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Original paper

Association between genetic polymorphism of IL-27 (rs153109) and toxoplasmosis in Iraqi women with recurrent abortion

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ABSTRACT. Single nucleotide polymorphisms (SNPs) are predictive markers for diseases, also cytokines are undergoing genetic controls and their genetic polymorphisms have a functional role in regulating the levels of cytokine gene expression. This study aims to reveal the association of toxoplasmosis with serum levels and SNP of IL-27 in aborted women. Total, 200 blood samples of patients and controls were collected from Al-Alawiya Maternity Teaching Hospital/Baghdad/Iraq from 2019–2020 for detecting the level of IL-27 by ELISA while the allelic discrimination method was used for SNP IL-27 (rs153109). The results indicated the IL-27 serum concentration elevated with significant differences in recurrent abortion with toxoplasmosis group compared to healthy women, pregnant women, and recurrent abortion. Also, recurrent abortion had significant differences compared to healthy women and pregnant women ($P<0.05$). Moreover, SNP results of IL-27 showed no significant association between patients and controls. Considering the distribution of serum levels for IL-27 by SNP, it was observed that IL-27 serum levels for TT, TC, and CC genotypes elevated in the patient group versus the control group. In addition, it was observed elevation serum level of IL-27 for the genotypes TT, TC, and CC in recurrent abortion with toxoplasmosis in contrast to healthy women, pregnant women, and recurrent abortion ($P<0.05$). Also, in recurrent abortion, the level of IL-27 for TC, and CC genotype showed significant differences comparing to healthy and pregnant women ($P<0.05$). In conclusion, the level of IL-27 in recurrent abortion women with toxoplasmosis was higher than the recurrent abortion women, which may be due to the inflammatory response to toxoplasmosis. SNP of IL-27 has not represented as a risk factor in recurrent abortion women with toxoplasmosis.

Keywords: toxoplasmosis, women with recurrent abortion, IL-27 (rs153109), single nucleotide polymorphism

Introduction

Cytokines are a group of immunomodulatory proteins leading a variety of immune reactions in the human body, which play a significant role in the development of appropriate immune responses against *Toxoplasma gondii* [1]. The majority of women infected with toxoplasmosis have asymptomatic; primary infection during pregnancy can result in disease transmission through the placenta and lead to hazardous consequences such as abortion, stillbirth, mental or physical retardation, hydrocephalus, and blindness [2]. Strikingly, cytokines are among a large number of miscarriage-related factors, those involved in abnormal immune reactions [3].

IL-27 is secreted by antigen-presenting cells and has been shown to regulate inflammation during pregnancy to control the intensity and duration of immune responses and to induce Th1 differentiation of CD4+ T cells, while attenuating proinflammatory cytokine production through STAT (Signal transducer and activator of transcription) transcription factors [4]. Further, signaling of IL-27 enhances a subset of T regulatory cells that restrict intestinal pathology and ameliorate host survival in *T. gondii* infection [5]. With consideration, the cytokines are undergoing genetic controls, and

recent studies suggest that genetic polymorphisms of cytokines have a functional role in regulating the levels of cytokine gene expression and their receptor [6]. Moreover, single nucleotide polymorphisms (SNPs) are predictive markers in diseases like a differential response to therapy [7]. Recently, it has been notified that mutations of IL-27 were shown to be susceptible to a diversity of diseases, such as asthma, colorectal cancer, rheumatoid arthritis, ovarian cancer, Crohn's disease, esophageal cancer, and nasopharyngeal carcinoma [8–10].

This study aimed to investigate the serum levels of IL-27 and its polymorphism in spontaneous abortion women with toxoplasmosis.

Materials and Methods

The samples collection and study groups design

The women's blood of 200 samples were collected from November 2019 until March 2020 from Al-Alawiya Maternity Teaching Hospital/ Baghdad/Iraq. The study groups were designed into two main groups, 100 samples of patients and 100 samples of controls for Iraqi women; these main groups divided into four subgroups. The control were divided to 50 healthy women (non-pregnant) and 50 healthy pregnant women. While the patient groups were divided into 50 recurrent abortion women without toxoplasmosis and 50 recurrent abortion women with toxoplasmosis (which diagnosed previously with anti-*Toxoplasma* IgG and IgM). According to a pre-prepared questionnaire, information was collected about control and patients group. The inclusion criteria were a woman age (more and and equal 35 and less than 35), number of miscarriages, number of abortion, children number. The exclusion criteria were autoimmune dysfunction, genetic anomalies, inflammatory disease, and other systemic disorders.

