Synergistic Effect of Some Molecular Biomarkers in The Development of Preeclampsia and Abortion in Pregnant Women Suffer from Toxoplasmosis

Fulk Abdulhafed Khattab[1*], Najah S. Nayef[2], Saad Ghanim Salih[3]

[1,2]*Department of Biology, College of Science, University of Mosul, Mosul, Iraq.*
[3]*Department of Biophysics, College of Science, University of Mosul, Mosul, Iraq.*
[1]*Fulsbio@uomosul.edu.iq,* [2]*najhsbio26@uomosul.edu.iq,* [3]*Saadghanim52@gmail.com.*

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ABSTRACT

The current study summarized the effect of toxoplasmosis, which is caused by parasitic infection with protozoan known as *Toxoplasma gondii*, on pregnant women who suffer from repeated miscarriages, especially in the first trimester of pregnancy, this research documented the reduction of sex hormones values in healthy pregnant women, association with down *ROP16* regulation(*T.gondii*-I virulence factor). While the average of the same parameters was increased in pregnant women who suffered from toxoplasmosis, which in turn led to inhibition of the transcription factor *NFκB* in parasite`s infected women, and inhibition of the patient's immune response. On the other hand, both of *ROP16* and *NFκB* genes play a critical role in an increase of *miR146* transcription, it consider a significant factor that activate epigenetic effect perhaps play role in reducing of host's immune system. At the end the placental cell signaling pathway was modified and increased atrophy of placental trophoblast with association of serious complications led at the end to emergence of early abortion.

1. Introduction

Apicomplixa is a phylum that belong to animal kingdom, which have many obligate intracellular parasites like *T.gondii* (Knoll *et al*.,2019). Infection with this parasite clearly associated with appearance of clinical signs and complications during pregnant period lead to loss of embryo (Attias *et al*.,2020).

Sexual hormones participate in regulating host´s Immune response and create a suitable environment for fetus development and growth and reduce host immune rejection, during pregnancy (Wu *et al*.,2022). Proges-terone play a critical role in stimulating T-helper lymphocyte that secret interlukin-4(IL-4),which in turn suppress other pro-inflammatory cyto-kines, and lead to regress host immune response(Menard *et al*.,2019).On the other hand, *T.gondii*(genotype-I)can penetrate placental tissue then embryonic organs and causes numerous sequels by secretion of Rhoptary Protein (*ROP16*) that directly reprograming host immune response or indirect by remodeling and modifying of some molecular markers like *miR-146* and *NFκB* gene(Mammari *et al*.,2019).Furthermore, placental *miR-146* is a critical ncRNA,that regulate epigenetic cell signaling(Li *et al*.,2019), the significant correlation between *miR146* and *NFκB* gene expression was documented in many projects(Gou *et al*.,2022 and Rojas-pirela *et al*.,2022),that showed reduction of *NFκB* gene expression with up *miR-146* transcription, and *NFκB* is turn suppress immune status in pregnant women who suffer from toxoplasmosis(Raghupathy and Szekeres- Bartho,2022). It was suggest that there are a certain synergistic effect act on suppressing of pregnant immune status during toxoplasmosis infection (Ahmed *etal*.,2023). This article aim was to investigate the effect of genotype-I *T.gondii* infection in pregnant women and the relation between subtype-I *T.gondii* and emergence preeclampsia signs and early miscarriage in these patients.

2. Methodology

Samples

One hundred samples were collected from aborted women as well as ten samples that utilized as a control from gynecologic and obstrics hospitals (AL-Batool and AL-Khansa) in Mosul City(3/2022-1/2023), five ml of blood and five gram of placental tissue were kept at-20 after processing until to

use. Sandwich ELISA a technique was utilized for demonstration of antibodies of *T.gondii*(long.-Biotech/China), specific mean(1)was consider as control,negative one that less mean value and more than one represent a positive value.

Deoxyribonucleic acid, coding and ncRNA were extracted from target tissues to detect interested genes and their expression average in both host and parasite, extraction procedures were achieved according to manufacturer instructions for DNA, total RNA and miRNA extraction (Geneaid/Taiwan). complementary strand of mRNA was synthesized by preparing of specific mixture composed of11µl mRNA,2µl of reaction buffer(10X),1µl of dNTP(20X),hexa and oligo primers(1.0)µl,1µl of RNase,0.5µl RNase silencer, 3.5µl NFW, cDNA synthesis steps was involved 55°c for300sec, 85°C for3600sec and 4°C for 300sec. Whereas miRMA-cDNA synthesis mixture was consisted of the same above mixture except of using of specific primer 1µl(10µml),the same synthesis steps were used in this field, amplification mixture for detection NF_kB expression was2µl of sense and antisense primer(10µml),14µl of enzy- mes free water and fourµl of cDNA strand with specific amplification conditions included unwinding 95°C for 300 sec, 57 °C for 45sec and 55- 90 °C for 5sec as melting curve. In addition, the same steps and mixture was performed for *miR-146* expression, except annealing temperature was 60 for the ncRNA. Progesterone hormone was measured using direct Elisa technique for detection parasite antigen(ST AIA-PACK PROG III kit/China).

