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# Detection of Genetic Variability within the Rop-17 Gene in Women Infertility with Toxoplasmosis and its Relationship to Levels of Some Antioxidants

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**ABSTRACT**

*Toxoplasma gondii* is considered an obligatory intracellular parasite that causes toxoplasmosis. This study included 100 infertile women. Serum and whole blood were taken from the included subjects. The DNA was extracted from the whole blood by using a commercial kit. Real-time PCR was done for all the samples to detect the presence of *T. gondii*. PCR was done for the positive samples to amplify a target sequence within the rop-17 gene. The PCR product was sequenced to reveal the variations within the rop-17 gene. The ELISA test was done to measure the serum level of SOD and GPX. The real time-PCR showed 43% frequency of the samples. The conventional PCR was used to amplify the rop-17 gene in the positive samples but showed a successful amplification in 92% of the real-time PCR positive samples. The results of the sequences showed different variations within the rop-17 gene. GPX and SOD were both higher in patients (93.76±9.6 and 89.6±9.4, respectively) than in control (76.5±31.4 and 85.6±7.43, respectively).

**Key words :** GPX, SOD, rop-17 gene, variations, sequence, real time, PCR

## INTRODUCTION

The inability to perceive in 12 months of unprotected sex is known as infertility. It is estimated that 84% of couples conceive after one year of intercourse. Additionally, after two years, 92% of the couples succeed to conceive (Al-Qadi *et al.*, 2019).

Infection by *Toxoplasma gondii* is related to various clinical problems in man, including from asymptomatic infection to unembellished central nervous system disease. Recently, it has been established that toxoplasmosis is a fundamental risk for pregnant women and immunocompromised patients (Feleke *et al.*, 2019; Malihe *et al.*, 2020). The risk of toxoplasmosis extends further from just pregnancy difficulties and congenital infections but also includes the negative effects on reproductive capacity in both men and women (Saadatnia, 2017). The relationship between toxoplasma and infertility has been confirmed by numerous studies done on animals and infertile couples (Chi *et al.*, 2014; Yamamoto-Furusho *et al.*, 2016).

The fact, that toxoplasmosis causes infertility, can be concluded by the fact that *T. gondii* in females can develop endometritis and can also cause fetal rejection which can be caused by a release of *T. gondii* from latently located cysts (El-Sherbini *et al.*, 2019).

This study was done to reach the following aims :

- The comparative evaluation of Toxoplasma infection rates of infertile females,
- To compare the different detection techniques against the *T. gondii,*
- To reveal the sequence variation within the rop-17 gene, and
- To treasure trove the effect of toxoplasmosis on the serum level of both SOD and GPX.

## MATERIALS AND METHODS

This study included 100 women who recorded no births after two years of marriage and were visiting an IVF clinic in Baghdad (The High Institute for Infertility Diagnosis and Assisted Reproductive Technologies). The samples suffering from PCOS and fibrosis were excluded from this study in order to obtain the aim of the study. Three ml of each sample was

collected and placed in an EDTA tube and then stored at -80°C, until the working day. DNA was extracted from all the samples by using the commercial kit Quick-DNA™ Miniprep plus Kit (cat#; D4068T).

All the samples were tested by real-time PCR for detection of *T. gondii* B1 gene. To amplify the B1 gene, a pair primer and a probe was used which was manufactured by Macrogen Company. The primer, reverse primer and probe sequences were 5′-TCCCCTCTGCTGGC GAAAAGT-3′; 5′-AGCGT TCGTGGTCAACT ATCGATTG-3′ and FAM TCTGTGCAACTTTGGT GTATTCGCAG-TAMRA. The final volume of reaction that inserted into the RT-PCR machine was 20 µl and contained the following components : 10 µl of Kappa master mix, 0.5 µl of each primer and probe, 3 µl of the eluted DNA the volume completed to 20 µl by nuclease-free water. The PCR was performed by using the Sa-cycler96 (SACACE, Italy). The instrument then was programmed as follows : initial denaturation at 96°C for 10 min, 40 cycles of 95°C for 20 sec and 60°C for 20 sec and then 72°C for 20 sec. The samples that showed a sigmoid curve in the resulting graph were considered positive.

