**The risk factor of polymorphism of interleukin-6 (-174 G⁄C) relationship to toxoplasmosis infection in Wasit province, Iraq**.

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**Abstract**

The present investigation was carried out to identify if there is a risk factor supplied by the occurrence ofthe single nucleotide polymorphism (SNP) of the interleukin-6 (-174 G⁄C) on thepresence of toxoplasmosis infection. Herein, 100 blood samples (50 samples from seropositive aborted women patients and 50 samples of seronegativeparticipants) were sampled from Al-Kut Hospitals and private clinic, Wasit province, Iraq. The blood specimens were exposed to a RFLP-polymerase chain reaction (RFLP-PCR) method. The polymorphism incidence was detected as GG (homozygous) in8 (16%) and 14 (28%) of the samples from patients and healthy individuals, respectively. Moreover, the polymorphism was revealed as G/C (heterozygous) in 24 (48%) and 14 (28%) of the samples from patients and healthy individuals, respectively. In addition, the occurrence of the polymorphism was identified as CC (homozygous) in 18 (36%) and 22 (44%) of the samples from patients and healthy individuals, respectively. The presence study reveals high relationship of the interleukin-6 polymorphism G/C (heterozygous) to the infection by *Toxoplasma gondii*.

**Keywords**: Interleukin-6 polymorphism, RFLP-PCR,SNP,*Toxoplasmagondii*, toxoplasmosis.

**Introduction**

The intracellular parasite of phyla Apicomplexa, the compulsory *T*.*gondii*, is most recognized infelids as final hosts.However, *T*. *gondii* has a variety ofintermediatehostsof the majority of warm-blooded animals such as rodents, birds, and human beings. In the majority of hosts, the parasite infects a wide-range oftissues,leading to prolonged latent infections, affectingthebrain, spinal cord, retina, and cardiac and skeletal muscles (Mendez and Koshy, 2017).

The main source of *T. gondii* is food or water that contains the infective stage of the parasite or vertical transmission. Seroprevalence incidence rates are observed globally and recognized as from <10% to >60%. In acute infections, *Gondii* spreads the quickly-replicating stage of the protozoan as a tachyzoite via the host, which is the target of the host immune machinery. As the disease progresses, the parasite is transported to a slowly replicating stage, bradyzoite, encyst form, generating the chronic infection. There are several transformations between the two forms making difficulties for the immune system to recognize the bradyzoite/cyst(Kim and Boothroyd, 2005; Pappas, Roussos and Falagas, 2009).

IL-6 is known for its important in the toxoplasmosis reinfection as an anti-parasite immune response.However, disturbances of thesignaling of the IL-6 via the receptor subunit, gp130, could introduce IL-6 as adiseaseriskprecursor instead of its protective role (Händel *et al.*, 2012). Inwomantoxoplasmosisinfection, IL6 expression level was calculated to be double as high when a comparison was madewith the control individuals(Matowicka-Karna, Dymicka-Piekarska and Kemona, 2009).

The present investigation was carried out to identify if there is a risk factor supplied by the occurrence ofthe interleukin-6 (-174 G⁄C) SNP on thepresence of toxoplasmosis infection.

**Materials and methods**

**Samples**

Herein, 100 blood samples (50 samples from seropositive aborted women patients and 50 samples of healthy individuals) were collected in EDTA-contained tubes from Al-Kut Hospitals and private clinic, Wasit province, Iraq.

**Extraction of the DNA**

Using Genetic DNA Mini Pack, Geneaid. USA, DNA has been obtained from the preserved blood samples. Depending on the guidance of the extraction steps provided by the manufacturer, the extraction was carried out using Proteinase K (frozen blood extraction protocol method). The concentration of the DNA and its quality were examined by working on aNanodrop spectrophotometer, and it was -20C-stored to be used in the PCR step.

**PCR RFLP**

PCR-RFLP was introduced for the detection of IL6 (-174 G⁄C) SNP from seropositive patients and healthy individual blood samples. The technique was performedrelying ona method by (Cordeiro et al., 2013). The primers, table 1, were ordered from MacrogenCompany (Korea).

