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Research Article

Polymorphism of IL-17A related to toxoplasmosis among pregnant and aborted women in Baghdad province

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Abstract

The intracellular parasite *Toxoplasma gondii*, responsible for toxoplasmosis, poses a significant zoonotic threat to both humans and homeothermic animals. Recent findings highlight the pivotal role of T helper 17 (Th17) cells, a subset of CD4+ T cells, in producing the cytokine IL-17A, a key component of the immune response. In this study, 96 blood samples were collected from pregnant and aborted women, with 5 milliliters utilized for Toxoplasma IgG and IgM detection using the VIDAS analyzer. Additionally, 2 milliliters were collected for IL-17A molecular analysis, and the samples were stored at -20°C until required. The investigation uncovered a significant prevalence of the A allele at the homozygous AA genotype in SNP rs2275913 IL-17A. Statistical analysis revealed a noteworthy odds ratio (OR) of 1.7 (0.40-7.3) with a p-value of 0.4 for genotype AA. Notably, allele A occurred at a higher frequency (30%) in the patient cohort with an OR of 1.71 (0.61-4.8) and a p-value of 0.3, contrasting the lower frequency (80%) observed in the control group. In conclusion, the frequency of IL-17A rs2275913 and allele A differed significantly between patients and controls, emphasizing the elevated occurrence of the risk allele A in the patient group.

Keywords: Interleukin-17A, Toxoplasmosis, IgG, IgM

Introduction

The protozoan parasite *Toxoplasma gondii*, responsible for the infection known as toxoplasmosis, targets warm-blooded animals and humans and must live inside cells to survive (Dubey, 2016; Ybañez et al., 2020). This microbe was identified independently in 1908 by Nicolle and Manceaux in Tunisia, as well as by Splendored in Brazil (Ferguson, 2009). The earliest documented incident

of congenital toxoplasmosis dates back to 1923, with Janků providing a comprehensive autopsy analysis of a young boy, aged 11 months, who succumbed to hydrocephalus without recognizing *T. gondii* as the cause (Weiss & Dubey, 2009). Humans contract this parasite primarily by consuming bradyzoite-infested undercooked meats and fish, a common pathway for toxoplasmosis transmission. Additionally, ingestion of oocyst-contaminated vegetables, water, and milk, along with receiving infected blood through transfusion or contaminated organs during transplantation, are recognized as significant transmission vectors of *T. gondii* in humans (Kolören & Dubey, 2020). *T. gondii* is transmitted mostly through the eating of undercooked meat with tissue lesions, as well as through the consumption of tainted vegetables/water containing oocyst (Bouchard et al., 2019). All animals and birds are susceptible to the disease, and fowls are a major source of zoonotic infection (Polley & Thompson, 2009). Globally, zoonotic parasite infections continue to be a major socioeconomic, veterinary, and public health concern (Ben-Harari & Connolly, 2019).

Recently identified as predominantly emanating from a CD4+ T cell subset known as Th17 cells, IL-17A is a cytokine initially discovered through the cloning of the cytotoxic T lymphocyte-associated antigen 8 (Ctla8) gene from a murine CTL hybridoma cDNA library (Wright et al., 2007). The murine version of IL-17A, a 21 kDa glycoprotein consisting of 147 amino acids, displays 63% sequence identity with its human counterpart, which is composed of 155 amino acid. Both the murine and human variants are secreted as disulfide-linked homodimers. Structurally akin to IL-17A, five cytokines—IL-17B, IL-17C, IL-17D, IL-17E (also named IL-25), and IL-17F—have been recently identified, expanding the family of IL-17 (Kolls & Lindén, 2004; Weaver et al., 2007). These members share 16%–50% amino acids individuality with IL-17A; IL-17A and IL-17F exhibit the highest similarity at 50%, while IL-17E shows the least at 16%. The amino acid resemblance is more pronounced in the C-terminal region and across five conserved cysteine residues. Notably, four of these residues contribute to a unique cysteine knot fold that distinguishes it from the typical cysteine knot found in the bone morphogenic protein, TGF-beta, and superfamilies of nerve growth factor, due to a deficit of two cysteine residues (Gerhardt et al., 2009; Hymowitz et al., 2001). The focus of this investigation is to assess the genotyping and allele frequency, evaluating the Odds Ratio (OR) for polymorphisms in the IL-17A gene among pregnant patients and women who have had miscarriages, all of whom were infected with toxoplasmosis, and then comparing these findings with a control group.