The positive samples were used for amplifying the rop-17 gene by conventional PCR. A set of primers was used forward primer, 5′-AGGACAACACTAGGTAGCGAGAACC-3′, and a reverse primer, 5′-TGGCGAAGTCAAGAGACG ACGCAG-3′. The reaction contained 12.5 µl of master mix (Promega, USA), 1 µl of each sense and anti-sense primer, 5 µl of the sample DNA, then finally the volume completed to 25 µl by 5.5 µl on nuclease-free water. The PCR was then programmed as the previous study that designed the primer (Zhang *et al.*, 2014). The PCR products then went through gel electrophoresis of 2% agarose and showed a sharp single band on the gel. The PCR products were sent to Macrogene Company for sequencing.

The results of sequencing were analyzed by using the software Mega-6 and NCBI-Blast alignment tools to reveal the genetic variations.

The serum level of GPX was estimated by commercial immunosorbent enzyme-linked assay (ELISA) Human Glutathione peroxidase (GPx) Kit, which applies the competitive enzyme immunoassay technique utilizing a polyclonal anti-GPx antibody and a GPx-HRP conjugate (Cat#; KT-64295). The serum level of SOD was determined by Superoxide Dismutase (SOD) Colorimetric Activity Kit (Cat. No. KT-745). This Kit was designed to quantitatively measure SOD activity in a variety of samples. The assay measured all types of SOD activity, including Cu/Zn, Mn and FeSOD types.

## RESULTS AND DISCUSSION

The real-timePCFR was done for all the subjected samples, the positive samples showed a sigmoid curve in the real-time PCR plot (Fig. 1). The results in Table 1 represent the positive samples for each test. The serological test showed all 100 samples positive. The real-time PCR showed 43% frequency of the samples. The conventional PCR was used to amplify the rop-17 gene in the positive samples, but showed a successful amplification in 92% of the real-time PCR positive samples.

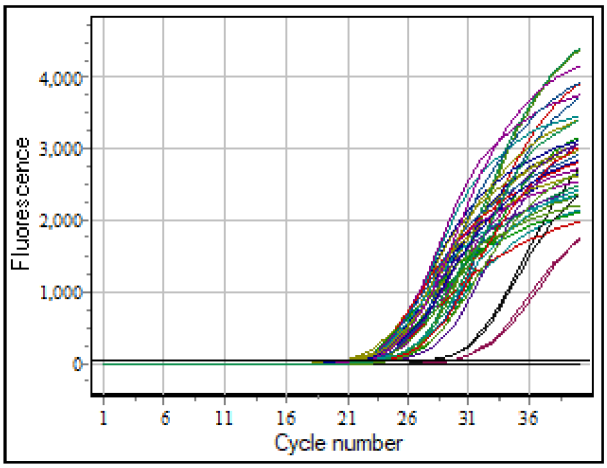


Fig. 1. Real time PCR amplification curves of the *Toxoplasma gondii* positive samples.

**Table 1.** Toxoplasma gondii positive samples by serological, real time PCR and cPCR

| Test | No. of positive samples | Frequency |
|---|---|---|
| Serologic | 100 | 1.00 |
| Real time-PCR | 43 | 0.43 |
| Conventional PCR | 39 | 0.92 |

After amplification of the the rop-17 gene, the PCR product was sent to the macro gene for sequencing (Fig 2). The sequence alignment showed different variations within the rop-17 gene of 18 isolates. The rest of four isolates showed no variations within the rop-17 gene (Table 2).