Detection of IL-27 serum level

IL-27 was detected in human serum depending the manufacturer instructions of kit (Abcam, USA, catalog number: ab83695).

Genetic analysis

Frozen whole blood in EDTA tube was utilized for extraction DNA by using Quick-gDNA™ MiniPrep Zymo kit. The allelic specific discrimination of SNP IL-127 (rs153109) was conducted via the use of TaqMan custom SNP genotyping assay from Thermo Fisher Scientific Company, USA (Catalog No. 4351379; Assay ID: C_176104812_10). In addition, TaqMan master mix used from Kapa Biosystems Company, USA (Catalog No: KK4702). SNP estimated by using Real-Time PCR System (Real-time thermal cycler Sa Cycler-96, Sacace Bio technologies, Italy).

Statistical analysis

The outcomes conducted with Graph Pad Prism version 8.0.1 for Windows (GraphPad Software, San Diego, California, USA). The concentration of IL-27 was presented as mean ± standard deviation and significant differences were analyzed by one-way ANOVA analysis. The genotype and alleles frequencies for SNPs were calculated directly counting method. Hardy-Weinberg equilibrium (HWE) for SNP was investigated via the use of the online calculator of Michael H. Court (2005–2008). Where if P-value more than 0.05, the population consistent with HWE. Whereas the odds ratio (OR) was estimated for evaluating the risk related to genotypes and alleles; it is calculated by chi-square, and Fischer's exact probability via utilizing the statistical software epidemiological (WINPEPI) version 11.65. Also, P-values are statistically significant when less than (0.05).

Ethical approvals

The assent was obtained from the participants, and also the study was consented by ethical committees of Biology Department/College of Science/University of Baghdad and was approved for collection of blood by the ethics committee of the Iraqi Ministry of Health and Environment.

Results

Serum level of IL-27 in the studied groups

The results pointed to significant differences in recurrent abortion with toxoplasmosis group comparing with healthy women, pregnant women, and recurrent abortion with probability ($P<0.05$). Additionally, recurrent abortion had significant differences when compared to healthy women, and pregnant women $P<0.05$) as shown in table 1.

SNPs of IL-27 in the studied groups

The results of the genotypic frequencies of IL-27 are demonstrated in Tab. 2. There was appeared genotypic distributions of IL-27 (rs153109T/C) accommodated to Hardy-Weinberg equilibrium in healthy women, pregnant women, recurrent

Table 1. Comparison serum level of IL-27 among the studied groups (mean±SD)

Groups	Concentration of IL-27	P-value with healthy women	P-value with pregnant women	P-value with recurrent abortion
Recurrent abortion with toxoplasmosis	243.1±27.73	<0.0001	<0.0001	0.0206
Recurrent abortion	215.1±36.52	<0.0001	0.0182	–
Pregnant women	186.5±10.08	0.1219NS	–	–
Healthy women	165.4±15.53	–	–	–

Explanations: P-value <0.05 mean significant; NS: non-significant

Table 2. The percentage frequencies of genotype and Hardy-Weinberg Equilibrium (HWE) of IL-27 (rs153109) in the studied groups

Groups			Genotype frequency			
			TT	TC	CC	HWE (P≥0.05)
Controls (N=100)	Observed	N	45	45	10	0.8
		%	45	45	10	
	Expected	N	45.6	43.9	10.6	
		%	45.6	43.9	10.6	
Healthy women (N=50)	Observed	N	18	26	6	0.464
		%	36	52	12	
	Expected	N	19.2	23.6	7.2	
		%	38.4	47.2	14.4	
Pregnant women (N=50)	Observed	N	27	19	4	0.799
		%	54	38	8	
	Expected	N	26.6	19.7	3.6	
		%	53.2	39.4	7.2	
Patients (N=100)	Observed	N	49	39	12	0.34
		%	49	39	12	
	Expected	N	46.9	43.2	9.9	
		%	46.9	43.2	9.9	
Recurrent abortion (N=50)	Observed	N	26	19	5	0.585
		%	52	38	10	
	Expected	N	25.2	20.6	4.2	
		%	50.4	41.2	8.4	
Recurrent abortion with toxoplasmosis (N=50)	Observed	N	23	20	7	0.442
		%	46	40	14	
	Expected	N	21.8	22.4	5.8	
		%	43.6	44.8	11.6	

