MOLECULAR DIAGNOSIS FOR SURFACE ANTIGENS GENES (SAG1, SAG2, & SAG3) OF TOXOPLASMSIS IN BASRAH PROVINCE-IRAQ

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ABSTRACT
The current study was done in Basrah province during the period from January 2017 to September 2017. The results show that 8 (68.5%) were positive by conventional PCR for SAG1 gene and 12 (17.1%), 13 (25.7%) were positive by nested-PCR for SAG2, SAG3 genes respectively, whereas 4 (20%) of negative latex (control group) was positive by PCR for SAG1 gene only. No statistically significant difference between PCR positive for SAG1 and gender but there are significant role with age for same gene, also there are a relationship between infection with toxoplasmosis and time of abortion in aborted women, no positive case were seen in 3th trimester of pregnancy in all genes.

KEYWORDS: Toxoplasma, surface antigen, SAG1, SAG2, SAG3, Basrah, Iraq.

INTRODUCTION
Toxoplasmosis is a disease caused by an obligate intracellular protozoan parasite called Toxoplasma gondii which is appears to have broad host specificity and capable of infecting warm-blood animal including humans.[1] Felines act as intermediate and definitive hosts, all non-feline vertebrates including humans serve as intermediate hosts.[2] There were a several virulence factors that make T. gondii one of the most successful parasitic protozoa, one of these are surface antigens (SAG1, SAG2, SAG3 and SAG4) and they are very interesting in recent years, some researchers have used this surface antigens as modified molecules for vaccine[3], other used them as recombinant antigens for serodiagnosis to detection the antibody IgM and IgG by ELISA because they are the main target of the host immune response and they have no cross-reactivity with protein from other microorganisms.[4,5,6]

Nowadays, PCR is the most important and sensitive tool in diagnosis of toxoplasmosis infection and genotyping in clinical samples of human, and main attention has been attracting toward the surface antigen (SAG) of the parasite in both tachyzoite and bradyzoite stages named as SAG1, SAG2, SAG3 & SAG4.[7,8,9,10] These antigens are important in determine the acute infection also its consider immune target which provides a strong immune response against the invasive or act as an adhesion during host-cell attachment through is binding to proteoglycans.[3] The aims of this study were detection the prevalence of toxoplasmosis among random samples (female, male, children) by using conventional and nested-PCR for genes which encodes for surface antigen (SAG1, SAG2, SAG3) and determination the relationship between toxoplasmosis with age, gender and state of women (aborted, un aborted, un married).

MATERIALS AND METHODS
Regions, time and location of Study
A total of 200 venous blood samples were collected from different groups of patients whom retreated Basra laboratories during January 2017 to September 2017. The work was done in unit of PCR lab in Biology department/ College of Education for Pure Sciences / University of Basra.

Collection of blood samples
Different group of patients including of 76 of aborted women, 37 of un aborted women, 21 of un married women, 44 men and 14 child (<10 years). For each patient complete information were taken using questionnaire, patients ages ranged from two up to 40 years. Four ml of venous blood has divided into two volumes, 2 ml drawn into gel tube containing clot activator for serum separate. Gel tubes containing blood sample were left for about 10 minutes, then transported into centrifuge (fixed on 3000 rpm for about 5 minutes) to use in subsequent latex test, 2 ml of whole blood drawn into EDTA vial then kept in 20- C for longer period to use in subsequent molecular studies. All samples were tested by latex agglutination test then
chosen the positive latex cases only and 15 negative samples as control in molecular study.

Genomic DNA extraction
The DNA was extracted from the positive cases (70 blood samples) of latex test and 15 negative latex as control using genomic DNA extraction kit (Geneaid, USA) with accordance to the manufacturer's instructions.

Detection of *T. gondii* by PCR
Polymerase Chain Reaction (PCR) used to amplified the gene SAG1 for DNA genomic of *T. gondii* which extracted from whole blood sample by standard PCR for 30 cycles with using specific primers which obtained from Bioneer company as SAG1F (5'-TTGCGCGGCCCACACTG ATG-3') and SAG1R (5'-CGCGACAC AAGCGCGGATAG-3'). Nested-PCR using to detection the SAG2 gene using two separately amplified by standard PCR for 40 cycles with external primers SAG2a (5'-GCTA CCTCGACAGGAACAC-3') and SAG2aR- (5'-GCATCAACAGTCCGTTGC-3') at 62°C annealing temperature, the resulting amplification products were diluted 1/10 in DDW, and a second amplification of 40 cycles was performed with the internal primers SAG2b F (5'-GAAATGTTCAGTTGCTGC-3') and SAG2b (5'-GCAAG AGCGAACTTCAAGTTCCAG-3') R by using 1 µl of the diluted product as the template.[11] Also SAG3 gene was amplified by standard PCR for 35 cycles with external primers SAG3aF (5'-CAACTTCACACCATTCCACC-3'), SAG3aR (5'-GCCGTTGTTAGAC AAGACA-3') at 60°C and a second amplification of 35 cycles was performed with the internal primers SAG3bF, (5'-TCTTGTCGGGTCTTCACTA-3'), SAG3bR, (5'-CACAAG GAGACCAGAAAGA-3') by using 1 µl of first amplified product as the template[12] then the products of all genes were electrophoreosed on 2% agarose gel.

