A	6(12%)	4(2.67%)		**5.0(1.34-18.43)**

A comparison between women with PCOS with those with controls revealed no significant difference in the frequency of genotypes and alleles (Table -3).

TABLE -3: GENOTYPES AND ALLELES TLR-5 RS5744168 IN WOMEN WITH PCOS/ AND CONTROLS.

rs5744168	PCOS (n=25)	Controls (n=75)	P-values	OR(95%CI)
Genotypes				
GG	22(88%)	71(94.67%)	0.270	1.0
AG	3(12%)	4(5.33%)		2.42(0.50-11.65)
HWE	0.438	0.804		
Alleles				
G	47(94%)	146(97.33%)	0.279	1.0
A	3(6%)	4(2.67%)		2.33(0.50-10.79)

C. TLR-5 rs5744174 polymorphism

This polymorphism had three genotypes: CC, CT and TT in all study groups (Figure -3).

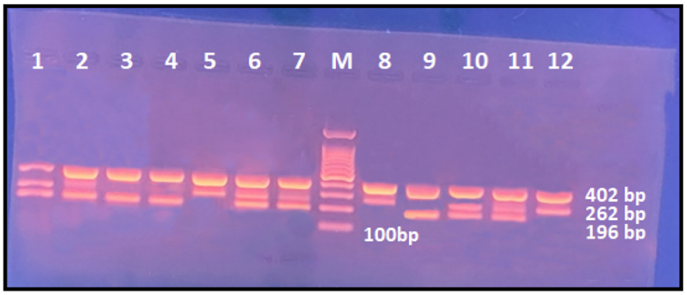

Figure -3: Gel electrophoresis of TLR- rs5744174 gene amplified ARMS method. The PCR products were stained with ethidium bromide. Lanes 1,2,6,10,and 11: CT genotype, lanes 3,4,and 9: TT genotype, lanes 5,8 and 12: CC genotype. M: molecular marker.

The frequency of different genotypes and allele were comparable between: women with PCOS+toxoplasmosis and those with PCOS; women with PCOS+toxoplasmosis and those toxoplasmosis; women with PCOS+toxoplasmosis and controls; and women with PCOS and controls with no significant difference (tables -4, 5, 6 and 7).

TABLE -4: GENOTYPES AND ALLELES TLR-5 RS5744174 IN WOMEN WITH PCOS+TOXOPLASMOSIS AND THOSE WITH PCOS.

Rs5744174	PCOS+Tox (n=25)	PCOS (n=25)	*P*-value	OR(95%CI)
Genotypes				
CC	9(36%)	12(48%)	0.504	1.0
CT	13(52%)	12(48%)	0.262	4.0(0.35-45.1)
TT	3(12%)	1(4%)	0.405	2.77(0.25-30.38)
HWE	0.605	0.341		
Dominant model				
CC+CT	22(88%)	24(96%)	0.320	1.0
TT	3(12%)	1(4%)		3.27(0.32-33.83)
Recessive model				
CC	9(36%)	12(48%)	0.391	1.0
TT+CT	16(64%)	13(52%)		1.64(0.53-5.09)
Alleles				
C	31(62%)	36(72%)	0.289	1.0
T	19(38%)	14(28%)		1.58(0.68-3.65)

TABLE -5: GENOTYPES AND ALLELES TLR-5 RS5744174 IN WOMEN WITH PCOS+TOXOPLASMOSIS AND THOSE WITH TOXOPLASMOSIS.

Rs5744174	PCOS+Tox (n=25)	Toxo (n=25)	*P*-value	OR(95%CI)
Genotypes				
CC	9(36%)	7(28%)	0.536	1.0
CT	13(52%)	12(48%)	0.790	1.19(0.34-4.19)
TT	3(12%)	6(24%)	0.277	2.57(0.47-14.1)
HWE	0.605	0.332		
Dominant model				
CC+CT	22(88%)	19(76%)	0.278	1.0
TT	3(12%)	6(24%)		2.32(0.51-10.54)
Recessive model				
CC	9(36%)	7(28%)	0.545	1.0
TT+CT	16(64%)	18(72%)		1.45(0.44-4.78)
Alleles				
C	31(62%)	26(52%)	0.313	1.0
T	19(38%)	24(48%)		1.51(0.68-3.34)

TABLE -6: GENOTYPES AND ALLELES TLR-5 RS5744174 IN WOMEN WITH PCOS+TOXOPLASMOSIS AND CONTROLS.