Statistical analysis

For traditional bio-analysis spss was applied Spss v.16,

while modified livank equation was depended for estimate of gene expression(Livak and Schmittgen (2001).

3. Results and Discussion

Diagnosis and detection of toxoplasmosis:

The results of current study revealed(table1)high values of infections with toxoplasmosis in pregnant women under 30 years with low occurence in pregnant over than previous age. this outcomes were agreed with many documented data overall the world like Almeria and Dubey (2021)and Dawood(2023) who noted high prevalence of infection in pregnant women during first decade of childbearing age that associated with their life style, and the modification of sexual hormones secretion during pregnancy, especially estrogen and progesterone (Muh ammed and Mohammed,2022),these hormones affects in immune response of infected pregnant woman, which is lead to fetus' damage after transmitting of parasite through placenta and causes numerous defects and complication may develop to abortion(Al-Hadraawy *et al*.,2019;Aziz, 2023),it suggest that age group under 30 considered as an active period for women, there-fore they exposed to sources of infection like dealing with meat, prep-aring food, dealing with pets like cats or breeding sheep or cattle(Aguirre *etal*.,2019;Al-Malki,2021;Hatam- Nahavandi *etal*.,2021),or dealing with soil that have a rich source of parasite phase for *T.gondii*(Atiaas *et al*., 2020;Morais *et al*.,2020).

Table (1): Toxoplasmosis values in infected women

Childbearing age	Sample no.	positive outcome(%)
< 30 years	63	5(7.9%)
>30 years	37	0(0%)
P>0.05		

Molecular and Immunological Diagnosis-

The study results showed an agreement between molecular and Immunological diagnosis, five samples were positive for anti *T.gondii* antibodies, and *B1* gene detection using PCR technique, and these results in turn were agreed with that achieved by Al-said *et al*.(2019) and AL-marsoomy,2020.

Table (2): ELISA and PCR techniques for detection of toxoplasmosis.

Techniques	positive values(%)	Sample no
ELISA	(5%) 5	100
PCR	(5%) 100	5
P>0.01		

Molecular classification of *T.gondii* strains

The molecular diagnosis using PCR technique showed the presence of specific amplicons of the *B1*gene at 115 bas pair(figure1) of *T.gondii* cell surface receptor, and the molecular diagnosis was confirmed as *T.gondii* subtype-I after aligned the sequences(NCBI, accession no. PP72 3076.1). Sequence alignment using Basic Local Alignment Search Tool consider as a gold standard bioinformatics application for specific confirmative of organisms depending of nucleotides sequence alignment (AL-marsoomy,2020 and Daoowd,2023).

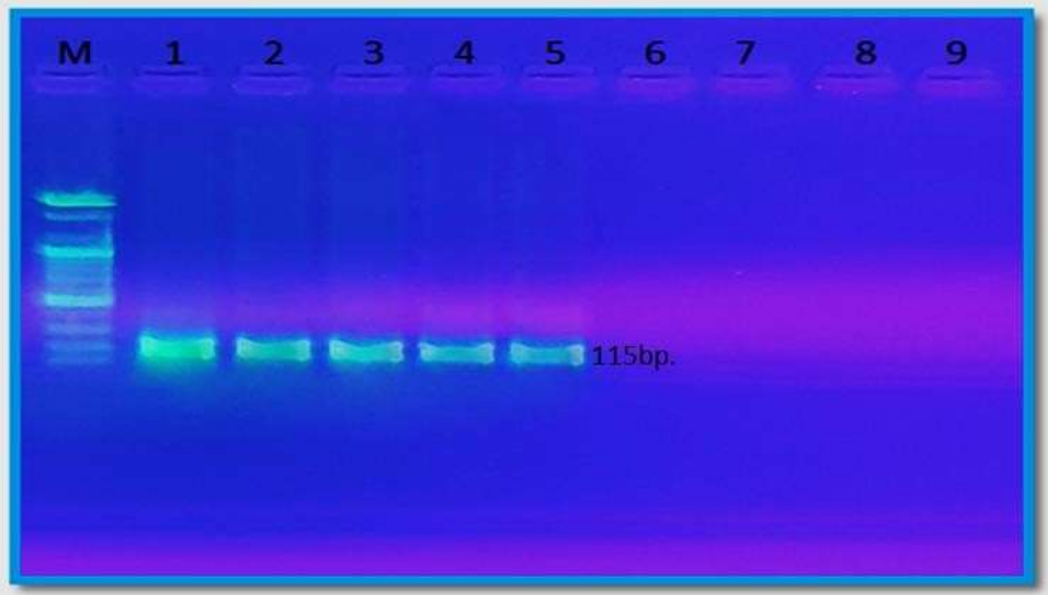

Figure (1): Electrophoresis of *B1* gene amplicons for diagnosis of *T.gondii* parasite, Path M was molecular scale:100 base pairs, wells 1-5were Positive results(115 base pairs).