Statistical analysis
The data obtained was analyzed using Chi-square test to find out the significant differences among the data, the differences were recorded as significant whenever the probability (p) was less than 0.05.

RESULT
PCR of Toxoplasmosis for SAG1, SAG2 and SAG3 genes
The amplification of SAG1 gene by conventional PCR showed products at 913 bp in agarose gel electrophoresis analysis (Fig.1) and the separate amplification of SAG2 gene by n-PCR showed products at 241 bp, whereas the amplification of SAG3 gene showed products at 290 bp in first amplification, and 241 bp in second amplification (Fig.1).

Out of 70 positive latex patients, 48 (68.5%) were positive by conventional PCR for SAG1 gene and 12 (17.1%), 13 (25.7%), were positive by nested-PCR for SAG2, SAG3 genes respectively, whereas 4 (20%) of negative latex (control group) was positive by PCR for SAG1 gene only (Table1).

According to the gender, the high percent of positive SAG1, SAG2 and SAG3 genes were seen in female but no significant effect (P< 0.05) was observed, whereas result revealed that there was a positive relation between the age group and SAG1 gene (Table 2).

The high percent of SAG1 gene positive was seen in un aborted women whereas the high percent for SAG2, SAG3 gene were seen in aborted women with significant effect, despite recording the highest percentage of SAG1 gene positive in un aborted women but there are no significant effect (P< 0.05). (Table 3).

![Figure 1: Agarose gel electrophoresis analysis of SAG1 gene](image-url)

M. 100 bp marker, 1,2,3,4, product 913 bp.
Table 1: Prevalence of PCR positive cases of toxoplasmosis in patients according to the genes (SAG1, SAG2, SAG3).

<table>
<thead>
<tr>
<th>Genes</th>
<th>Patients</th>
<th>Control</th>
</tr>
</thead>
</table>
|       | No.      | Ve+     | No. | Ve+ | %  |%
| SAG1  | 70       | 48      | 15  | 3   | 20 |
| SAG2  | 70       | 12      | 15  | 0   | 0  |
| SAG3  | 70       | 13      | 15  | 0   | 0  |

(P ≤ 0.05)
The highest percent of \( SAG1 \) gene positive (100%) was seen in women with double abortions, and the highest percent of \( SAG2 \) and \( SAG3 \) genes positive were seen in women with triple and more than three abortions with significant effect (\( P \leq 0.05 \)) for all genes, according to the time of abortion, the highest percent of \( SAG1 \) and \( SAG2 \) genes positive were seen in women abortion in first trimester of pregnancy with no significant effect, whereas the highest percent of \( SAG3 \) gene positive was seen in women whom abortion in 2th trimester of pregnancy. No positive case were seen in 3th trimester of pregnancy in all test (Table 4).

**DISCUSSION**

The conversion between, tachyzoite and bradyzoite forms is associated with morphological and molecular biological changes, including stage-specific gene expression and alterations in metabolism\(^{[14]}\) so some genes are specific in tachyzoite stage like \( SAG1 \) gene and another is specific in bradyzoite stage such as \( SAG4 \) gene\(^{[15]}\), others are expression in both stage like \( SAG2 \)\(^{[16]}\) and \( SAG3 \) gene\(^{[17]}\). The use of molecular diagnostics for these antigens are most effective, not only to detect the parasite but also to determine the acute infection and the techniques which is used to detect these surface antigens is PCR which is used to detect DNA of *T. gondii* in various biological samples and has showed higher sensitivity and specificity in diagnosis compared to serological tests\(^{[18,19]}\).

