Sequencing of Zona Pellucida 4 (ZP4) Gene in Patients with Polycystic Ovary Syndrome Infected with Toxoplasmosis

Maysara Samer Khalaf¹, Hanaa N. Abdullah²*, Suzan Adil Rashid³

¹Health and Medical Technical College /Baghdad, Middle Technical University/Iraq.
³Kirkuk technical Institute/Kirkuk, Northern Technical University/Iraq.

*Corresponding author: dr.hanaa_genetic2010@rocketmail.com

Abstract

Polycystic ovary syndrome is an endocrine disorder in women which leads to reproductive and metabolic abnormalities. The zona pellucida 4 gene is a glycoprotein which is a part of the extracellular matrix of an ovum. Twenty blood samples were taken from women with PCOS. Also, 20 blood samples were taken from the infected women, polycystic ovary with toxoplasmosis and healthy women groups. Whole blood samples were used to perform a polymerase chain reaction, while was ELISA used to measure Anti-Toxoplasma IgG antibody levels in the sera of both the studied and control groups. The biochemical test was used to determine the follicle-stimulating hormone, luteinizing hormone, and Testosterone. There is a highly significant difference regarding the levels of anti-Toxoplasma IgG antibody among PCOS patients who were infected with Toxoplasma gondii (1.5865±.42295), Toxoplasmosis control group (1.4097±.46171) and healthy control (6380±.3330). The hormones levels of PCOS showed a highly significant difference (P<0.01) FSH; (22.260±3.8692) compared with healthy control (8.362±1.1149) and Toxo. Control group LH; (32.080 ±2.7523), compared to Toxo. Control group (9.939±1.3910) and healthy control (9.445±1.8159) and Testosterone; (2.0470±.55663) compared to Toxo. The control group (.6580±.13575) and healthy control (.6365±.12512) respectively.

Single nucleotide polymorphism T6235C indicated that T to C conversion was observed at nt 6325 in the ZP4 gene, and this evolution was experiential in one PCOS. This SNP is a synonymous substitution, which occurred in the fifth position of the proline codon, changing the codon ATA to ACA.

Introduction

About 10% of women are affected with Polycystic ovary syndrome (PCOS) which is one of the most common endocrinopathies lead to no reproductive [1] a symptom of PCOS there is intolerance of glucose and dyslipidemia [2]. Toxoplasmosis is one of the common injuries occurring within our bodies and may lead to complications in most of the human infections [3]. It may be one of the effective factors on the human ovary and lead to polycystic ovary syndrome (PCOS) [4]. The zona pellucida 4 (ZP4) genes which encodes glycoprotein is a part of the extracellular matrix of the oocyte [5].

These genes play an important role during sperm-egg interaction but also in folliculogenesis. Many studies have confirmed that toxoplasmosis caused many recurrent miscarriages of women of different ages or congenital malformations [6]. Studies have also confirmed that infertility for both sexes is due to the T. gondii and its problems within the tissues of couples [7]. The verification of those phenomena and cases causes the loss of fetuses due to those parasites as well as non-occurrence of the procreation [8]. The polycystic ovary is a phenomenon that occurs in the ovarian tissue and this change is caused by the occurrence of genetic mutations and there must be a causative T. gondii [9]. The aim of this study is to identify sequencing of zona pellucida 4 (ZP4) in patients polycystic ovary syndrome and investigate the position of the ZP4 protein in the ovaries of patients with PCOS infected with T. gondii.

Methods

Twenty whole blood samples were collected from polycystic ovary female patients who were suffering from toxoplasmosis. Each sample was stored in a sterile container sealed with a plastic cover, and incubated at -
Patients infected with Toxoplasma gondii complained from eumenorrheic and oligomenorrheic in the late follicular phase, whose aged between 20-40 years and attended Al-Illaweaa Hospital/Baghdad during the period from 1st August 2016 to 1st March 2017.

The age of the participants, was ranging between 20 and 40 years. Biochemical tests were done to detect the FSH, LH and Testosterone hormones. Enzyme liked immune sorbent (ELISA) was applied for the detection of the Anti-Toxoplasma IgG antibodies parameter (ELISA TOXO IgG Biotik, USA). PCR application of Bio System Big Dye TM termination V 3.1 cycle sequencing kit and twenty PCOS of patient’s samples were sequenced for the detection of ZP4 gene to extraction and isolation of DNA were carried out by using DNA isolation kit (QIAGEN).

Statistical Analysis

SPSS Microsoft Office Excel program was used for statistical analysis of data. The values were represented as mean ± SEM (standard error of the mean). The comparison of the numeric data for healthy control and patient groups was clone by using the ANOVA test [10].

Results

Table 1: shows that mean age of patients who are infected with Toxoplasma gondii (25.55±6.894). P²=0.741 with no significant differences compared to the healthy control group (6.870±6.870), P¹=0.98, also showed no significant difference between Toxoplasmosis patients and PCOS patients compared to healthy control. There is a highly significant difference regarding the levels of anti-Toxoplasma IgG antibody, among PCOS patients who were infected with Toxoplasma gondii(1.586±.42295) compared to Toxo. Control group (1.409±.46171) and healthy control group (.6380±.33308).

The hormone levels of PCOS revealed a highly significant difference (P<0.01) FSH; (22.260±3.8692) according to healthy control (8.362±1.1149) and Toxo. Control group LH (32.080 ±2.7523), compared to Toxo .Control group (9.939±1.3910) and healthy control (9.445±1.8159) and Testosterone; (2.0470±.55663) compared to Toxo. Control group (.6580±.13575) and healthy control group (6365±.12512).