Material and methods

Samples Collection

In the Baghdad province, at the Baghdad Hospital for pregnant and aborted women, 96 blood specimens were randomly collected from women who were either pregnant or had experienced abortions. These women were between the ages of 18 and 45 and the collection took place from October to December of 2022. From each subject, five milliliters of blood were extracted and subsequently stored in a frozen state at a temperature of -20°C until they were needed for use. Two distinct kits were utilized in this analysis: the first to identify IgG antibodies to Toxoplasma and the second to detect specific IgM antibodies to Toxoplasma antigens present in the serum of the patient.

Molecular assay, The extraction of DNA from blood

1. Collected 2 ml frozen human blood in an anticoagulant-treat collection tube and waited at room temperature until it thawed and gently mixed.
2. Transferred 300µl of blood to a microcentrifuge tube for each sample .
3. Added 30ul of Proteinase K for each sample and mixed gently .
4. Incubated for 20 minutes at 56 C^o temperature .
5. A 200µl FABG Buffer was added, and the vortex was combined.
6. Incubated at 70 C^o for 15 minutes. Invert the sample every 3 ~ 5 minutes, during incubation .
7. The samples were supplemented with 200µl ethanol (96 ~ 100 percent). Mix vigorously for 10 seconds overtaxing 30 min.
8. Pick up spin column tubes in their collection tubes
9. Transverse all lysate into the spin column of samples for each.
10. Spin the microcentrifuge for one minute discard the filtrate lysate and replace the collection tubes.
11. The FABG Column was rinsed by adding 400µl of W1 Buffer with ethanol, followed by centrifugation for 30 seconds.
12. A subsequent rinse of the FABG Column was completed using 600µl of ethanol-containing Wash Buffer and centrifugation for another 30 seconds.
13. The column was then centrifuged for an additional three minutes to ensure it was thoroughly dried.
14. Each FABG Column then transported into a new 1.5 ml microcentrifuge tube.

15. To the center of the membrane in the FABG Column, 100µl of Preheated Elution Buffer or TE was added, letting the column stand for 3 to 5 minutes or until the membrane fully absorbed the buffer.
16. Centrifuged for thirty seconds to elute the pure DNA.
17. Stored the DNA fragment at $4C^0$ or $-20C^0$ (AL-JUBORY & IMRAN, 2020).

Interpretation of sequencing data

About 18-20 µl of PCR products of all genes under interest in this study specimens were sent to the macrogen company in South Korea to perform the sequence of DNA for IL-17A for the detection of SNPs under interest. Through 15 days the data of sequencing was received by email in three formulas; pdf file, text document sheet, and AB1 file which requires a sequencing reading program by Geneious Prime purchased version. In addition, the NCBI data tools were used for the alignment of the gene sequence by the BLAST tool of NCBI.

Statistical analysis

The SPSS software (IBM Corp., 2012; IBM SPSS Statistics for Windows, Version 21.0; Armonk, NY: IBM Corp.) and Microsoft Excel (2010, Microsoft Corp.) were utilized for all statistical analyses. Statistical significance was conventional at $p < 0.05$. The Chi-square test was employed to evaluate the associations between categorical variables and genetic linkage as outlined in the reference (Kang et al., 2004). Gene allele frequencies were determined through direct counting methods. Deviations from the Hardy-Weinberg (H-W) equilibrium were measured with an online H-W calculator for two alleles, accessible at "http://www.had2know.com/academics/hardy-weinberg-equilibrium-calculator-3-alleles.html".

Results

DNA extraction results for IL-17A

The profile gel-electrophoresis 20 blood samples for patients (Fig.1), 12 samples for the control group Figure (2), huge bright bands of DNA for IL-17a except some samples shown faint bands, this may result due to clotting blood samples through processing.

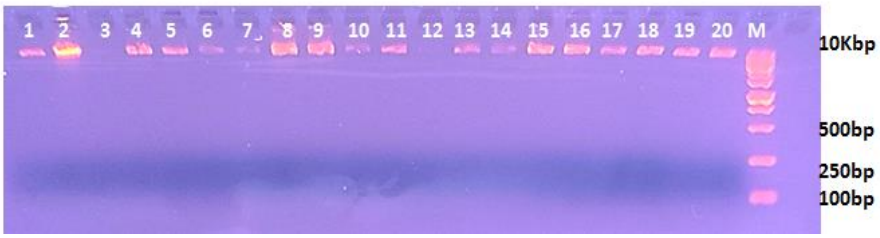

Figure 1. Gel-electrophoresis illustration quality of DNA for IL-17a extracted from patients group, 1-20 patient samples, M=molecular marker (size10kbp), First step 100bp.

