



THE GENETIC SEQUENCE OF THE HUMAN *TOXOPLASMA GONDII* – IN BAGHDAD

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ABSTRACT

Toxoplasma gondii considered one of the most common and dangerous protozoan parasites for human, this due to the ability to invade all parts of the body and may lead to the physiological changes known as toxoplasmosis. Determination the genotyping and genetic sequence for *T. gondii* isolated from aborted women through cultivated and inoculated into mice. This study lasted for the period from the beginning of December/ 2015 to the end of August/ 2016 at technical analytical department/ health and medical technology college. Seventy isolates of *T. gondii* were identified from the amniotic fluids of aborted women; these isolates were inoculated in mice. All samples Were subject to Nested polymerase chain reaction and restriction fragment length polymorphisms for genotyping determination while, gene sequence was performed for 17 sample depending on site of surface antigen for protein on SAG2 gene for each of the I , II and III genotype. Twenty four isolates was observed for I genotype, 42 for II genotype and 4 for III genotype in all 70 samples of human *T. gondii* isolates. Gene sequence technology was conducted for 17 samples out of 70. The results indicated the presence of a partially mutation in genotype I at the site gaggggtggg which changed into gaggcctggg compared with the wild strain. This study presents the original data of *T. gondii* genotype in Baghdad. The value of molecular diagnostic methods, due to their high specificity and sensitivity, was comforted according to the result of current study in addition to conventional microscopical methods, It is shown that the predominance genotype was type II and much less of genotype III. Two of the type I isolates showed a point mutation at the gaggggtggg locus that altered to gaggcctggg in rapprochement with the wild strain.

KEYWORDS: Human *Toxoplasma gondii*, DNA amplification, Gene sequencing.

INTRODUCTION

Toxoplasma gondii a unicellular obligatory protozoan which is not like any other scrounger, it is the most insidious types of parasites and there are subtle injuries get by this parasite^[1,2]. Transmission of infection may occur orally(Both waterborne and foodborne) which is called the horizontal transmission or from the mother to her fetus which is called vertical transmission, also sexual transmission has become a common presence around the world^[1,3]. Recent studies revealed that this parasite is one of the carcinogen factors^[4]. Occurrence of genetic mutations makes the parasite more virulent^[5]. There are several surface proteins that represent genes and used to determine the type of *T. gondii*, such as SAG2 gene which plays an important role in adhesion on the membrane^[6]. Therefore, to determine the presence of gene mutations have significance important role in increasing the virulent of the parasite^[7]. Such mutations are the reason for the occurrence of many pathological cases like abortion in pregnant women and cancers diseases^[8]. Some parasite proteins were extracted to manufacture as a vaccine including the excretory-Secretary dense granule proteins (GRAs), Rhoptry Proteins (ROPs), and Micronemal Proteins (MICs),^[6,9,10,11].

MATERIALS & METHODS

Collection of Samples

Seventy samples of amniotic fluids were collected from aborted women attended Hospitals for delivery (Baghdad Teaching Hospital and Al-Ilweia Hospital). These samples were treated as described by^[12,13] microscopical examination was done for the parasite detection by preparing two slides each one containing one drop of sediment, the first slid was examined directly under 40X and the second slid was stained with 10% Giemsa. All samples have been treated with 3 ml of preservative Ringer saline till use^[14].

Mice inoculation

Following the procedure described by Rasheed, 1984,^[15] briefly the suspension of prepared solution was centrifuged at 3000 rpm for 15 min then 10 ml of sterile normal saline (0.9 %) were added to the sediment and re-centrifuged .This process repeated for three times, 10 ml of sterile inoculum was prepared by adding (100 µg) of Streptomycin and (1000 IU) of Penicillin per 1 ml of the inoculums^[16]. Seventy Swiss albino mice aged 6-8 weeks and their weight range between 210 - 280 gm were injected using intra-peritoneal (ip) route with 0.2 ml of *T.*

gondii strain^[17], *T. gondii* isolation were obtained after 7-10 days after inoculation^[18].

DNA Extraction and Amplification

T. gondii DNA was extracted from mice peritoneal exudates by application of a ready kit of blood according to the internal newsletter of blood extraction kit supplier of the company (Promega, USA). Amplification of DNA was done using Nested Polymerase Chain Reaction (nPCR) to amplify the gene B1 using the set of primer F: GGAAGTGCATCCGTTTCATGAG and R: TCTTTAAAGCGTTCGTGGTC^[19,20].