abortion and recurrent abortion women with toxoplasmosis. The results showed that genetic models appeared no significant association between patients and controls (Tab. 3). Furthermore, the polymorphic genotypes TT, TC, CC have been shown no significant difference in recurrent abortion and recurrent abortion with toxoplasmosis comparing to healthy women and pregnant women as shown in the tables 4–8. In regard to the distribution of genotypes polymorphism for IL-27 by maternal age (Tab. 9), there were no significant differences between the genotypes and maternal age progressed and also no appeared relevant with the risk of progressed age.

Table 3. The genetic model of the association for alleles and genotypes of IL-27(rs153109) in co-dominant, dominant, recessive, and overdominant models

Genetic model	Genotype and allele	Patients (N=100)	Control (N=100)	OR (CI: 95%)	P-value
Codominant	TT ref	49(49%)	45(45%)	1(0.57–1.77)	1
	TC	39(39%)	45(45%)	0.8(0.44–1.43)	0.5
	CC	12(12%)	10(10%)	1.1(0.44–2.78)	1
Dominant	TT ref	49(49%)	45(45%)	1(0.57–1.77)	1
	TC/CC	51(52%)	55(55%)	0.85(0.49–1.48)	0.7
Recessive	TC/TT ref	88(88%)	90(90%)	1(0.66–1.51)	1
	CC	12(12%)	10(10%)	1.23(0.51–2.97)	0.8
Overdominant	TT/CC ref	61(61%)	55(55%)	1(0.60–1.67)	1
	TC	39(39%)	45(45%)	0.78(0.45–1.37)	0.5
Allele	T ref	137(68.5%)	135(67.5%)	1.1(0.71–1.40)	1
	C	63(31.5%)	65(32.5%)	0.96(0.63–1.45)	0.9

Explanations: OR: Odds ratio; CI: Confidence interval; P-value <0.05

Table 4. Comparison of the genotype and allele frequency of IL-27 (rs153109) between healthy women and recurrent abortion with toxoplasmosis

Genotype	Recurrent abortion with toxoplasmosis	Healthy women	P-value	OR (CI: 95%)
TT	23(46%)	18(36%)	0.416	1.5(0.68–3.35)
TC	20(40%)	26(52%)	0.316	0.62(0.28–1.35)
CC	7(14%)	6(12%)	1	1.19(0.38–3.80)
Total	50	50		
Allele	Recurrent abortion with toxoplasmosis	Healthy women	P-value	OR (CI: 95%)
T	66(66%)	62(62%)	0.659	1.19(0.67–2.11)
C	34(34%)	38(38%)	0.659	0.84(0.47–1.49)
Total	100	100		

Explanations: OR: Odds ratio; CI: Confidence interval; P-value <0.05

Table 5. Comparison of the genotype and allele frequency of IL-27 (rs153109) between healthy women and recurrent abortion

Genotype	Recurrent abortion	Healthy women	P-value	OR (CI: 95%)
TT	26(52%)	18(36%)	0.158	1.9(0.87–4.26)
TC	19(38%)	26(52%)	0.228	0.57(0.26–1.24)
CC	5(10%)	6(12%)	1	0.81(0.23–2.83)
Total	50	50		
Allele	Recurrent abortion	Healthy women	P-value	OR (CI: 95%)
T	71(71%)	62(62%)	0.231	1.5(0.83–2.70)
C	29(29%)	38(38%)	0.231	0.67(0.37–1.20)
Total	100	100		

Explanations: OR: Odds ratio; CI: Confidence interval; P-value <0.05

Table 6. Comparison of the genotype and allele frequency of IL-27 (rs 153109) between healthy women and pregnant women