The relationship between the virulence factor gene *ROP16* and the progesterone hormone

The results(table3)revealed that there was a significance variation in the expression values of the *ROP16* gene in infected women(8.5-9.4) compared with aborted(1.05-1.33)and pregnant(0.015-0.019)women, while the progesterone hormone values were decreased in parasite infec- ted women(4.7-27pg/ml) compared with healthy pregnant women who showed a high incidence in this hormone secretion(232.8-387.0 pg/ml).Therefore it was shown that there is an inverse proportion between the *ROP16* gene expression values and progesterone secretion, which deal that there a synergistic relationship between them at a value of P> 0.001. A local study that a chivied by Abd ali *et al*. (2023) revealed that the activity of the aforementioned gene was increased in women suffering from toxoplasmosis, which in turn play a critical role in regulating of placental trophoblast metabolic

pathway and assist to exhibit secretion of some hormones during the pregnancy process. The results of the current study also agreed with Wang *et al*. (2018) who confirmed the ability of *ROP16* to stimulates macrophages to secret of IL-10, which activate Th2 cells upon infection with the *T.gondii* genotype-1 during pregnancy, and lead to inhibit of host immune response and causes early miscarriage during the first trimester of pregnancy(Aziz,2023). Cui *et al*. (2020) were confirmed that secretion *ROP16* during *T.gondii* type-I infection and is more aggressive due to an increasing in TNF-α and IL-12 levels and decrease in TGF-B production that lead to inhibit of pregnant women immune response and activate of parasite, which in turn, leads to miscarriage after invasion of the placental cell by the parasite.

Table 3: Evaluation of *ROP16* gene activity and its relationship with *NF_KB* and *miR-146* genes and progesterone hormone level.

Samples	Diagnosis						
	Immunological	Genetic		Epigenetic		T. gondii	
	Progesterone hormone	c	NFκB	c	Mir146	c	Rob16
Infected	4.580	1.00	0.11	1.00	1.31	0.99	9.32
	3.860		0.12		1.25		9.16
	2.750		0.13		1.24		9.44
	4.080		0.11		1.26		8.573
	4.770		0.11		1.31		8.94
Pregnant	232.8	1.00	3.18	1.00	0.85	0.99	0.016
	294.0		3.50		1.06		0.019
	259.6		3.64		0.99		0.017
	387.0		3.21		0.95		0.016
	252.0		3.39		1.07		0.015
Miscarriage	6.240	1.00	1.57	1.00	0.85	0.99	1.09
	7.930		1.50		1.06		1.14
	6.540		1.56		0.99		1.34
	6.950		1.65		0.95		1.11

Relationship between progesterone and the immune response of pregnant women

The results of molecular investigation in table (3) showed a decrease in the gene expression values of the transcription factor *NF_KB* for infected women(0.11-0.13) compare with its an increasing values in pregnant group(3.18-3.64). *NF_KB* is the active form of the *IKB*gene, which is responsible for inhibiting the pro-inflammatory pathway of the immune response and thus inhibiting the immune response in pregnant women, there is a large number of research conducted to investigate the

interaction between the female hormone progesterone and immune- suppression in pregnant women,

it turns out that increased hormone values are directly proportional to the effectiveness of the STAT3 pathway,which stimulates the inflammatory pathway of the pro-inflam- matory response, as the progesterone hormone enhances the expansion or increase of Th2 cells and stimulates the production of IL-4 which in turn can reduce damage to maternal trophoblasts during a rejection reaction caused by parental antigens, progesterone can induce the production of cytostatic factors such as IL-4 to inhibit IFN-γ production and activate the NF_KB pathway, which can reduce tolerance parasite infection with *T. gondii* and this was confirmed by Wu and others (2022).The present outcomes were agreed with many researches around the world such as (Mammari *et al*.,2019 and Faria Junior *et al*.,2021).

Relation between epigenetic biomarkers and host immunity

The results of the current study in table (3) showed that there were a direct correlation between *miR-146*(1.23-1.31)with activity values of *ROP16*gene(9.44-8.57) in infected women, comparing with the activity values of the gene *miR-146* (0.845-1.047), and values of *ROP16*(0.0152- 0.0192) in pregnant women, thus, the current results showed that there is an effect of*T.gondii* on the reorganization of gene activity of microRNAs and genes in the host. This effect is due to the presence of genetic correspondence between parasite and human microRNA nucleotides sequences, and the difference does not exceed one nitrogenous base, and this permit this parasite to reregulateion the expression of these genetic molecules according to its requiring, for that,it is very likely that *T.gondii* uses endogenous miRNAs to inhibit apoptosis and manipulating the defense system signaling pathways and activate the biosynthetic pathways of some molecules needed to support it metabolism necessary for growth and reproduction(Rojas-Pirela *et al*.,2022). Faria Jonior *et al*. (2021) also demonstrated the existence of a close relationship between the activity of microRNA that represents epigenetic inheritance and target genes that represent genetic inheritance by recording micro-nucleic acid values during toxoplasmosis infection.

4. Conclusion and future scope

The present study outcomes documented the synergestic effect of toxoplasmosis by secretion of virulence factor *ROP16* on reducing of progesteron hormone, that assist to inhibit of immune response of the infected pregnant women through inhibition of NF_kB and *miR-146* which in turn lead to develop of preeclampsia symptoms and abortion.

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