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Alteration of genetic sequence of *Toxoplasma gondii* by using the bands missing in ppGalNAc strains as a treatment

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Abstract

The study was conducted on 48 serums, placental and umbilical cord samples taken from pregnant women, who have undergone abortion in Alawiya Obstruction and Gynecology and Al-Imam Ali Hospital from the period 15th January, 2018 to 1st September, 2018. Twelve out of 48 samples have been diagnosed by gene expression technique. The results regarding the distribution of *T. gondii* infection according age groups, showed that 18(37.5%) is more frequent in both aborted and healthy women. While the age groups 14(29.2%) was found in (26-35) years and 16(33.3%) in the age group (36-46) years. *T. gondii* strains were identified by Real Time PCR technique. For deleting of some bands of ppGalNAc of parasite to throw or shed a light on the mechanisms of *O*-GalNAc glycosylation in *T. gondii*, by verifying *T. gondii* tachyzoite *in vitro* augmented under (pH 7.1 with 5% CO_2). The pathogenic toxoplasmosis was identified by Real Time PCR technique.

INTRODUCTION

Toxoplasma gondii invades the host body by a mechanism which has a multi-organ, like roptries and micronimes, and the bradyzoite have a rigid glycoprotein wall. The Bradyzoite stage have a rigid cyst wall from glycoproteins which is a thick structure with compact layers [1]. The structure of the tissue cyst stage wall of *Toxoplasma gondii*, which is compact and rigid, measures between (250- to 500-nm), and composed of two layers outer, and an inner sponge-like layer [2]. The outer rigid layer of this bradyzoite composed of glycosylated with carbohydrate and various lectins [3]. For the purpose of changing the severity and invasion characteristics of this parasite, the rigidity of this wall has been altered [4]. Experimental data contained several such as those that are currently known to be situated. The *O*-GalNAc glycosylation is consider as a common post-translational modification [5], or mega karyocyte/platelet glycoprotein GPIbα [6]. The modification is essential for the protein function [5]. To develop *Toxoplasma gondii* in vitro, enrichment materials like protein and vitamin-free plantations must be added to provide a suitable environment for growth [7]. This automated modulation gets the carbohydrates to the wall of the cyst to hide the wall proteins and makes it less rigid and vulnerable to the host immune system [8]. CST1 protein exists in the cyst wall of *T. gondii* which composed of mucin-like domain, to which the parasites' rigidity and stability are attributed to. Hence, any changes in the cyst's wall ultra-structures are critical for their functions. However, the exact mechanisms by which this posttranslational modification changes the functions of these different proteins is unclear [8]. The domain CST1 is, therefore, a major cyst wall component involved in main function of this structure [9]. The current study aimed to clarify the mechanisms of *O*-GalNAc glycosylation in *T. gondii* functioning.

MATERIALS AND METHODS

Forty-eight serum, placental and umbilical cord samples were taken from pregnant women who were suspected to have toxoplasmosis from Alawiya Obstetrics and Gynecology and Al-Imam Ali Hospitals, during the period from 15th January 2018 to 1st September 2018. Eighty Four serum samples were taken from aborted women who were suspected for *T. gondii* infections, IgM and IgG for detection of acute and chronic injury were performed by ELISA technique. 12 out of 48 samples have been diagnosed with toxoplasmosis. The samples of placental and umbilical cord tissues were collected during abortion and taken by Petri dishes. These tissues were minced to small pieces and digested by acid pepsin solution. To the solution, approximately 10 times the size of partially extracted placental umbilical cord tissue has been added in a clean dry beaker and stirred in the magnetic stirrer for 30 min, then left at room temperature. In many sterile gauze layers, the digested material has been filtered; the filtrate has been pleased in a clean test tube and spun 5 minutes at 3000 rpm. Direct microscopic examination has been done to detect the parasite. The filtrate has then been discarded and one drop of the precipitate has been put in a clean class slide by a micropipette, mounted under a cover slip and examined under x40 lens microscope.

Another smear sample was prepared and left to dry and fixed by methyl alcohol for two minutes, and stained for 30 min with 10% Giemsa stain to see the colored parasite. The sediment of each sample was added to 3 ml of Ringer Saline(with concentration: sodium ion = 130 mmol/L chloride ion = 109 mmol/L, lactate = 28 mmol/L, potassium ion = 4 mmol/L, 3 mEq of calcium ion = 1.5 mmol/L), and then centrifuged at 3000 rpm at 5 minutes. The filtrate is discarded, and the sediments were washed by the same volume of Ringer saline, the process was repeated three times, the solution re- suspended by 3

ml of Ringer saline till use, [10]. DNA was extracted and pathogenic *T. gondii* strains were identified by Real Time PCR technique. For deleting of some bands of ppGalNAc of parasite to show the role of *O*-GalNAc glycosylation in *T. gondii*, by *in vitro T. gondii* tachyzoite cultivation under (pH 7.1 with 5% CO_2).