**Table (1): The RFLP-PCR primers.**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Primers (5'-3')** | | **Size of the product (bp)** |
| **IL-6 gene** | **F** | CAGAAGAACTCAGATGACTG- | 431 |
| **R** | GTGGGGCTGATTGGAAA |

The master mix of the PCR was generated by using (AccuPower® PCR PreMix kit. Bioneer. Korea) as in the following table 2:

**Table (2): Master mix components of the PCR.**

|  |  |  |
| --- | --- | --- |
| **PCR master mix** | | **Volume (µl)** |
| **DNA (5-50ng)** | | 5 |
| **Primers (10pmol)** | **Forward** | 1 |
| **Reverse** | 1 |
| **PCR water** | | 13 |
| **Total** | | 20 |

The PCR premix tubes that contained1UDNA polymerase, 250µMdNTPs, 10mMTris-HCl (pH 9.0), 30mMKCl, 1.5mMMgCl2, and a dye were used to place the master mix in and vortex-mixed briefly (Bioneer, Korea). A thermocycler (T100 Thermal cycler Bio-Rad,USA) was employed to generate the PCR reaction using the conditions provided in the table 3

**Table (3): The Thermocycler PCR conditions**

|  |  |  |  |
| --- | --- | --- | --- |
| **PCR step** | **Temp. (°C)** | **Time (min)** | **Repeat cycle (No.)** |
| **Initial denaturation** | 95 | 5 | 1 |
| **Denaturation** | 95 | 0.5 | 35 |
| **Annealing** | 56 | 0.5 |
| **Extension** | 72 | 1 |
| **Final extension** | 72 | 5 | 1 |
| **Hold** | 4 | Forever | - |

Electrophoresis using an agarose gel at 1.5%mixed with ethidium bromide was introduced to examine the presence of the PCR products(431bp)visualized utilizing a UV transilluminator.

UsingHsp92II restriction enzyme (Biolabs, UK), the RFLP step was performed generating 229 + 122 + 51 + 29bp and 229 + 173 + 29bp fragments out of the PCR product digestion for the C allele and for the G alleles, respectively. Employing an agarose gel at 3% concentration inoculated with ethidium bromide,anelectrophoresis process was produced. The fragments were visualized using a UV transilluminator.

**Statistical analysis:**

The statistical differences in the genotype/allele frequencies between toxoplasmosis patients and control group were done by the Fishers exact test. Statistical significant was set at p value ≤ 0.05 . Odd ratio (OD) , relative risk (RR), etiologic fraction (EF), and preventive fraction (PF) were done following the Woolf ҆s method (Al-Okaily*et al.,*2015)

**Results**

The presence of the polymorphism was detected as GG (homozygous) in 8 (16%) and 14(28%) of the samples from patients and healthy individuals, respectively. Moreover, the polymorphism was revealed as G/C (heterozygous) in 24 (48%) and 14 (28%) of the samples from patients and healthy individuals, respectively. In addition, the occurrence of the polymorphism was identified as CC (homozygous) in 18 (36%) and 22 (44%) of the samples from patients and healthy individuals, respectively, table 1 , figure 1,2,3.

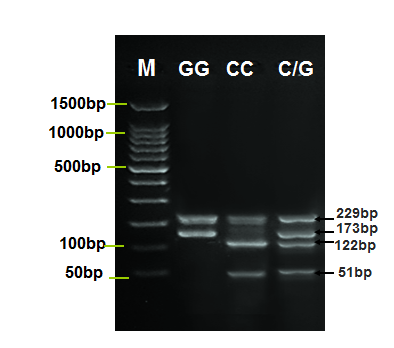
**Table (4) Genotype and allele frequencies of IL-6 variant in toxoplasmosis patients and matched control.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Genotype / allele | Patients  N=50 | | Control  N=50 | | P value | OR | 95% CI | RR | EF | PF |
| No. | % | No. | % |
| **Genotype :** | | | | | | | | | | |
| GG | 8 | 16 | 14 | 28 | 0.15 | 0.48 | 0.1845 to 1.3000 | 0.489 | ----- | 0.273 |
| GC | 24 | 48 | 14 | 28 | 0.04 | 2.37 | 1.035 to 5.4436 | 2.373 | 0.36 | ---- |
| CC | 18 | 36 | 22 | 44 | 0.41 | 0.71 | 0.3206 to 1.5986 | 0.715 | ----- | 0.152 |
| **Allele :** | | | | | | | | | | |
| G | 40 | 40 | 42 | 42 | 0.77 | 0.92 | 0.5239 to 1.6177 | 0.920 | ----- | 0.040 |
| C | 60 | 60 | 58 | 58 | 0.77 | 1.08 | 0.6182 to 1.9087 | 1.086 |  | ---- |