Genotype	Pregnant women	Healthy women	P-value	OR (CI: 95%)
TT	27(54%)	18(36%)	0.107	2.09(0.94–4.62)
TC	19(38%)	26(52%)	0.228	0.57(0.26–1.24)
CC	4(8%)	6(12%)	0.741	0.64(0.17–2.38)
Total	50	50		
Allele	Pregnant women	Healthy women	P-value	OR (CI: 95%)
T	73(73%)	62(62%)	0.131	1.66(0.91–3.01)
C	27(27%)	38(38%)	0.131	0.6(0.33–1.09)
Total	100	100		

Explanations: OR: Odds ratio; CI: Confidence interval; P-value <0.05

Table 7. Comparison of the genotype and allele frequency of IL-27 (rs153109) between pregnant women and recurrent abortion with toxoplasmosis

Genotype	Recurrent abortion with toxoplasmosis	Pregnant women	P-value	OR (CI: 95%)
TT	23(46%)	27(54%)	0.549	0.73(0.33–1.58)
TC	20(40%)	19(38%)	1	1.09(0.49–2.41)
CC	7(14%)	4(8%)	0.525	1.87(0.52–6.76)
Total	50	50		
Allele	Recurrent abortion with toxoplasmosis	Pregnant women	P-value	OR (CI: 95%)
T	66(66%)	73(73%)	0.357	0.72(0.39–1.31)
C	34(34%)	27(27%)	0.357	1.39(0.76–2.54)
Total	100	100		

Table 8. Comparison of the genotype and allele frequency of IL-27 (rs153109) between pregnant women and recurrent abortion

Genotype	Recurrent abortion	Pregnant women	P-value	OR (CI: 95%)
TT	26(52%)	27(54%)	1	0.92(0.42–2.01)
TC	19(38%)	19(38%)	1	1(0.45–2.22)
CC	5(10%)	4(8%)	1	1.28(0.33–5.00)
Total	50	50		
Allele	Recurrent abortion	Pregnant women	P-value	OR (CI: 95%)
T	71(71%)	73(73%)	0.875	0.91(0.49–1.67)
C	29(29%)	27(27%)	0.875	1.10(0.60–2.04)
Total	100	100		

The association between serum levels for IL-27 and SNP (rs153109)

The results demonstrated the distribution of serum levels for IL-27 by SNP (rs153109) as shown in table 10. The genotypes TT, TC, and CC in the patient groups had significant differences with high serum level for IL-27 comparing to these genotypes in the control groups. Whilst there were no differences between genotypes within each group. Moreover, the results in table 11 showed significant differences in the genotypes TT, TC, and CC where

Table 9. Distribution of genotypes of SNP IL-27 (rs153109) by maternal age

	Groups	Genotype			
		TT	CT	CC	Total
Age ≤35	Patient	45	31	11	87
	Control	42	38	9	89
	OR(CI:95)	1.2(0.67–2.16)	0.7(0.41–1.36)	1.4(0.54–3.5)	
	P-value	0.651	0.385	0.635	
Age >35	Groups		Genotype		
		CC	CT	TT	Total
	Patient	5	7	1	13
	Control	4	5	2	11
	OR(CI:95)	1.09(0.22–5.4)	1.4(0.30–6.54)	0.38(0.03–4.3)	
	P-value	1	1	0.576	
Overall	Groups		Genotype		
		CC	CT	TT	Total
	Patient	50	38	12	100
	Control	46	43	11	100
	OR(CI:95)	1.2(0.68–2.04)	0.8(0.46 –1.43)	1.1(0.46– 2.6)	
	P-value	0.671	0.565	1	

OR: Odds ratio; CI: Confidence interval; P value <0.05

Table 10. Distributions of serum level for IL-27 in patients and controls by SNPs genotypes

Groups	SNP genotypes		
	TT	TC	CC
Controls	181.8±15.28 A	171.1±18.77 A	175.2±15.51 A
Patients	230.1±27.82 B	230.6±25.53 B	225.6±22.19 B

Explanations: means with different letter within each column are significantly different (P<0.05); means with similar letter within each row are non-significantly different (P>0.05)

Table 11. Distributions of serum level for IL-27 in healthy women, pregnant women, recurrent abortion and recurrent abortion toxoplasmosis by SNPs genotypes

Groups	SNP genotypes		
	TT	TC	CC
Healthy women	170.9±14.05Aa	156.8±16.28Aa	168.6±17.78Aa
Pregnant women	192.6±5.731Aad	185.4±4.658Ab	181.8±10.05Aa
Recurrent abortion	213.1±12.83Abd	218.6±20.01Ac	212.1±4.734Ab
Recurrent abortion with toxoplasmosis	247.0±28.94Ac	242.6±25.84Ad	239.0±24.90Ac