RESULTS

Effect of age groups on *T. gondii* infection

The distribution of patients according to age groups under study summarized in (table 1) it was found that the age group of (16-20) years 18(37.5%) are more frequent in both of aborted women and healthy categories. While the age groups (26-35), 14(29.2%) and (36-46) years 16(33.3%) more than second group of aborted women respectively.

Table 1: Distribution of *T. gondii* infection according to age groups

Age groups / Year		Aborted women	Chi-Square (P-value)
16 - 25	N	18	P<(0.05)
	%	37.5%	
26 - 35	N	14	
	%	29.2%	
36 - 46	N	16	
	%	33.3%	
Total	N	48	
	%	100.0%	

Correlation of IgM Anti-*Toxoplasma gondii* and Gestational trimesters

It is clear from table 2 that most abortion cases occurred at the first trimester 53 (73.6%), of them were positive for IgM anti *Toxoplasma gondii* with difference in comparison with only 14 (58.3%) at the second trimester.

Table 2: Correlation of IgM Anti-*Toxoplasma gondii* &Gestational trimesters.

Anti-Toxoplasmosis IgM Ab.		Gestational trimesters		Total	Chi-Square (P-value)
		First	Second		
Negative	N	9	5	29	P=0.158 NS (P<0.05)
	%	25%	41.7%	30.2%	
Positive	N	27	7	67	
	%	75%	58.3%	69.8%	
Total	N	36	12	96	
	%	100.0%	100.0%	100.0%	

Association of IgG Anti-*Toxoplasma gondii* &Gestational trimester's periods.

It seems from table 3 that the chronic infection enhanced by progression of pregnancy hence the positivity of IgG elevated from 77.8% at first trimester up to 91.7% during the second trimester.

To determine the pathogenesis of *Toxoplasma gondii* by the Real Time technique for 12 isolates of this parasite by JOE (Yellow)/HEX/Cy3 channel; IC is detected on the FAM (Green) channel, of by Real Time PCR as shown in figure 1.

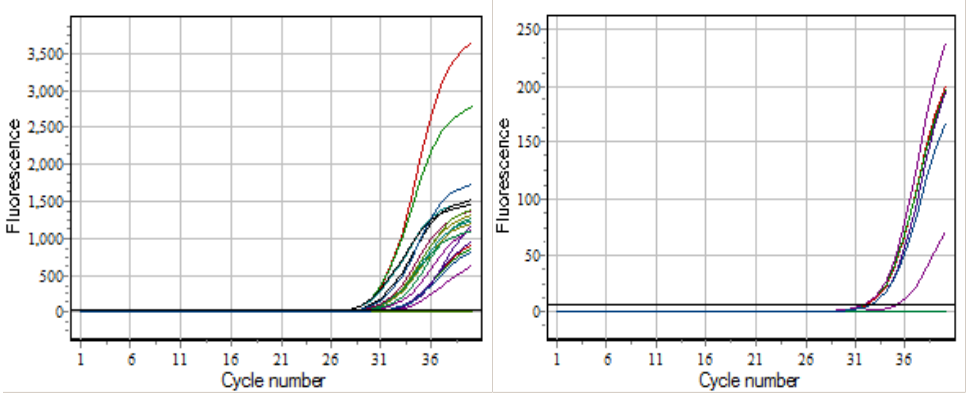
Table 3: Correlation of IgG Anti-*Toxoplasma gondii* &Gestational trimester's periods.

Anti-Toxoplasmosis IgG Ab.		Gestational trimesters		Total	Chi-Square (P-value)
		First	Second		
Negative	N	8	2	9	P=0.131 NS (P>0.05)
	%	22.2%	8.3%	18.8%	
Positive	N	28	22	37	
	%	77.8%	91.7%	81.3%	
Total	N	36	24	48	
	%	100.0%	100.0%	100.0%	

Treated of *T. gondii* genes by missing *O*-glycosylated protein bands.

