Interleukin-6 gene polymorphism (-174 G/C) association with seropositive Toxoplasma gondii infection patients in Al-Diwaniyah hospital

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ABSTRACT

The present study aims to detection Interleukin-6 gene polymorphism (-174 G/C) from seropositive Toxoplasma gondii pregnant women patients and healthy control from individuals reviewers to AL-Diwaniyah hospital. 80 samples were collected for the period from October 2018- April 2019, including two groups of 30 samples healthy control and 50 samples from pregnant women infected with Toxoplasma gondii. The results of the present study indicated the presence of IL-6 (-174 G/C) polymorphism in pregnant women infected with Toxoplasma gondii and healthy control. Where was observed that CG genotype more frequent in patients indicating the association of this genotype with the disease compared to the control group where CG genotype appears less. Also, the results of the current study indicate the appearance of allele C more frequently in pregnant women patients compared to healthy control. We can conclude from the present study there association of appearance allele C with Toxoplasma gondii infection.

INTRODUCTION

Toxoplasma gondii is widespread parasite infects the human belongs to the phylum protozoa (Murray and Rosenthal, 2013). Congenital toxoplasmosis transmitted from the mother to fetus by transplacental and cause abortion or severe damage to the fetus if it occurs in early pregnancy (Robert-Gangnru and Darde, 2012). T. gondii, in their life cycle, needs two hosts are intermediate and definitive felids represents the definitive host where occurs the sexual phase in its intestine. All the warm-blooded animals represent the intermediate host that infected with the parasite by ingested drink or food contaminated with oocysts from feces of infected felids and tissue cysts exist in other intermediate hosts (Montoya and Liesenfeld, 2004). Acute infection depends on the immunity of the host and the dose with the double speed of the tachyzoites that convert to bradizoites. An intermediate host that carried tissue cysts or tachyzoites are responsible for the spread T. gondii, as well as felids (Dubey, 2010).

The installation and maintenance of pregnancy and birth success depend directly on the immune system of the mother (Munoz-Suano et al., 2011). This is associated with many hormonal changes and changes in the concentrations of the cytokines within the uterus (Robert et al., 2001).

In studies done on immunity to T. gondii showed Toxoplasma induces a strong innate immune response and role of T cells in resistance to this parasite that provides resistance during influence subsequent adaptive response and acute infection (Gazzinelli et al., 1992).
Interleukin 6 is produced by macrophages, fibroblasts, monocyte, endothelial cells, neoplastic cells and myelomatous. It is associated with glucocorticoids, IL-1 and TNF-α, in acute inflammatory phase proteins, IL-6 consider the main mediator responsible for hepatocytic production.

IL-6 increase the cytotoxic activity of Natural killer cells then induces differentiation of cytotoxic T lymphocytes and differentiation of B lymphocytes into antibody sereting cells (Beaman, 1992). T cells has an important role in embryo development and pre-implantation the phenomenon of fetal allograft tolerance and implantation process (Piccinni et al., 2015). Th2 cytokines block activation of Th1 cells, and Th1 cytokine inhibits Th2 cells expansion (Nair et al., 2013). IL-6 may have beneficial and detrimental effects on the events of early pregnancy and abnormal pregnancies and other disease such as Autoimmune disease, Rheumatiod Arthrits, obesity and preeclampsia (Zenclussen et al., 2003; S. and A, 2016; A, 2016).

Increased of IL-6 levels in the plasma occurs during sporadic miscarriage (Calleja-Agius et al., 2012) and in pregnancies complicated which include early rupture of membranes, prematurity and intrauterine infection, noticed high levels of IL-6 in the deciduous, amniotic cells and placenta (Unfried et al., 2003).

MATERIALS AND METHODS

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Materials and Methods

Samples collections

50 blood samples were a collection in EDTA tubes from seropositive toxoplasma gondii pregnant woman patients and healthy control individual from Al-Diwanyiah Hospital, then the samples storage in the refrigerator until use in Blood DNA extraction.

Genomic DNA Extraction

The extraction process of DNA from frozen blood samples was performed using (Genomic DNA Mini Kit, Geneaid. the USA). The extraction was done according to the company instructions by using frozen blood extraction protocol method with Proteinase K. then, examination detected the DNA extracted from samples of blood and through the use of device Nanodrop spectrophotometer and then storage to -20°C while in use in PCR amplification.

PCR RFLP

PCR-RFLP was carried out for detection Interleukin-6 gene polymorphism (-174 G/C) from seropositive toxoplasma gondii infection patients and healthy control blood samples. The technique was carried out according to (Cordeiro et al., 2013). The primers were provided by (Bioneer company. Korea). Then PCR master mix was prepared by using (AccuPower® PCR PreMix kit. Bioneer. Korea). The reaction was performed in a thermocycler (T100 Thermal cycler Biorad. USA). The 431 bp PCR products were examined by electrophoresis in a 1% agarose gel, stained with ethidium bromide, and visualized under UV transilluminator.