Rs5744174	PCOS+Tox (n=25)	Controls (n=75)	*P*-value	OR(95%CI)
Genotypes				
CC	9(36%)	33(44%)	0.493	1.0
CT	13(52%)	38(50.67%)	0.235	2.75(0.52-14.58)
TT	3(12%)	4(5.33%)	0.343	2.19(0.43-11.12)
HWE	0.605	0.097		
Dominant model				
CC+CT	22(88%)	71(94.67%)	0.270	1.0
TT	3(12%)	4(5.33%)		2.42(0.51-11.65)
Recessive model				
CC	9(36%)	33(44%)	0.484	1.0
TT+CT	16(64%)	42(56%)		1.4(0.55-3.56)
Alleles				
C	31(62%)	104(69.33%)	0.339	1.0
T	19(38%)	46(30.67%)		1.39(0.71-2.7)

TABLE -7: GENOTYPES AND ALLELES TLR-5 RS5744174 IN WOMEN WITH PCOS AND CONTROLS.

Rs5744174	PCOS (n=25)	Controls (n=75)	*P*-value	OR(95%CI)
Genotypes				
CC	12(48%)	33(44%)	0.923	1.0
CT	12(48%)	38(50.67%)	0.765	1.15(0.46-2.91)
TT	1(4%)	4(5.33%)	0.748	1.45(0.15-14.34)
HWE	0.341	0.097		
Dominant model				
CC+CT	24(96%)	71(94.67%)	0.792	1.0
TT	1(4%)	4(5.33%)		1.35(0.14-12.7)
Recessive model				
CC	12(48%)	33(44%)	0.728	1.0
TT+CT	13(52%)	42(56%)		1.17(0.47-2.91)
Alleles				
C	36(72%)	104(69.33%)	0.722	1.0
T	14(28%)	46(30.67%)		1.14(0.56-2.31)

DOI: http://doi.org/10.24086/biohs2024/paper.1256

However, women with toxoplasmosis demonstrated higher frequency of TT genotype (24%) than those with PCOS (4%) with a significant difference (OR= 10.3, 95%CI= 1.02-103.9, p= 0.048). At allelic level, the T allele was more common among women with toxoplasmosis than those with PCOS (48% vs. 28%) with a significant difference (OR= 2.37, 95%CI= 1.03- 5.44, p=0.041) as shown in table-8.

TABLE -8: GENOTYPES AND ALLELES TLR-5 RS5744174 IN WOMEN WITH TOXOPLASMOSIS AND WITH PCOS.

Rs5744174	PCOS (n=25)	Toxo (n=25)	*P*-value	OR(95%CI)
Genotypes				
CC	12(48%)	7(28%)	0.137	1.0
CT	12(48%)	12(48%)	0.390	1.71(0.50-5.86)
TT	1(4%)	6(24%)	**0.048**	**10.3(1.02-103.9)**
HWE	0.341	0.332		
Dominant model				
CC+CT	24(96%)	19(76%)	0.071	1.0
TT	1(4%)	6(24%)		7.58(0.84-68.46)
Recessive model				
CC	12(48%)	7(28%)	0.251	1.0
TT+CT	13(52%)	18(72%)		1.96(0.62-6.19)
Alleles				
C	36(72%)	26(52%)	**0.041**	**1.0**
T	14(28%)	24(48%)		**2.37(1.03-5.44)**

Finally, women with toxoplasmosis demonstrated higher frequency of TT genotypes than controls (24% vs 5.33% with a significant difference (Or= 4.75, 95%CI= 1.15-19.69, p =0.032). This polymorphism seems to act in dominant model as the frequency of CC+CT genotype was more common among women with toxoplasmosis than controls with a significant difference (OR= 5.6, 95%CI= 1.43-22, p= 0.013). At allelic level, the T allele was more common among women with toxoplasmosis than controls (48% vs. 30.67%) with a significant difference (OR= 2.09, 95%CI= 1.08- 4.02, p=0.028) as shown in table-9.

TABLE -9: GENOTYPES AND ALLELES TLR-5 RS5744174 IN WOMEN WITH TOXOPLASMOSIS AND CONTROLS.