Explanations: mean with different capital letter within each row and different small letter within each column are significantly different (P<0.05); means with similar capital letter within each row and similar small letter within each column are non-significantly different (P>0.05)

it was observed elevation serum level of IL-27 in recurrent abortion with toxoplasmosis in contrast to healthy women, pregnant women, and recurrent abortion. Also, in recurrent abortion the level of IL-27 for TC, and CC genotype showed significant differences comparing to healthy and pregnant women whereas genotype TT in recurrent abortion had no significant differences comparing to pregnant women. Besides, pregnant women have shown a significant difference in the genotype TC comparing to healthy women.

Discussion

One of the studies suggested an elevated level of IL-27 in usual miscarriage women in comparison to healthy pregnant with significant differences ($P<0.05$), therefore, it was observed the synchronic raise in the level of anti-inflammatory and pro-inflammatory cytokines. This can be interpreted through a raise in immune response and stimulation of fetal defense mechanism. Imbalance of cytokines leads to developing the loss of actual pregnancy in women with usual miscarriage [18]. Consequently, the results of serum level for IL-27 in this study have agreed with another study in the elevated level of IL-27 in aborted women infected with toxoplasmosis compared with recurrent abortion women which are infected with another microorganism with a P-value of 0.016 [19].

Dogruman-Al et al. [20] demonstrated that *Toxoplasma* infection relies on the balance of signals between pro-inflammatory cytokines (IL-12, IFN-γ, TNF-α), and anti-inflammatory cytokines (IL-10, lipoxin A4, IL-27) that repress the proliferation of parasite and the control of an inflammatory response. In addition, it has been observed that the levels of IL-27 are elevated through the interactions of the host cell with a virus, bacteria, and parasites [21]. Likewise, the signaling of IL-27 inhibits the neurological harm development during chronic toxoplasmosis [22]. On the other hand, another study indicated an increase in the level of IL-27 in healthy pregnant and women with preeclampsia comparing to healthy women (non-pregnant), the raise was significantly different in preeclampsia cases (P-value <0.05) [23]. As well as, studies showed that a pregnancy's success is reliant on immune balance, involving the immune response, immune tolerance, and proportional cytokine levels [24].

Through the results of this study, it was found that they agreed with some studies and contradicted other studies, and this may be due to ethnic differences and the hereditary predisposition to some diseases. One of the studies performed on the aborted Iraqi women showing there are significant differences in the association between SNP of IL-27 (−964A>G) and recurrent abortion [11]. While another study indicated that no significant differences between genetic polymorphisms of IL-27 (−964A>G) and recurrent miscarriage in Iranian women [12].

Recent studies of genetic correlations have evidenced that polymorphism of IL-27 (rs153109) influences the expression of mRNA and affects patients susceptibility to different inflammation associated of diseases [13–16]. Liu et al. [17] suggested that CC genotype of rs153109 was a risk factor in preeclampsia cases (OR=1.54), whilst CT genotype was a protective factor in preeclampsia cases (OR=0.74).

Nevertheless, it appears in other studies for SNP rs153109 of IL-27 and another SNP rs17855750 that the rs17855750 was related to IL-27 levels in bladder carcinoma. The genotype GG recorded a minimum level for IL-27 than the detecting threshold. While the genotype of the IL-27 (rs153109) not recorded significant differences with plasma levels of IL-27 [25]. Where the SNP (rs17855705) has located in the region of missense codon, which changes the allele T to G lead to the alteration of the amino acid (serine to alanine), whereas SNP (rs153109) in the IL-27 gene has existed in the promoter region. The promoter region has a critical role in regulating the process of transcription and the expression of a protein [26]. In addition, it has been observed no significant relevance between mRNA expression of IL-27 and genotype of SNPs (rs153109 and rs17855750) in carcinoma of epithelial ovarian for the Chinese population, also the diminished mRNA expression of IL-27 may point to the antitumor efficacy for this cytokine [10].