The results showed that the \( SAG1 \) gene was the most prevalence (50.7%) in molecular diagnosis of the acute infection of toxoplasmosis in blood samples, followed by \( SAG3 \) then \( SAG2 \) genes (19.8%, 16.6%) respectively, and the positive case of these genes mean that the patients have acute stage of toxoplasmosis. Recorded of \( SAG1 \) gene as a most gene prevalence in this study comparison with \( SAG2 \) and \( SAG3 \) may be due \( SAG1 \) antigen constitutes the most abundant and predominant antigen in surface of *Toxoplasma*\(^{[20]}\).

Recording of highest prevalence in female is agree with other previous studies\(^{[21]}\) found that *T. gondii* in female was highest compared with male in some Iraqi individuals. In Baghdad, no difference in the rate of infection between male and female.\(^{[22]}\) But

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**Table 2: Prevalence of PCR Positive cases of toxoplasmosis in patients according to the Age and gender.**

<table>
<thead>
<tr>
<th>Genders</th>
<th>( SAG1 )</th>
<th></th>
<th>( SAG2 )</th>
<th></th>
<th>( SAG3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Ve+</td>
<td>%</td>
<td>No.</td>
<td>Ve+</td>
</tr>
<tr>
<td>Female</td>
<td>47</td>
<td>35</td>
<td>74.4</td>
<td>47</td>
<td>10</td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>10</td>
<td>58.8</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>children</td>
<td>6</td>
<td>3</td>
<td>50</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>48</td>
<td>68.5</td>
<td>70</td>
<td>12</td>
</tr>
</tbody>
</table>

(P \( \leq 0.05 \))

**Table 3: Prevalence of PCR Positive cases of Toxoplasmosis in female and relation with aborted, un aborted and un married.**

<table>
<thead>
<tr>
<th>State of female</th>
<th>( SAG1 )</th>
<th></th>
<th>( SAG2 )</th>
<th></th>
<th>( SAG3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Ve+</td>
<td>%</td>
<td>No.</td>
<td>Ve+</td>
</tr>
<tr>
<td>Aborted</td>
<td>29</td>
<td>21</td>
<td>72.4</td>
<td>29</td>
<td>9</td>
</tr>
<tr>
<td>Un aborted</td>
<td>11</td>
<td>9</td>
<td>81.8</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Un married</td>
<td>7</td>
<td>5</td>
<td>71.4</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>35</td>
<td>74.4</td>
<td>47</td>
<td>10</td>
</tr>
</tbody>
</table>

(P \( \leq 0.05 \))

**Table 4: Relationship between number and time of abortion and toxoplasmosis.**

<table>
<thead>
<tr>
<th>Number of abortion</th>
<th>( SAG1 )</th>
<th></th>
<th>( SAG2 )</th>
<th></th>
<th>( SAG3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Ve+</td>
<td>%</td>
<td>No.</td>
<td>Ve+</td>
</tr>
<tr>
<td>Single</td>
<td>12</td>
<td>7</td>
<td>58.3</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Double</td>
<td>7</td>
<td>7</td>
<td>100</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>triple &gt;3</td>
<td>7</td>
<td>5</td>
<td>71.4</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>66.6</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>21</td>
<td>72.4</td>
<td>29</td>
<td>9</td>
</tr>
</tbody>
</table>

(P \( \leq 0.05 \))
seroprevalence of toxoplasmosis in females was more than male[23] the positive cases of Real-Time PCR of SAG1 in female were significantly higher than those of male.[24] But its disagreement with[25] whom recorded Toxoplasma in male highest than female, this difference variation due to several factors such as sex differences in immune responses, social activities and different occupational, and differences in exposure of toxoplasmosis infection.[26]

The variation between adults and young human beings in deferent country may be due to the difference in environmental conditions, different nutrition, behavioral patterns of life immune status of the persons, socio-cultural differences, and topography which expose the population to the infective form of parasite in different ages.[27]

It seem that the highest positive PCR for surface antigen was seen in 21-30 age group by SAG1 and SAG2 genes, the study was in disagreement with some local studies such as[28] which recorded highest percentage of toxoplasmosis was seen in[29, 30, 31] category of women suspected infection by this parasite, researcher recorded the highest percentage of infection in[32, 29, 30, 31] age group. The varying results from those regions may be due to differences in patients number, type samples, demographics, history and used techniques.

It is appeared that all groups of women are susceptible to T. gondii infection and the risk of this parasite is not limited to women with a history of abortion, but even to women who are not abortions for the chance of subsequent abortion, also the present study demonstrate that the parasite can be dangerous on un married women this agree[29] in Kirkuk province who recorded the highest positive toxoplasmosis in unmarried women compared with married women.

REFERENCES