RFLP step was done by using (Hsp92I, Biolabs, UK) restriction enzyme. THE PCR products were digested into 229 + 122 + 51 + 29 bp for C allele and 229 + 173 + 29 bp for G alleles. The fragment was separated by 3% agarose gel electrophoresis containing ethidium bromide and visualized under UV transilluminator.

RESULTS AND DISCUSSION

the result of the current study showed emergence IL-6 (-174 G/C) polymorphism in both healthy control group and pregnant women patients infected with T.gondii.

CG genotype observed is more frequent in pregnant women infected with T. gondii this indicating the association of this CG genotype with disease compared to a healthy control group where it appears less as the Table 1

The result of the current study showed that allele C was more frequent in pregnant women patient infected with T.gondii compared to the healthy control group these indicates the association of the appearance of the C allele with infection of T.gondii as the Table 2

Toxoplasma gondii is a protozoan intracellular parasite that infects all warm-blooded mammals and birds, including humans throughout the world (Webster, 2010).

This study indicated that GC genotype is more frequent in patients compared with control. This means presence association between IL-6 gene polymorphism (-174 G/C) and T.gondii.

The major function of interleukin-6 is the contribution in the immune response during the action of lymphocytes and is responsible for increased cytotoxic activity of NK cells and acute-phase protein where interleukin-6 consider an early and sensitive marker of inflammation (Matowicka-Karna et al., 2009). In women with recurrent spontaneous
Table 1: Genotype frequencies of IL-6 gene polymorphism (-174G/C) in pregnant women infected with T. gondii and healthy control

<table>
<thead>
<tr>
<th>P-value</th>
<th>95CL%</th>
<th>Odd ratio</th>
<th>Groups</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3225 to 3.1006</td>
<td>1</td>
<td>10</td>
<td>GG</td>
</tr>
<tr>
<td>0.8087</td>
<td>0.3457 to 2.903</td>
<td>0.8898</td>
<td>17</td>
<td>CC</td>
</tr>
<tr>
<td>0.8165</td>
<td>0.4477 to 2.7715</td>
<td>1.1140</td>
<td>23</td>
<td>GC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50</td>
<td>total</td>
</tr>
</tbody>
</table>

Table 2: Allele frequencies of IL-6 gene polymorphism (-174 G/C) in pregnant women infected with T. gondii and healthy control

<table>
<thead>
<tr>
<th>P-value</th>
<th>CL 95%</th>
<th>Odd ratio</th>
<th>Groups</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8688</td>
<td>0.5523 to 2.0194</td>
<td>1.0561</td>
<td>43(43)</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td>0.4952 to 1.8105</td>
<td>0.9468</td>
<td>57(57)</td>
<td>C</td>
</tr>
</tbody>
</table>

abortion, peripheral blood lymphocytes secrete high level of IL-6 may explain the role IL-6 in the recurrent spontaneous abortion and pathogenicity (Prins et al., 2012).

Also found IL-6 increase in recurrent spontaneous abortion more than healthy pregnant (Bakir et al., 2010).

(Hua et al., 2013) Showed that in the rats, IL-6 concentration was higher in a repeated miscarriage than normal pregnant, while (Makhseed et al., 2000) recorded that IL-6 in the serum level was higher in pregnant women than spontaneous abortion. Also (Koumantaki et al., 2001) showed reduced IL-6 in plasma levels may be related to the underlying etiopathogenetic mechanisms in a woman with spontaneous abortion. Found that IL-6 increased in plasma levels in sporadic miscarriage (Calleja-Agius et al., 2012), (Ahmed, 2008) showed that IL-6 in plasma levels was lower in women with repeated spontaneous abortion compared with women undergoing normal delivery.

CONCLUSIONS

Interleukin-6 produced at the site of inflammation, it’s pro-inflammatory cytokine and plays in acute phase response as key role by a variety biological features and clinical such as the production of acute-phase proteins (Gabay, 2006). The level of interleukin 6 was significantly higher in miscarriages women with acute infection compared with non-miscarriage women and without toxoplasmosis (Zohairy and Qadhi, 2015), this is dictated that T. gondii may lead to increasing the level of IL-6 in infected women than in uninfected one (Atici et al.,...
When IL-6 increased significantly in acute infection more than chronic infection, this cytokine may be used as an indicator for disease activity. The control of immune response associated with the anti-inflammatory mechanism involves the release of an anti-inflammatory cytokine such as (IL-10, IL-13 and IL-4) (Paris et al., 1995). The major function of IL-6 participates in the immune response during the action on lymphocytes B also IL-6 consider a mediator responsible for the production of acute-phase proteins and increased cytotoxic activity of NK cells (Matthias et al., 1997).

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