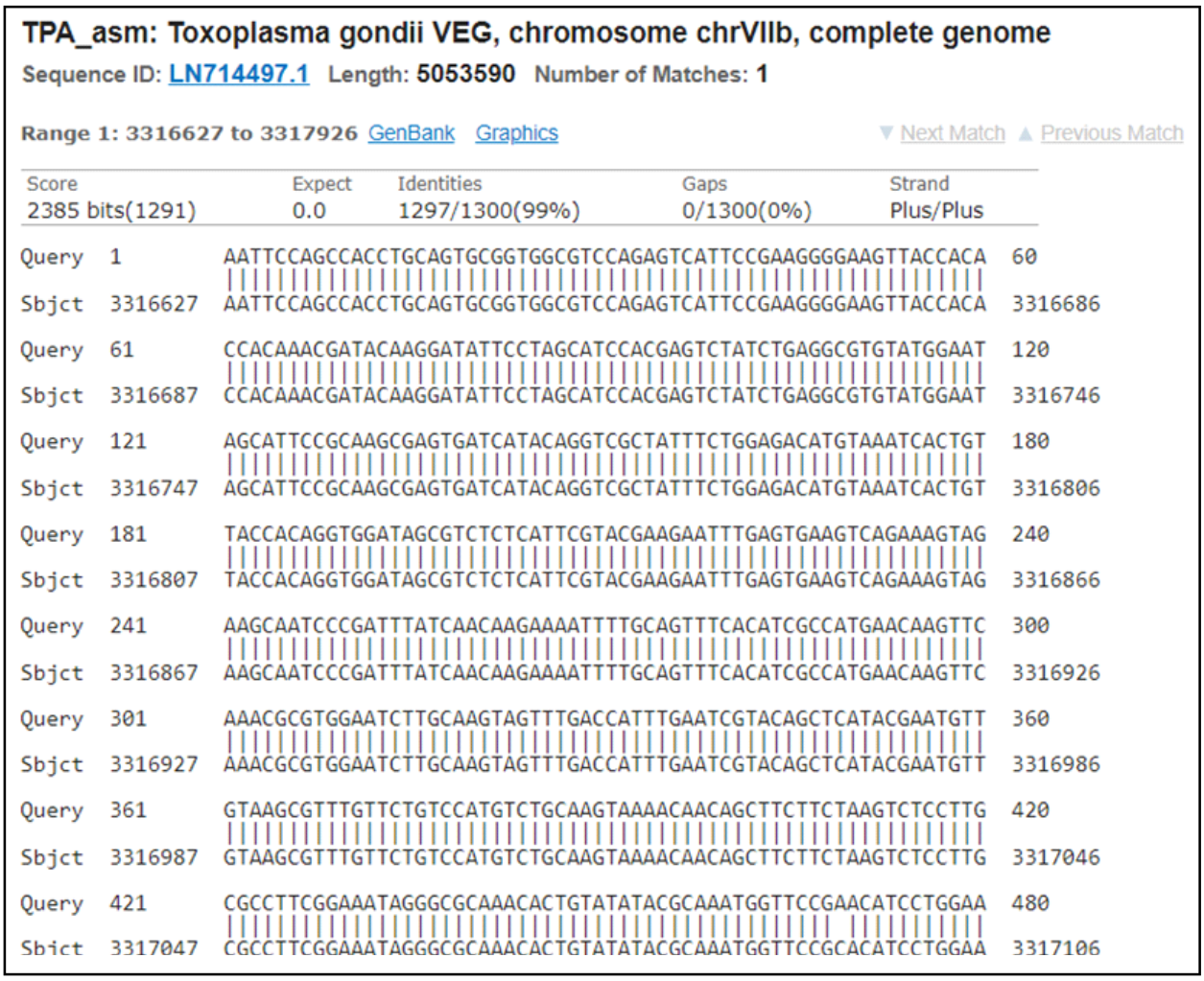


Fig. 2. Sequence alignment of the rop-17 gene.