It was noted that the level of IL-27 in recurrent abortion women with toxoplasmosis was higher than the recurrent abortion women, which may be due to the inflammatory response to toxoplasmosis. The SNP of IL-27 has not appeared as a risk factor in recurrent abortion women with toxoplasmosis despite appeared significant differences in the concentration of IL-27 in the genotypes. Although, serum level of IL-27 elevated in recurrent abortion with toxoplasmosis in contrast to healthy women, pregnant women, and recurrent abortion in the genotypes TT, TC, and CC.

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References

[1] Abdollahi S.H., Ayoobi F., Khorramdelazad H., Hassanshahi G., Ahmadabadi B.N., Rezayati M., Arababadi M.K. 2013. Interleukin-10 serum levels after vaccination with *in vivo* prepared *Toxoplasma gondii* excreted/secreted antigens. *Oman Medical Journal* 28: 112.

[2] Saki J., Mohammadpour N., Moramezi F., Khademvatan S. 2015. Seroprevalence of *Toxoplasma gondii* in women who have aborted in comparison with the women with normal delivery in Ahvaz, southwest of Iran. *The Scientific World Journal* 2015: 764369. doi:10.1155/2015/764369

[3] Zhang M., Xu J., Bao X., Niu W., Wang L., Du L., Sun Y. 2017. Association between genetic polymorphisms in interleukin genes and recurrent pregnancy loss – a systematic review and meta-analysis. *PLoS ONE* 12: e0169891. doi:10.1371/journal.pone.0169891

[4] Enninga E.A.L., Nevala W.K., Creedon D.J., Markovic S.N., Holtan S.G. 2015. Fetal sex-based differences in maternal hormones, angiogenic factors, and immune mediators during pregnancy and the postpartum period. *American Journal of Reproductive Immunology* 73: 251-262. doi:10.1111/aji.12303

[5] Hall A.O., Beiting D.P., Tato C., John B., Oldenhove G., Lombana C.G., Hunter C.A. 2012. The cytokines interleukin 27 and interferon-γ promote distinct Treg cell populations required to limit infection-induced pathology. *Immunity* 37: 511-523. doi:10.1016/j.immuni.2012.06.014

[6] Kaur G., Mehra N. 2012. Cytokine gene polymorphisms: methods of detection and biological significance. In: Immunogenetics: methods and applications *in clinical practice.* (Eds. F.T. Christiansen, B.D. Tait). Totowa, NJ: Humana Press: 549-568.

[7] Stavric K., Peova S., Trajkov D., Spiroski M. 2012. Gene polymorphisms of 22 cytokines in Macedonian children with atopic dermatitis. *Iranian Journal of Allergy, Asthma and Immunology* 11: 37-50.

[8] Huang Z.Q., Wang J.L., Pan G.G., Wei Y.S. 2012. Association of single nucleotide polymorphisms in IL-12 and IL-27 genes with colorectal cancer risk. *Clinical Biochemistry* 45: 54-59. doi:10.1016/j.clinbiochem.2011.10.004

[9] Tao Y.P., Wang W.L., Li S.Y., Zhang J., Shi Q.Z., Zhao F., Zhao B.S. 2012. Associations between polymorphisms in IL-12A, IL-12B, IL-12Rβ1, IL-27 gene and serum levels of IL-12p40, IL-27p28 with esophageal cancer. *Journal of Cancer Research and Clinical Oncology* 138: 1891-1900. doi:10.1007/s00432-012-1269-0

[10] Zhang Z., Zhou B., Wu Y., Gao Q., Zhang K., Song, Y., Xi M. 2014. Prognostic value of IL-27 polymorphisms and the susceptibility to epithelial ovarian cancer in a Chinese population. *Immunogenetics* 66: 85-92. doi:10.1007/s00251-013-0753-2

[11] Humadi E.H., Hamad L.H., Al Azzawie H.F., Hamad S.H. 2018. Single-nucleotide polymorphism of interleukin-27 gene: a risk factor of recurrent pregnancy loss in Iraqi women. *Mustansiriya Medical Journal* 17: 47-51.

[12] Nematollahi Z., Hadinedoushan H., Aflatoonian A., Eslami G., Ghasemi N. 2015. The association between single nucleotide polymorphism in interleukin-27 gene and recurrent pregnancy loss in Iranian women. *Iranian Journal of Reproductive Medicine* 13: 209.