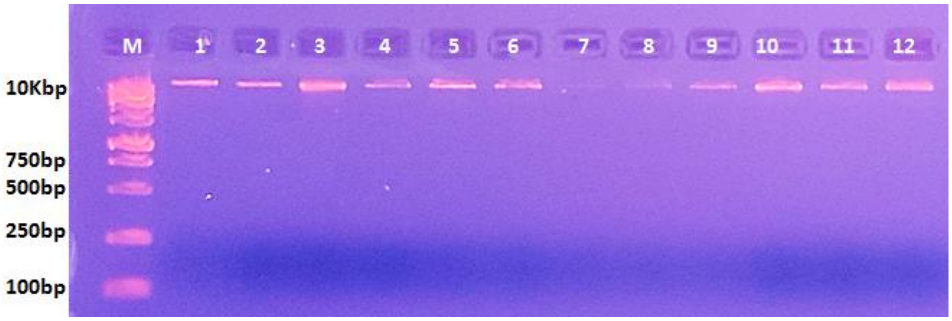

Figure 2. Gel-electrophoresis illustration quality of DNA for IL-17a extracted from the control group, 1-12 samples, M=molecular marker (size10kbp), First step 100bp.

Amplification of partial region of IL17A gene:

Figure 3 shows the targeted region of the partial sequence of IL-17A amplified by a primer pair covering the amplicon target of the patient and control group (Fig. 3 and 4).

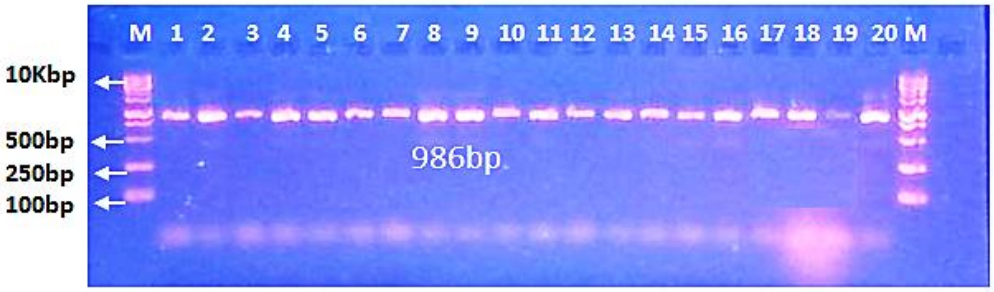

Figure 3. Gel electrophoresis 52185415-52186396 as target DNA region of IL17A amplification region with flanking primers, 1-20 patient group, PCR products 986bp, M=molecular marker 100bp for each step.

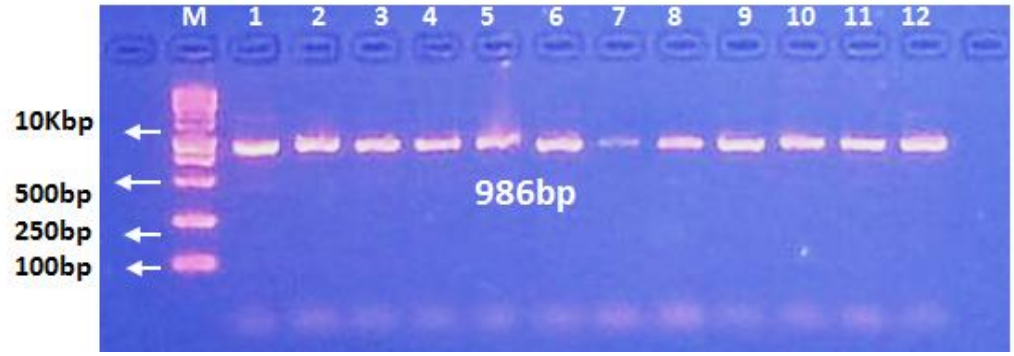

Figure 4. Gel electrophoresis 52185415-52186396 as target DNA region of IL17A amplification region with flanking primers, 1-12 control group, PCR products 986bp, M=molecular marker 100bp for each step.