Rs5744174	Toxo (n=25)	Controls (n=75)	*P*-value	OR(95%CI)
Genotypes				
CC	7(28%)	33(44%)	**0.036**	**1.0**
CT	12(48%)	38(50.67%)	**0.011**	**7.07(1.57-31.86)**
TT	6(24%)	4(5.33%)	**0.032**	**4.75(1.15-19.69)**
HWE	0.332	0.097		
Dominant model				
CC+CT	19(76%)	71(94.67%)	**0.013**	**1.0**
TT	6(24%)	4(5.33%)		**5.61(1.43-22.0)**
Recessive model				
CC	7(28%)	33(44%)	0.162	1.0
TT+CT	18(72%)	42(56%)		2.02(0.75-5.41)
Alleles				
C	26(52%)	104(69.33%)	**0.028**	**1.0**
T	24(48%)	46(30.67%)		**2.09(1.08-4.02)**

IV. DISCUSSION

The current study showed a significant association between the TLR-5 gene polymorphism rs5744168 and women with PCOS+toxoplasmosis/or toxoplasmosis only that displayed higher frequency of AG genotype than controls (24% vs. 5.33%) (OR= 5.61, 95%CI= 1.43-21.9, p =0.013). Moreover, allele A was more common in patients with PCOS+toxoplasmosis/or toxoplasmosis only (12%) than controls (2.67%) with a significant difference (OR= 5.0, 95%CI= 1.34-18.43, p =0.016).

These results disagree with Al-Baldawy AN et al 2022 who revealed that there is no significant association between the TLR -5 gene polymorphism rs5744168 with the susceptibility to toxoplasmosis [16]. As a novel outcomes of studied SNP there were no previous similar studies to compare the current our results with PCOS patients.

TLR-5 signaling is inhibited when the SNP rs5744168 encodes a stop codon at codon 392 (TLR5r392x) of the TLR-5, resulting in truncation of the TLR5 transmembrane signaling domain. Because TLR5 is normally a homodimer, the TLR5r392x variation may also inhibit TLR5 from assembling and localizing, limiting immunological responses [17].

On the other hand, SNP rs5744174 in women with toxoplasmosis demonstrated higher frequency of TT genotype (24%) on both those with PCOS (4%) and controls (5.33%) with a significant difference (OR= 10.3, 95%CI= 1.02-103.9, p= 0.048) and (Or= 4.75, 95%CI= 1.15-19.69, p =0.032) respectively. Regarding to this SNP it was the first study in Iraq and globally that investigate the correlation of this SNP (rs5744174) with both toxoplasmosis and PCOS.

The TLR5 rs5744174 locus is situated within the coding region of the gene, causing a Non-synonymous missense mutation. This mutation replaces phenylalanine at the 616th position of the TLR5 protein with leucine. Polymorphisms within the genes encoding Toll-like receptors (TLRs) can have diverse impacts, including influencing gene expression levels, modifying binding affinities, or altering downstream signaling pathways. Consequently, these alterations in the primary defense mechanisms can result in diminished immune responses and potentially elevate vulnerability to infections [18].

V. CONCLUSION

It was concluded no significant correlation between the heterozygous genotype (AG) and mutant allele (allele A) of TLR-5 gene polymorphism rs5744168 and susceptibility to PCOS in both two disease groups (women with PCOS+ toxoplasmosis and PCOS only). While there was a significant correlation between the heterozygous genotype (AG) and mutant allele (allele A) of TLR-5 gene polymorphism rs5744168 and susceptibility to Toxoplasmosis. Furthermore, there was no significant correlation between the heterozygous genotype (TT) and mutant allele (allele T) of TLR-5 gene polymorphism rs5744174 and susceptibility to PCOS in both two disease groups (women with PCOS+ toxoplasmosis and PCOS only). Finally, there was a significant correlation between the heterozygous genotype (TT) and mutant allele (allele T) of TLR-5 gene polymorphism rs5744174 and susceptibility to Toxoplasmosis.

VI. REFERENCES

1. Dardé ML, Mercier A, Su C, Khan A, Grigg ME. Molecular epidemiology and population structure of Toxoplasma gondii. InToxoplasma gondii 2020 Jan 1 (pp. 63-116). Academic Press.
2. Deng H, Cummins R, Schares G, Trevisan C, Enemark H, Waap H, Srbljanovic J, Djurkovic-Djakovic O, Pires SM, van der Giessen JW, Opsteegh M. Mathematical modelling of Toxoplasma gondii transmission: A systematic review. Food and Waterborne Parasitology. 2021 Mar