Determination of *Toxoplasma gondii* genotypes

By using restriction fragment length polymorphisms method, two types of endonucleases enzyme was used in splitting process, *Sau3A1* enzyme which digest the 3rd allele at 5' end (type III) and *HhaI* enzyme which digest the 2nd allele at 3' end (type II). If the fragmentation or splitting doesn't occur by any of these two enzymes it will referred to presence of Type I strain.

Prepare of antigen

A soluble antigen was prepared by destroyed the vegetative phase of the parasite. By using repeatedly

freezing and thawing process, a total of 5×10^6 per ml were mix up and centrifuged at 12,000 rpm for 45 min at 4°C. After conducting the steps of preparation and dissimulation it is stored at -20°C until use^[21].

Prepare the sample for Sequencing

The DNA is fragmented into a lot of Pieces by restriction enzymes. As the molecular detection methods for individual DNA molecules sequencing are not sensitive enough, an in vitro cloning steps are used for DNA amplification^[22].

Loading of sample

PCR products which cared on agarose gel plate that submerged in 1X TBE solution and pass the voltage value of 95 volts degree at 95 V for 40 min. for base pairs determination^[23].

Sequence reaction:

Procedure:

The application of the instructions issued by the manufacturer was followed using V3.1 cycle dye of sequencing kit. (Perkin-Elmer, Foster city, CA) number 4336817:

TABLE1: Thermal pattern used in sequencing reaction:

Number of cycle	Time	Temperature ° C
1	1 min.	96
25	10 sec.	96
	5 sec.	50
	2 min.	60

The version BIOEDIT 7.0.4.1.

The biological sequence alignment editor written for Windows 95/98/NT/2000/XP.

MEGA 4.0.2 software

MEGA: is an essential tool used to perform automatic and manual sequence betoken of phylogenetic trees. By estimating the rates of molecular evolution by online databases.

Sequence FASTA files

Which are used in the phylogenetic tree, it has been submitted to the bank's General.

Provide genome sequences

It was presented sequence according to the guidance provided by the Internet bank of Gene tool.

PCR products/ Cloning and sequencing

Through the amplification of the (241 and 221bp) of excess primers with nucleotides using the Min El extraction kit (QIAGEN), and then reproduced in PCR_4 TOPO Several carriers using TOPO TA cloning in sequence. The sequence analysis in comparison with the late transcription factor (VLTF-1) gene sequences from different pox Parra in databases using Blast Online^[24].

RESULTS

The forward and reverse of sequence results were received via mail as text, BLAST reports and AB1 files.

The amplification of DNA by B1 gene primers

Amplify sized pieces 221 and 241bp from B1 gene of the DNA extracted by nested PCR by Daport deported electrically reaction solution with a concentration of 1.5% agarose gel to use DNA size ladder 100bp Figure (1).¹¹

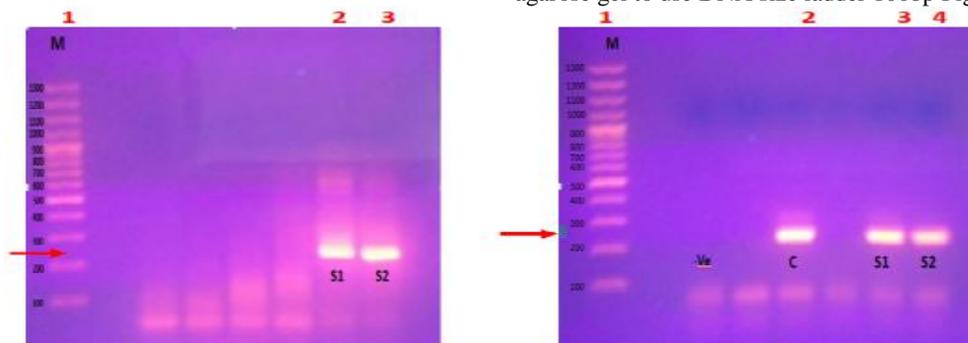


FIGURE 1: Amplification of *Toxoplasma gondii* genome by nested PCR using *Toxoplasma* gene B1¹¹

A: 5 Amplification Product, B: 3 Amplification Product Lane1:, ladder, Lane1: ladder, Lane2: Sample 1, Lane 2: Control, Lane 3: Sample 2, Lane 3: Sample 1, Lane4: Sample 2

When comparing the nucleotide sequence and BLAST analysis of *T. gondii* isolates with what exists in Gen Bank, we find that there are 100% homology with genotype II.

TPA-asm *Toxoplasma gondii* VEG, chromosome chrVIII, complete genome

Sequence ID: tpe [N7144984498, 1] Length: 6937759
Number of Matches: 1 Range 1: 4761662 to 4761831 Gen Bank Graphic.