The SDS-PAGE, was used for the separation of parasite lysates from the Δ*t1* and knockout strains, then they were transferred on a "poly-vinylidene di-fluoride" (PVDF) membrane, and the following lectins were used probed them: JACALI, VVA, HPA, DBA, A (ConA), (s-WGA, SNA-I I), and GSL-1 Three specific glycoprotein bands of 95 kDa were determined by labeling at 37, 49, and 60 and were present in Δ*t1* strains but lack the ppGalNAc-Δ*t2* and -Δ*t3* parasites. In general, these data conclude either ppGalNAc-type2 alone or both type2 and type3 are needed to form these lectins binding protein (figure 2).

Because of the variation that took place in the three specific glycoproteins at 37, 49 and 95 kDa which were shown in the ppGalNAc-Δ*t1* type whereas disappeared in ppGalNAc-Δ*t2* and -Δ*t3*strains. All the lectin binding bands were restored by T2 complementation and T3 complementation was also restored. The change occurred in the wall, in character the rigidity and invasion mechanism. The gene expression for the exons primers was showen in figure 3.



Determination of pathogenic *T. gondii on* the JOE (Yellow)/HEX/Cy3 channel; IC is detected on the FAM (Green) channel, of by Real Time PCR.

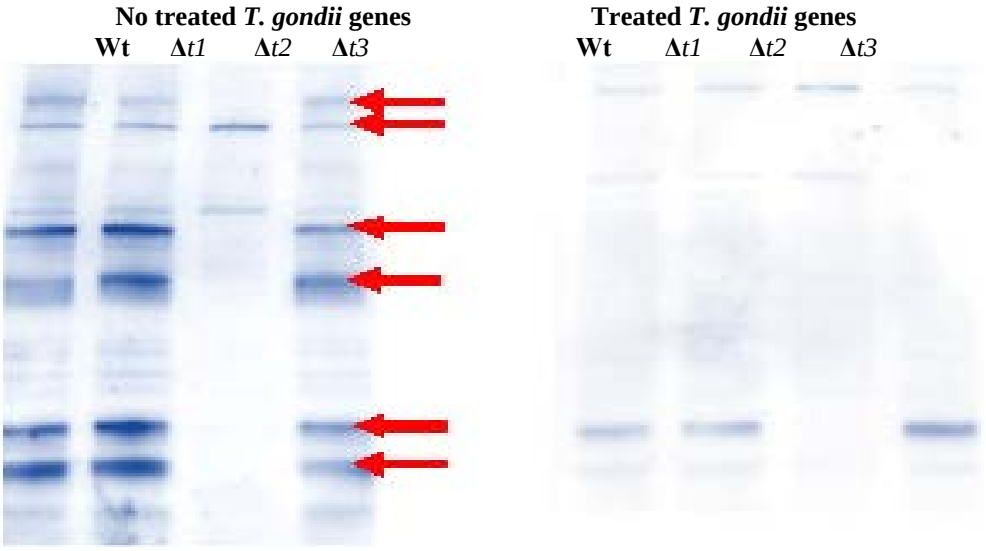


Figure2: Lectin profiling of ppGalNAc- *T. gondii* strains. The SDS-PAGE were used for separation of *T. gondii* strains purified lysates, which were then transferred on a PVDF membrane then after addition of biotin-conjugated and incubation. In both ppGalNAc-Δ*t2* and -Δ*t3*strains bands were missing, and for detection of these bands nonspecific (Streptavidin) was added. The blot showed activities of ppGalNAc-T2 and -T3 glycosyl transferase that are needed for multiple *T. gondii* glycoproteins. The blots duplicate on the right side which were undergone β-elimination, revealed that O-linked action.