Sequence results for IL-17A

To perform a sequence of PCR products, about 18-20µl of PCR products were sent to the macrogen laboratory (South Korea). After receiving the sequence, the results were subjected to bioinformatics analysis by generous prime software**.**

The multiple alignments of chromatograms of patient cases

The results of the targeted region show one SNP: rs2275913 G>A. (Fig. 5).

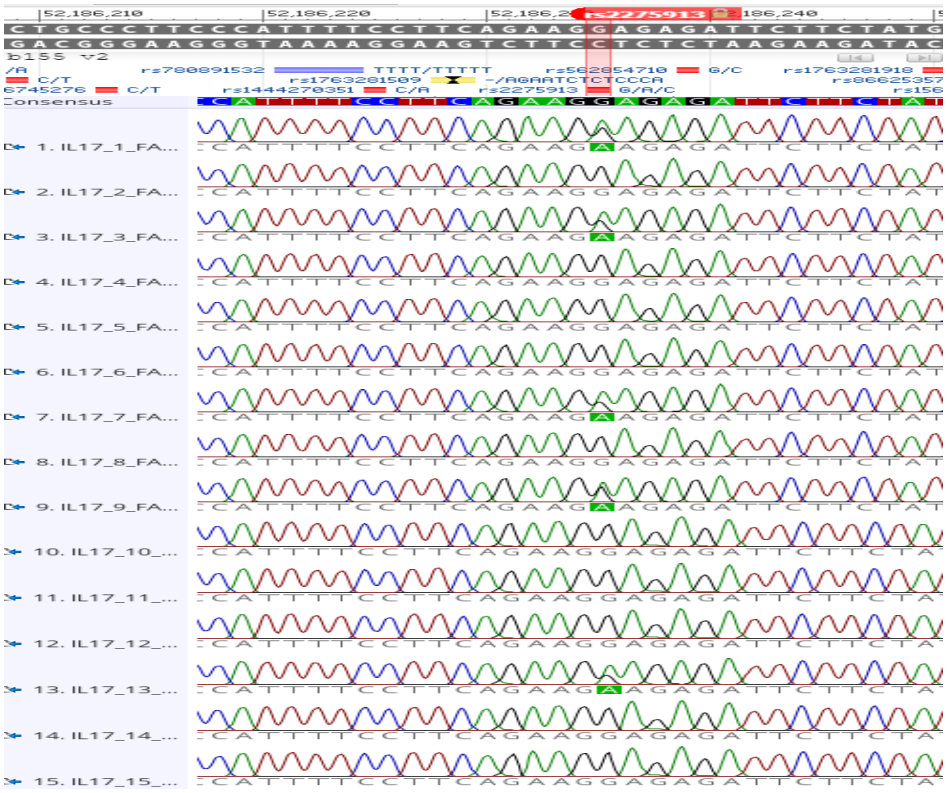

Figure 5. Multiple alignments of 15 partial IL17A sequences of the patient group, based on Chromatogram peaks, showed the combination of SNP: rs2275913G>A between its position in chromosome 6 and its position in some patient groups. The multiple alignments performed by Geneious Prime software.

The multiple alignments of chromatograms of the control group: The results of the targeted region show one SNP: rs2275913 G>A. (Fig. 6)