Fourteen samples showed two variations when compared to the most sequence that showed similarity (ID: LN714497.1) within the NCBI. The first variation was missense in the bp 469, the second one in the 1024bp was also missense (Ala >ser). One sample showed three variations similar to the two previous variations but the third variation was located in the nucleotide 1204bp and it was missense. On another hand, three samples showed three variations : the first one was located at 361bp and it was non-sense, the second one was shown within all the varied samples located at 1024, the third one was located at 651bp and it was missense.

The GPX level recorded a higher level in patients that were positive for *T. gondii* than the patients negative to the parasite (89.6±9.4 and 76.5±31.4, respectively; Table 3). Similarly, the SOD showed a higher level in positive samples than the negative samples (93.76±9.6 and 85.6±7.43, respectively).

Until now-a-days, the ELISA technique that detects the IgM antibodies against *T. gondii* seemed to be a steadfast technique. However, this test was not enough for all kinds of patients, such as patients with AIDS. For this reason, numerous PCR types were developed to detect toxoplasmosis. These techniques made use of the most conserved gene sequences among different strains of *T. gondii* including the B1 gene repetitive sequence, the P30 (SAG1) gene, and ribosomal DNA (Hamad *et al.*, 2020). This study focused on the use of the rop-17 gene to detect the infection.

This study showed that 46% of the samples showed a varied sequence of the rop-17 gene, the variations that appeared in this study were completely different than the previous study

**Table 2.** Sequence variations of the isolated *Toxoplasma gondii*

| No. of samples | Type of substitution | Location | Nucleotide | Nucleotide change | Amino acid change | Predicted effect | Sequence ID | Score | ID | Source |
|---|---|---|---|---|---|---|---|---|---|---|
| 14 | Transversion | 469 | A>C | GAA>GCA | Glu>Ala | Missense | ID : LN714497.1 | 1291 | 99% | *Toxoplasma gondii* veg (rop-17) |
| | Transversion | 1024 | G>T | GCG>TCG | Ala>ser | Missense | | | | |
| 1 | Transversion | 469 | A>C | GAA>GCA | Glu>ala | Missense | ID : LN714497.1 | 508 | 98% | *Toxoplasma gondii* veg (rop-17) |
| | Transversion | 1024 | G>T | GCG>TCG | Ala>ser | Missense | | | | |
| | Transversion | 1204 | G>A | GGT>GAT | Gly>asp | Missense | | | | |
| 3 | Transition | 361 | A>T | GGT>GGC | Gly>gly | Non-sense | ID : LN714497.1 | 574 | 99% | *Toxoplasma gondii* veg (rop-17) |
| | Transversion | 1024 | G>T | GCG>TCG | Ala>ser | Missense | | | | |
| | Transition | 651 | T>A | ATT>AAT | Ile>asn | Missense | | | | |

**Table 3.** Serum level of GPX and SOD comparison between positive and negative subjects to *Toxoplasma gondii*

| Test | Group | | p-value |
|---|---|---|---|
| GPX | Negative for *Toxoplasma gondii* | 76.5±31.4 | 0.032 |
| | Positive for *Toxoplasma gondii* | 89.6±9.4 | |
| SOD | Negative for *Toxoplasma gondii* | 85.6±7.43 | 0.004 |
| | Positive for *Toxoplasma gondii* | 93.76±9.6 | |

that also focused rop-17 gene (Zhang *et al.*, 2014).

This study also demonstrated that each test had different specificity and sensitivity. The results of this study disagreed with the study that showed the serologic test was the most sensitive test for detection of the *T. gondii* (Salih *et al.*, 2019).

The GPX and SOD levels were higher in patients than in control. These results also agreed with a previous study by Aydin Türkoglu *et al.* (2018) that observed increased SOD and GPX level in women after 30 days of infection. The previous studies suggested that higher antioxidant defense on the acute phase can be useful to diagnose the parasite which is difficult to diagnose in this phase (Salih *et al.*, 2019).

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