The main sequence of SAG2

Tgcctccgct_tcgaaattgt_ctgcaaaaga_aaacgttgtt
gaggggtg gg _ attgcgattg

In figure 2, similarity in all the seventeen *T. gondii* isolates at the site of SAG2 gene were recorded as shown in the phylogram map.

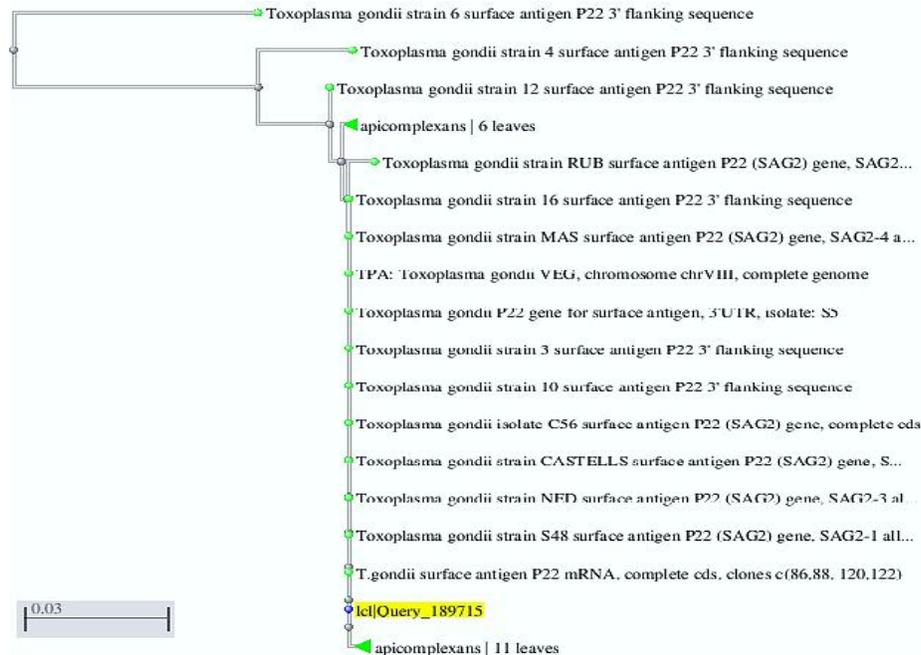


FIGURE 2: Phylogram Map of 17 *Toxoplasma gondii* strains determined by analysis of the entire sequences of the SAG2 genomic region

DISCUSSION

Many of the research in the field of molecular technology included a study of *T. gondii* surface antigens for the purpose of causative agent diagnosis, study of virulence factors and investigate the presence of mutations^[25,26]. Toxoplasmosis became threatened human life due to high morbidity rate and the possibility of infection without clinical signs which may lead to development of other human pathologies^[27]. Many studies have investigated preventive immune from the various immune components of *T. gondii*^[28]. In immunization field, the new strategy of encoding antigenic proteins of pathogenic agent by plasmid will enhance cellular and humeral immunity against many of infectious and non-infectious pathogens^[29]. SAG2 consider to be the major surface antigen among all *T. gondii* antigenic components which was identified using monoclonal antibody^[9]. Thus, an excellent immunogenicity and antigenicity of SAG2 protein will provide an effective diagnosis method or vaccines^[10]. In addition, the sequence of SAG2 scored a high degree of homology among different strains of *T. gondii* in both type I (pathogenic) and type II, III (cytogenic) strains^[30]. Therefore, many researchers in recent years have tried to use recombinant SAG2 protein in serological diagnostic method or in experimental animals through express SAG2 in different hosts including *E. coli*, *Pichia pastoris*, baculo-virus and insect^[31]. It was necessary to refold or

use the truncated SAG2 in serological methods as this recombinant protein loses specific immunogenicity when produced in *E. coli*^[32]. The expression of a fragment (957 bp) of SAG2 / RH *T. gondii* that cloned in *E.coli* was studied by *Kazemi et al.* (2007) in order to use this antigen for Toxoplasmosis detection using Enzyme Linked Immuno-sorbent Assay (ELISA)^[33]. The extracted genome of the loci of SAG2 was applied in DNA sequencing^[32]. In this study, the sequence analysis and comparison to the wild-type showed a point mutation in the isolated strain of Type I at the locus gaggggtggg which mutated to gaggcctggg mean while , compatibility was recorded for each of the Type II and Type III, these results were similar to the proven by *Farrell et al.* (2014) through his conclusion in that the *T. gondii* has a chemical mutagenesis to change the sequence of nucleotides, and this shall be more virulent in the incidence of infection^[34].

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