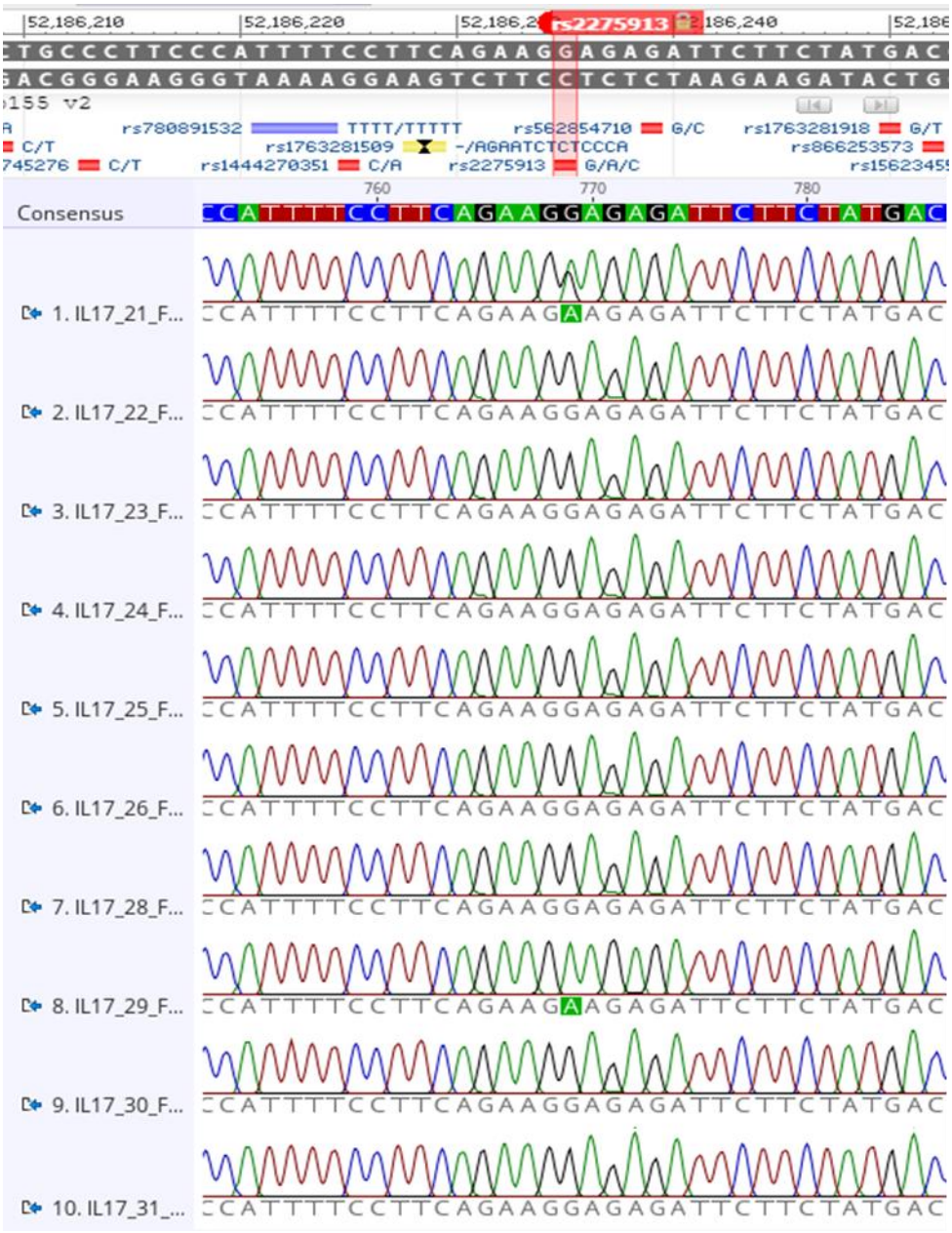

Figure 6. Multiple alignments of 10 partial IL17A sequences of the control group, based on Chromatogram peaks, showed the combination of SNP: rs2275913G>A between its position on chromosome 6 and its position in some patient groups. The multiple alignments performed by Geneious Prime software.

Table 1. Genotypes number and allele frequency of SNP rs2275913 of IL-17A between patients and control groups , shown P-value (0.05) and the Odd Ratio values.

Rs2275913 G>A	Patients group .N=20		Control N=20	OR(95%CI)	P-value
Genotypes	GG	14(70%)	16(80%)	Reference group	
	GA	0(0%)	0(0%)	1(0.019-52.03)	1
	AA	6(30%)	4(20%)	**1.7(0.40-7.3)**	0.4
Allele Frequency	G	28(70%)	32(80%)	0.58(0.21-1.63)	0.3
	A	**12(30%)**	8(20. %)	**1.71 (0.61-4.8)**	0.3

Note if OR value for mutant allele (A) is more than one, means this allele is considered as risk allele and caused disease. In this table A allele in the homozygous AA genotype has OR= **1.7**, it's a risk allele and causes disease. As seen in Figures (5), (6), the IL-17A SNP polymorphism the presence of GG genotype of rs2275913 of IL-17A allele is found mentally lower in the control group than in patient groups.