DOI: http://doi.org/10.24086/biohs2024/paper.1256

1;22:e00102References should be double-spaced and have a hanging indent: The first line of a reference is set flush, and subsequent lines are indented witn 0.25".
3. Hillion S, Arleevskaya MI, Blanco P, Bordron A, Brooks WH, Cesbron JY, Kaveri S, Vivier E, Renaudineau Y. The innate part of the adaptive immune system. Clinical reviews in allergy & immunology. 2020 Apr;58:151-4.
4. Duan T, Du Y, Xing C, Wang HY, Wang RF. Toll-like receptor signaling and its role in cell-mediated immunity. Frontiers in Immunology. 2022 Mar 3;13:812774.
5. Deng H, Cummins R, Schares G, Trevisan C, Enemark H, Waap H, Srbljanovic J, Djurkovic-Djakovic O, Pires SM, van der Giessen JW, Opsteegh M. Mathematical modelling of Toxoplasma gondii transmission: A systematic review. Food and Waterborne Parasitology. 2021 Mar 1;22:e00102.
6. Hamie M, Najm R, Deleuze-Masquefa C, Bonnet PA, Dubremetz JF, El Sabban M, El Hajj H. Imiquimod targets toxoplasmosis through modulating host toll-like receptor-MyD88 signaling. Frontiers in Immunology. 2021 Mar 9;12:629917.
7. Fitzgerald KA, Kagan JC. Toll-like receptors and the control of immunity. Cell. 2020 Mar 19;180(6):1044-66.
8. Pazoki H, Mirjalali H, Niyyati M, Tabaei SJ, Mosaffa N, Shahrokh S, Ahdaei HA, Kupz A, Zali MR. Toxoplasma gondii profilin induces NLRP3 activation and IL-1β production/secretion in THP-1 cells. Microbial Pathogenesis. 2023 Jul 1;180:106120.
9. Sameer AS, Nissar S. Toll-Like Receptors (TLRs): Structure, functions, signaling, and role of their polymorphisms in colorectal cancer susceptibility. Biomed Res Int. 2021; 2021: 1157023. doi:10.1155/2021/1157023.
10. Liao X, Xiao Y, Elbelt U, Weylandt KH, Li K, Deng J, Zeng N, Xue C. Association of interleukin-1 beta and interleukin-1 receptor antagonist gene polymorphisms and plasma levels with diabetic nephropathy. BioMed Research International. 2022 May 25;2022.
11. Alkhuriji AF, Al Omar SY, Babay ZA, El-Khadragy MF, Mansour LA, Alharbi WG, Khalil MI. Association of IL-1β, IL-6, TNF-α, and TGFβ1 gene polymorphisms with recurrent spontaneous abortion in polycystic ovary syndrome. Disease Markers. 2020 May 5;2020.
12. Ullah Khan , F. ., & Hussain , N. . (2020). NH Serological and Molecular Based Diagnosis of Toxoplasma gondii in Galliformes by using ToxPK1 gene. Journal of Scientific Research in Medical and Biological Sciences, 1(2), 116-122. https://doi.org/10.47631/jsrmbs.v1i2.58.
13. Zangeneh, F.Z., Naghizadeh, M.M., Masoumi, M. (2017). Polycystic ovary syndrome and circulating inflammatory markers. International Journal of Reproductive BioMedicine, 15(6): 375-382. doi: 10.29252/ijrm.15.6.375.
14. Mason HD, Dilaver N, Rice S. Ovarian Dysfunction in Polycystic Ovary Syndrome (PCOS). InPolycystic Ovary Syndrome: Current and Emerging Concepts 2022 Apr 14 (pp. 95-120). Cham: Springer International Publishing.
15. Chaudhary H, Patel J, Jain NK, Joshi R. The role of polymorphism in various potential genes on polycystic ovary syndrome susceptibility and pathogenesis. Journal of ovarian research. 2021 Dec;14:1-21.
16. Al-Baldawy AN, Al-Marsomy HD, Kahleel II. The Correlation Between Toll-Like Receptor-5 Gene Polymorphism with Serum Level of Toll–Like Receptor-5 and Interleukin-6 and Interleukin-12 Response to Toxoplasma Gondii in Pregnant Women. Pakistan Journal of Medical & Health Sciences. 2022 Aug 23;16(07):367-.
17. Chaichana, P., Chantratita, N., Brod, F., Koosakulnirand, S., Jenjaroen, K., Chumseng, S., et al. "A nonsense mutation in TLR5 is associated with survival and reduced IL-10 and TNF-α levels in human melioidosis." PLoS neglected tropical diseases 11.5 (2017): e0005587.
18. Törmänen S, Teräsjärvi J, Lauhkonen E, Helminen M, Koponen P, Korppi M, Nuolivirta K, He Q. TLR5 rs5744174 gene polymorphism is associated with the virus etiology of infant bronchiolitis but not with post-bronchiolitis asthma. Health Science Reports. 2018 Jun;1(6):e38.

DOI: http://doi.org/10.24086/biohs2024/paper.1256