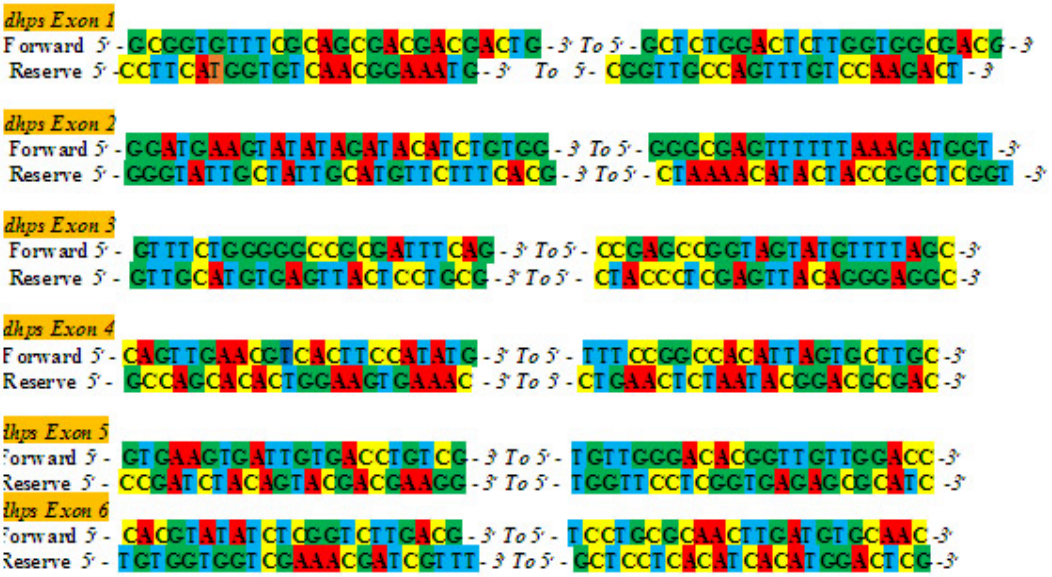


Figure 3: The gene expression for used to amplify the full length hydroxy methyledi-hidropterin pyrophosphokinase- droptiraote synthase (*dhps*) coding sequence cDNA.

DISCUSSION

The distribution of toxoplasmosis among age groups of aborted women between 16 -46 years was converged. These findings were agreed with Mohammed *et al.,* 2015 who reported that the distribution among these ages was converged [11]. The correlation of IgM Anti-*Toxoplasma gondii* &Gestational trimesters, about this study it was found that the acute infection of *T. gondii* was in the first trimester. This was true with Mohammed's 2015, finding that most of the acute parasitic infections were first trimester, but it was more chronic in the second trimester [12]. For identified the pathogenic infectious toxoplasmosis using Real time technology by gene expression on the JOE (Yellow)/HEX/Cy3 channel; IC is detected on the FAM (Green) channel. For identified of pathogenic *T. gondii* strains of gene expression on ROP21 protein gene [13]. By modified *O*-glycosylated proteins, the strains were separated onto a "poly-vinylidene di-fluoride" (PVDF) membrane, and probed with the above lectins: Tissue cyst wall protein, including the physico-chemical properties, transmembrane domain, sub-cellular localization, secondary and tertiary structure, B and T-cell potential epitopes, and other important characteristics of this protein. The findings showed that cyst wall protein had several potential post-translational modification sites, Foroutan, *et al*. (2018) [14]. This changes the specifications in the toxicity factors of *Toxoplasma* parasites and becomes less effective in cases of infections [5]. The purified lysates from *T. gondii* strains were separated by the missing bands in both ppGalNAc-Δ*t2* and -Δ*t3*strains led to change the

genetic sequence in a multi place on the starter by amplify the full length hydroxymethyledi-hidropterin pyrophosphokinase-droptiraote synthase (*dhps*) coding sequence cDNA, the change occurred by it that prominent in several sites with nitrogen bases which led to change the qualities of virulence in the parasite during the invasion [2]. This change in the genetic sequence of these exons made the parasite lost a lot of ferocity factors. These findings agreed with Tedford and McConkey (2017), who reported that the change occurrence on the bradyzoites after the missing of some the proteins, which is evident through the misses of bands and this alteration changes the virulence of the parasite [15]. Huang, *et al;* reported the transcriptional changes escort or took along the variations of transformation of the rapidly proliferating tachyzoites into the encysted bradyzoites, due to a mechanism still obscure underlying these alterations in gene expression [16]. By this modification of the parasite genes in changing the path of invasion which was done on the tissue cyst stage and it has become less harmful inside the host body [17]. The benign genetic mutation on these exons altered the pathogenesis of the parasite and showed that the nucleotides sequence may change markedly. In *dhps* exon 1, in forward primer the changed from GCGGTGTTTCGCAGCGACGACGACTG to GCTCTGGACTCTTGGCGACG. ATCGTTT to GCTCCTCACATGGACTCG. This is considered a benignant genetic mutation and makes the parasite less dangerous in the invasion mechanism of host tissue, these results are matched with Dass, and Vyas (2013), who declared that the *Toxoplasma gondii* infection established an epigenetic alteration in the DNA methylation of the arginine vasopressin promoter in the host tissue, that resulted in new inherited characteristic of the parasite [17]. As Bouchut, *et al.*, (2015), expressed that the changing impact in the protein localization, interactions, activity, and stability, and present the first proteome-wide survey of lysine acetylation in host cells [18].

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