Discussion

This study took up two important statua; the first one is pregnant and the second is aborted women with toxoplasmosis and polymorphism of IL-17A. Toxoplasmosis, caused by *T. gondii*, is a common food-borne opportunistic pathogen that may cause serious disease in immunocompromised patients. In strains from around the world, the pathology and immunological responses associated with the resulting illness have not been thoroughly documented (Lieberman & Hunter, 2002). The results of the current study are consistent with the study results (Ali et al., 2022). And consistent with study results (Daher et al., 2003). IL-17A promoter polymorphisms they have been engaged to a difference of disorders (Abdul–HadiCHabuk et al., 2016; Al-Ameer et al., 2020; Al-Hamairy, 2016; Al-Hasnawi et al., 2020, 2021; Almuhana & Al-Hamairy, 2021; Daher et al., 2003; Gasime et al., 2022; Mahdi et al., 2020; Manji & Al-Hamairy, 2015; Oliewi & Al-Hamairy, 2016). In the current study, the high frequency of allele risk allele A in homozygous genotype (AA) in SNP rs2275913 IL-17A based on the odd ratio was higher in genotype AA, OR=2.1(0.40-7.3) with high significance, and the allele frequency is higher with allele A=12(30%) in patient group with high value of OR=2.14(0.61-4.8)p-value=0.2, while the allele frequency is low 20(80%)in control group as shown in Table (1). These cause deviation of the role of IL-17A. This deviation was correlated with the function of IL-117A.In general, IL-17A levels are enhanced during intense and continuous disease, while immunosuppressed patients' IL-17A levels decrease.

This anti-inflammatory cytokine can stimulate T helper 17 (Th17) reactions, IL-17A is thought to be a suppressor of Th17, released by endothelial cells, epithelial, or macrophages make pull of neutrophils to the precious locations, insusceptible responses (Brinkmann et al., 2004; Wright et al., 2007) as cytokine production is genetically controlled, and IL-17A promoter polymorphisms they have been get engaged to a difference of disorders (Daher et al., 2003; Kamali-Sarvestani et al., 2005)**,** here IL-17A gene polymorphisms have come true in toxoplasmosis and pregnant and aborted women passion and control group. These results demonstrate a role for SNP cases under interest (Moore et al., 2001). The present results are consistent with the results of (Al-Dahmoshi et al., 2013), when they refer to the role of Interleukin-17A*(*IL-17A) which important anti-inflammatory cytokines, the genetic polymorphisms include two polymorphisms G>A (rs2275913), and the present results are consistent with results of (Kadhum et al., 2023). The final finding shows that the IL-17A polymorphisms analysis has a clear impact on any human diseases. In this study, the polymorphism of IL-17A shows high effect on occurrence of toxoplasmosis and pregnant and aborted women, and the results illustrated in Figures (3), (4), and Tables (1), show the role and polymorphism and distribution of IL-17A (rs2275913) G>A. The frequency of IL-17A(rs2275913) G>A and A alleles are similar between patients and controls (Zammiti et al., 2006). In contrast, the frequency of the IL-17A rs2275913 (mutant) G allele (OR of GA=1(0.019-52.03); AA=2.1(0.35-12.8), is higher among patients 11(45%) see Table (1).

Conclusions

Pregnant and aborted women patients have been highly exposed to toxoplasmosis because of depression of the immune system, and the age group (18-40) years has a high incidence of toxoplasmosis. Patients who bear toxoplasmosis show different percentages and have the same chance of infection with toxoplasmosis. The IL-17A is considered anti-inflammatory or regulatory chemokine. The IL-17A polymorphisms analysis had a clear impact on any human diseases and showed a high effect on the occurrence of toxoplasmosis, polymorphism, and distribution of IL-17A rs2275913 G>A. The frequency of IL-17A rs2275913 and alleles A were not similar between patients and controls. Risk allele A more frequent in patients than in the control group.The allele frequency of the IL-17A rs2275913 mutant allele A (OR of GA=1(0.019-52.03); AA=1.7(0.40-7.3), were higher among patients 32(80%).

References

Abdul–HadiCHabuk, F. R. H., Al-Saadi, H. K. Z., & Al-Hamairy, A. K. (2016). Effect of the Experimental Infection with *Toxoplasma gondii* on some Biochemical aspects and Histological Changes for the Liver and Spleen in Female Rats. *International Journal of PharmTech Research*, *5*(11), 142–150.

Al-Ameer, Z. I., Al-Hamairy, A. K., & Omran, D. G. (2020). Prevalence of Toxoplasmosis and Relationships With Estradiol Hormone and Vascular Cells Adhesion Molecular-1 (VCAM-1). *Indian Journal of Forensic Medicine & Toxicology*, *14*(3), 1434–1439.