[13] Dehghanzadeh R., Babaloo Z., Sakhinia E., Khabazi A., Shanehbandi D., Sadigh-Eteghad S., Gharibi T. 2016. IL-27 gene polymorphisms in Iranian patients with Behcet's disease. *Clinical Laboratory* 62: 855-861. doi:10.7754/Clin.Lab.2015.150843

[14] Paradowska-Gorycka A., Raszkiewicz B., Jurkowska M., Felis-Giemza A., Romanowska-Prochnicka K., Mańczak M., Olesinska M. 2014. Association of single nucleotide polymorphisms in the IL-27 gene with rheumatoid arthritis. *Scandinavian Journal of Immunology* 80: 298-305. doi:10.1111/sji.12209

[15] Yu W., Zhang K., Wang Z., Zhang J., Chen T., Jin L. 2017. Functional variant in the promoter region of IL-27 alters gene transcription and confers a risk for ulcerative colitis in northern Chinese Han. *Human Immunology* 78: 287-293. doi:10.1016/j.humimm.2017.01.002

[16] Zhang D., Ma M., Yang Y., Wan L., Yang Z., Lv J., Li L. 2016. Association between polymorphisms in IL27 and risk for CHD in a Chinese population. *Cardiology in the Young* 26: 237-243. doi:10.1017/S1047951115000037

[17] Liu B., Li Y., Yao Y., Li H., Liang H., Xin M., Liu S. 2016. Polymorphisms of the IL27 gene in a Chinese Han population complicated with pre-eclampsia. *Scientific Reports* 6: 1-6. doi:10.1038/srep23029

[18] Malyshkina A.I., Sotnikova N.Y. 2020. [Peculiarities of the content of peripheral blood cytokines in pregnant women with a habitual miscarriage]. *Klinicheskaia Laboratornaia Diagnostika* 65: 299-303 (in Russian with summary in English). doi:10.18821/0869-2084-2020-65-5-299-303

[19] Kadhim W.H., Tektook N.H., Khalaf A.K. 2020. Study the correlation between cytokines (IL-6, IL-27,

TGF-beta and IFN-gamma) levels and microbial abortion among woman in Thi-Qar Province. *International Journal of Innovations in Biological and Chemical Sciences* 13: 40-48.

[20] Dogruman-Al F., Fidan I., Celebi B., Yesilyurt E., Erdal B., Babur C., Kustimur S. 2011. Cytokine profile in murine toxoplasmosis. *Asian Pacific Journal of Tropical Medicine* 4: 16-19.

[21] Povroznik J.M., Robinson C.M. 2020. IL-27 regulation of innate immunity and control of microbial growth. *Future Science OA* 6: FSO588. doi:10.2144/fsoa-2020-0032

[22] Stumhofer J.S., Laurence A., Wilson E.H., Huang E., Tato C.M., Johnson L.M., Sehy D. 2006. Interleukin 27 negatively regulates the development of interleukin 17–producing T helper cells during chronic inflammation of the central nervous system. *Nature Immunology* 7: 937-945. doi:10.1038/ni1376

[23] Jahantigh D., Mousavi M., Forghani F., Javan M.R., Movahedinia S., Rezaei M. 2018. Association between maternal circulating IL-27 levels and preeclampsia. *Cytokine* 102: 163-167. doi:10.1016/j.cyto.2017.08.012

[24] Kwak-Kim J., Bao S., Lee S.K., Kim J.W., Gilman-Sachs A. 2014. Immunological modes of pregnancy loss: inflammation, immune effectors, and stress. *American Journal of Reproductive Immunology* 72: 129-140. doi:10.1111/aji.12234

[25] Zhou B., Zhang P., Tang T., Liao H., Zhang K., Pu Y., Zhang, L. 2015. Polymorphisms and plasma levels of IL-27: impact on genetic susceptibility and clinical outcome of bladder cancer. *BMC Cancer* 15: 433. doi:10.1186/s12885-015-1459-7

[26] Yu X., Zhang Z., Zhou B., Zhang L., Chen P., Duan R., Xi M. 2016. Genetic polymorphisms of interleukin-27 is associated with endometrial cancer susceptibility in Chinese Han women. *International Journal of Clinical and Experimental Pathology* 9: 2718-2725.

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