RR : Relative risk EF : etiologic fraction PF : Preventive fraction

**Figure (1) IL-6Genotype polymorphism in seropositive toxoplasmosis patients and healthy individuals**.

**Figure (2) IL-6 allele polymorphism in seropositive toxoplasmosis patients and healthy individuals**



**Figure (3). Agarose gel electrophoresis image that show the RFLP-PCR RFLP product analysis for IL-6 gene polymorphism using *Hsp92II* restriction enzyme. Where M: marker (2000-50bp). The lane (GG) mutant type homozygote that show digested by restriction enzyme into 229bp and 173bp band. The lane (CC) wild type homozygote, the product was digested by restriction enzyme digested into 229 bp, 122 bp, 51 bp, and invisible 29 bp band, and lane (C/G) heterozygote, the product digested by restriction enzyme into 229 bp, 173bp, 122 bp, 51bp, and invisible 29 bp band.**

**Discussion**

*T*.*gondii* is aglobally common parasites, partly since it is able to target and continue to be found in most animals of warm-blooded species. A distinctive feature of this parasite isits ability to remain latent, in a number of hosts, such as humans and rodents, in the central nervous system (CNS)(Koshy *et al.*, 2012; Alvarado-Esquivel *et al.*, 2015; Cabral *et al.*, 2016). Disturbances of thesignaling of the IL-6 via the receptor subunit, gp130, could introduce IL-6 as adiseaserisk precursor instead of its protective role (Händel *et al.*, 2012).

The results of the present investigation showed increases of the IL-6 G/C SNPs in seropositive patients. This is with high correlation with the presence of the toxoplasmosis infection especially when compared with those from the healthy individuals. It has been revealed that there arebig roles of thechanges occurred in the genetic materials of the IL6 gene, -174 G>C SNP, and that is associated with toxoplasmicretino-choroiditis infection (Cordeiro *et al.*, 2013).The strength of the immune system responses against infections are highly regulated by the important activities of chemokines, cytokines, and their receptors.Human toxoplasmic disease is distinguished by an elevated cytokine levels like IL-12 and TNF-α which are correlated with tissue infections. In addition to the participation of lymphocytes CD8+T and natural killer (NK) cells, a powerful Th1 reaction induced through the manufacturing of CD4 + T cell and is overtaken by the development of pro-inflammatory mediator production. While Th1 discourages the reproduction of parasites, a strong Th1 activity can also trigger organ damages caused by the responses of the immune systems(Cordeiro *et al.*, 2008; Garweg and Candolfi, 2009; Sauer *et al.*, 2012; Dutra *et al.*, 2013; Naranjo-Galvis *et al.*, 2018).

Cytokine genetic functional polymorphisms can engage with or strengthen cytokine expression and can play a major role in the regulation of the genetic materials of inflammatory activities and ability to resist an infectious diseases. The SNPs are helpful in identifying phenotypic changes that are related to a specific infection. Natural selection encourages the incorporation of cytokine SNPs of the biallelic type, which contributes to a difference in gene expression levels instead of the efficiency(Vasconcelos *et al.*, 2012).According to those factors, the IL-6 (G/C) SNPs may encouragethe development of toxoplasmosis infection.

The presence study reveals high relationship of the interleukin-6 polymorphism G/C (heterozygous) to the infection by *Toxoplasma gondii*.

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