Al-Dahmoshi, H. O. M., Al-Mammori, R. T. O., ShareefHasanain, K. I., & Al-Khafagee Noor, S. K. (2013). Study of IL-8 and IL-17 levels among certain group of Repeated Spontaneous Abortion Women with or without Toxoplasmosis, Iraq. *International Research Journal of Biological Sciences*, *2*(8), 37–41.

Al-Hamairy, A. K. (2016). Effect of Lipopolysaccharide extracted from Escherichia coli on the growth and development of promastigote of Leishmania tropica (In vitro study). *International Journal of PharmTech Research*, *9*(4), 377–386.

Al-Hasnawi, N. Z. S. S., Al-Hamairy, A. K., & Altameme, H. J. M. (2020). Evaluation Activity of Boiled Aqueous Extract of Broccoli Flower Plant (Brassica Oleraceae L. Var. Italic) And Albendazole Drug Against the Protoscolices in Vitro. *Indian Journal of Forensic Medicine & Toxicology*, *14*(4), 2461.

Al-Hasnawi, N. Z. S. S., Al-Hamairy, A. K., & Altameme, H. J. M. (2021). Haplotyping of Echinococcus granulosus Isolates From Human, Sheep and Cattles Hydatid Cysts in Some Central Euphrates Provinces, Iraq. *Indian Journal of Forensic Medicine & Toxicology*, *15*(3), 2363.

AL-JUBORY, R. J., & IMRAN, Z. K. (2020). Molecular Detection of Abundance of Uncultured Lavage Microbiota and Evaluated the SNPs in Partial Sequence of CARD9 Gene. *J. Res. Lepidoptera*, *51*(2), 256–269.

Ali, S., Majid, S., Ali, M. N., Banday, M. Z., Taing, S., Wani, S., Almuqbil, M., Alshehri, S., Shamim, K., & Rehman, M. U. (2022). Immunogenetic Role of IL17A Polymorphism in the Pathogenesis of Recurrent Miscarriage. *Journal of Clinical Medicine*, *11*(24), 7448.

Almuhana, W. H. Y., & Al-Hamairy, A. K. (2021). Incidence of Toxoplasma gondii and relationship with some inflammatory factors in babylon province. *Indian Journal of Forensic Medicine and Toxicology*, *15*(1), 1647–1658.

Ben-Harari, R. R., & Connolly, M. P. (2019). High burden and low awareness of toxoplasmosis in the United States. *Postgraduate Medicine*, *131*(2), 103–108.

Bouchard, É., Elmore, S. A., Alisauskas, R. T., Samelius, G., Gajadhar, A. A., Schmidt, K., Ross, S., & Jenkins, E. J. (2019). Transmission dynamics of Toxoplasma gondii in Arctic foxes (Vulpes lagopus): A long-term mark-recapture serologic study at Karrak Lake, Nunavut, Canada. *Journal of Wildlife Diseases*, *55*(3), 619–626.

Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D. S., Weinrauch, Y., & Zychlinsky, A. (2004). Neutrophil extracellular traps kill bacteria. *Science*, *303*(5663), 1532–1535.

Daher, S., Shulzhenko, N., Morgun, A., Mattar, R., Rampim, G. F., Camano, L., & DeLima, M. G. (2003). Associations between cytokine gene polymorphisms and recurrent pregnancy loss. *Journal of Reproductive Immunology*, *58*(1), 69–77.

Dubey, J. P. (2016). *Toxoplasmosis of animals and humans*. CRC press.

Ferguson, D. J. P. (2009). Toxoplasma gondii: 1908-2008, homage to Nicolle, Manceaux and Splendore. *Memorias Do Instituto Oswaldo Cruz*, *104*, 133–148.

Gasime, S., AL-Hamairy, A. K., & Al-Zubaidy, Z. H. (2022). Molecular Detection and Diagnosis of Pathogenic Escherichia Coli and Giardia Lamblia of Patients in Babylon Province. *HIV Nursing*,

22(2), 1902–1906.

Gerhardt, K. E., Huang, X.-D., Glick, B. R., & Greenberg, B. M. (2009). Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. *Plant Science*, *176*(1), 20–30.

Hymowitz, S. G., Filvaroff, E. H., Yin, J., Lee, J., Cai, L., Risser, P., Maruoka, M., Mao, W., Foster, J., & Kelley, R. F. (2001). IL-17s adopt a cystine knot fold: structure and activity of a novel cytokine, IL-17F, and implications for receptor binding. *The EMBO Journal*, *20*(19), 5332–5341.