Detection of the Genotype of Polycystic Ovary Syndrome

The genetic analysis was carried out in the department of Molecular and Human Genetics. Blood samples were collected in tubes containing EDTA and stored at 4°C. Genomic DNA was extracted from peripheral leukocytes by salting out procedure [11]. A T6235C variant of ZP4 gene was amplified using forward
primer 5’CAGTGAAGAGGTGAGCCGT3′ and reverse primer 5’TAGGGAGTCTTGTCTCATGCC3′ by polymerase chain reaction (QIAGEN). Initial denaturation at 94°C for 5 minutes was followed by 35 cycles at 94°C for 60 seconds, at 63°C for 60 seconds and extension at 72°C for 60 seconds followed by final extension at 72°C for 10 minutes and left overnight at 37°C. Subsequently, the PCR product was digested with msp1 restriction enzyme. 

PCR products were separated using 2% agarose gel electrophoresis followed by visualization under UV illuminator after ethidium staining. Genotypes were expressed TC for heterozygous mutant genotypes. A single band of 340 bp was produced. The two bands of 200 bp and 140 bp indicate homozygosity for T allele and presence of 3 bands of 340 bp, 200 bp, and 140 bp indicate heterozygosity (TC, Figure 1).

Sequencing of ZP4 Gene

For the application of Bio System Big Dye TM termination V 3.1 cycle sequencing kit, twenty PCOS strain of patients samples they were sequenced for the detection ZP4 gene PUC/Hinf1 [12]. The resulting sequences were compared with the reference sequence that was obtained from the NCBI database. To edit the sequences and determine the nature of mutations, a computer sequencing program TM was used. It was revealed that the base substitutions were pleased with 6235 nucleotides (nts) of PCOS as shown in (Table 2).

SNP T6235C

A T to C conversion was observed at nt 6325 in the ZP4 gene, and this evolution was experiential in one PCOS. This SNP, which is a synonymous substitution, occurred in the fifth position of the pro line codon, changing the codon ATA to ACA (Fig 2).
Table 2: Nucleotide changes in the ZP4 gene coding sequence from a total of 20 patient samples with PCOS and resulting amino acid changes in the ZP4 protein

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Exon number of ZP4 gene</th>
<th>Nucleotide change position in Exon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-9</td>
<td>5</td>
<td>T-C, position 6355</td>
</tr>
<tr>
<td>10-20</td>
<td>5</td>
<td>no change</td>
</tr>
</tbody>
</table>

Discussion

Polycystic ovary syndrome (PCOS) is a very common manifestation among women especially in younger aged and that causes a risk problems in pregnancy [13]. This study is the first one to be conducted in Iraq that consider the effect of *Toxoplasma gondii* on women with pregnancy problems.

The study demonstrated that there was a highly significant difference in the levels of anti-*Toxoplasma* IgG antibody, among PCOS patients who were infected with *Toxoplasma gondii*. The hormone levels of PCOS were a highly significant difference (P<0.01) regarding FSH; Toxo. Control group LH; and Testosterone compared to Toxo. Control group and healthy control group. The results were in a harmony with Suresh S. and Vijaya kumar T. 2014, [14].

Zona pellucida 4 (ZP4) genes encodes for the glycoprotein which is a part of the extracellular matrix of oocyte. The results shows that A T to C conversion was observed at nt 6325 in the ZP4 gene, this evolution was experiential in PCOS. The SNP which is a synonymous substitution, occurred in the five position of the proline codon, changing the codon ATA to ACA, this finding was agreed with Meczekalski, B. et al, 2015, who proved that there are several mutations on several gene sites [5]. Recent studies have shown that *T. gondii* have affected many on cases, such as cancer patients, prostate and uterine cancer AL-Gabbar 2016, [15].

Mohammad et al, 2017 proved that this parasite has caused genetic mutations in men with infertility [16]. As we mentioned previously, this study is the first one to be done in Iraq or worldwide and it proved that there is a direct effect of *T. gondii* on the polycystic ovary, and the absence of fertilization in married women. These findings were agreement with Amir Abdoli and Abdolhossein Dalimi who proved that *T. gondii* infection influencing factors have diverse effects on testosterone production, behavioral alterations, and PCOS [17].

Also these results agreed with Cristina S. et al, 2015, [18]. *T. gondii* invading every place in the human body causes serious malfunction and may lead to tissue damage [19]. The study also showed that there is a positive relationship in the increase in the proportion of hormones such as FSH, LH, and Testosterone in Toxoplasmosis, in addition to multiple mutations on the ZP4 gene located on the surface of the ovaries. This indicates that the pinch may be the cause of this disorder and the occurrence of polycystic ovaries, and consequently the fertility of married women does not occur.

This is what Mohammad et al, 2017 proved to be the basis of the universe of infertility in women and men [20]. Based on the presence of this parasite in the body of these cases and the occurrence of several mutations on the surface protein of oocyte of the ovaries, this condition was identified to cause dysfunction of certain sites of the body and possibly the occurrence of infertility.

In our study, We concluded that mutation in position at 6325 in the ZP4 gene, exon 5, result from synonymous substitution, occurred in the five position of the proline codon, changing the codon ATA to ACA. And maybe toxoplasmosis is the causative agent for these conditions.

References


10. Technical problems, contact, URL: http://www.its.msstate.edu/software/ Mississippi State University. All rights reserved. 2017.