Kadhum, L. S., Rabee, A. K. A., & Kadhim, A. J. (2023). Toxoplasma gondii infection correlated to Interleukin 12 in wasit Women. *Journal of Survey in Fisheries Sciences*, *10*(3S), 592–597.

Kamali-Sarvestani, E., Zolghadri, J., Gharesi-Fard, B., & Sarvari, J. (2005). Cytokine gene polymorphisms and susceptibility to recurrent pregnancy loss in Iranian women. *Journal of Reproductive Immunology*, *65*(2), 171–178.

Kang, H. C., Kim, I.-J., Park, J.-H., Shin, Y., Ku, J.-L., Jung, M. S., Yoo, B. C., Kim, H. K., & Park, J.-G. (2004). Identification of genes with differential expression in acquired drug-resistant gastric cancer cells using high-density oligonucleotide microarrays. *Clinical Cancer Research*, *10*(1), 272–284.

Kolls, J. K., & Lindén, A. (2004). Interleukin-17 family members and inflammation. *Immunity*, *21*(4), 467–476.

Kolören, Z., & Dubey, J. P. (2020). A review of toxoplasmosis in humans and animals in Turkey. *Parasitology*, *147*(1), 12–28.

Lieberman, L. A., & Hunter, C. A. (2002). The role of cytokines and their signaling pathways in the regulation of immunity to Toxoplasma gondii. *International Reviews of Immunology*, *21*(4–5), 373–403.

Mahdi, Z. M. S., Al-Hamairy, A. K., & Al-Rubaiey, H. M. (2020). Genotyping of Echinococcusgranulosus Isolates from Human, Sheep and Cattles Hydatid Cysts in Some Central Euphrates Provinces, Iraq. *Medico-Legal Update*, *20*(2), 570–575.

Manji, Z. F., & Al-Hamairy, A. K. (2015). Seroepidemiological and molecular study of toxoplasmosis in the blood donors and applicants for marriage peoples in the Babylon province, Iraq. *RESEARCH JOURNAL OF PHARMACEUTICAL BIOLOGICAL AND CHEMICAL SCIENCES*, *6*(6), 1106–1114.

Moore, K. W., de Waal Malefyt, R., Coffman, R. L., & O'Garra, A. (2001). Interleukin-10 and the interleukin-10 receptor. *Annual Review of Immunology*, *19*(1), 683–765.

Oliewi, M. K., & Al-Hamairy, A. K. (2016). Epidemiological and diagnostic study for diarrheic parasites (Entamoeba histolytica, Giardia lamblia, and Cryptosporidium sp.) among diarrheic infected patients by using multiplex polymerase chain reaction in the Babylon province, Iraq. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, *7*(1), 438–447.

Polley, L., & Thompson, R. C. A. (2009). Parasite zoonoses and climate change: molecular tools for tracking shifting boundaries. *Trends in Parasitology*, *25*(6), 285–291.

Weaver, C. T., Hatton, R. D., Mangan, P. R., & Harrington, L. E. (2007). IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu. Rev. Immunol.*, *25*, 821–852.

Weiss, L. M., & Dubey, J. P. (2009). Toxoplasmosis: A history of clinical observations. *International Journal for Parasitology*, *39*(8), 895–901.

Wright, J. F., Guo, Y., Quazi, A., Luxenberg, D. P., Bennett, F., Ross, J. F., Qiu, Y., Whitters, M. J., Tomkinson, K. N., & Dunussi-Joannopoulos, K. (2007). Identification of an interleukin 17F/17A heterodimer in activated human CD4+ T cells. *Journal of Biological Chemistry*, *282*(18), 13447–13455.

Ybañez, R. H. D., Ybañez, A. P., & Nishikawa, Y. (2020). Review on the current trends of toxoplasmosis

serodiagnosis in humans. *Frontiers in Cellular and Infection Microbiology*, *10*, 204.

Zammiti, W., Mtiraoui, N., Cochery-Nouvellon, E., Mahjoub, T., Almawi, W. Y., & Gris, J.-C. (2006). Association of-592C/A,-819C/T and-1082A/G interleukin-10 promoter polymorphisms with idiopathic recurrent spontaneous abortion. *MHR: Basic Science of Reproductive Medicine*, *12*(12), 771–776.