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DETECTED TOXOPLASMA GONDII IN WOMEN AND STUDY ITS ROLE IN INHIBITING CYTOCHROME C-INDUCED CASPASE ACTIVATION IN ITS HOST CELL

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Abstract:

The purpose of the current study is detecting the role in inhibiting cytochrome c-induced caspase activation in its host cell. 115 samples of placenta aborted women were collected from women whom attended to (Al –kut Hospital for Gyneclogy and obstetrics and pediatrics) from 15 October 2022 to 15 June 2023. The study showed the prevalence of Toxoplasma gondii among abortion women in Waist province was 32 (27.9%). DNA was extracted from the positive samples, then the PCR-RFLP method was applied in the GRA6 gene the examination showed three types of Toxoplasma, Type I was the most prevalent, and Type III was the least prevalent. The percentages were (59.3% , 37.5% and 3.1% for the Type I ,Type II , and Type III, respectively. immunohistochemistry technique used to to detect the intensity of expression of caspases 8 and caspases 3, and the study showed weak expression of caspases. 8 While the expression intensity of caspase-3 differed between low, medium and high and according to the difference of the Type, the study concludes the programmed cell death was by intrinsic effect in samples of the placenta of aborted women by using the immunohistochemistry method.

Keywords: immunohistochemistry, Toxoplasma gondii, inhibiting cytochrome c-induced.

Introduction

Toxoplasmosis is a common disease that increases its risk when transmitted from the pregnant mother to the fetus, and it was noted that the pregnant mother infected for the first time with Toxoplasmosis occurs in her fetus by 30% (1), and Toxoplasmosis constitutes 8% annually of deaths caused by diseases transmitted by Food borne illness (2). Most intracellular infections, such as T. gondii, have developed defense mechanisms to prevent their host cells from experiencing programmed cell death (PCD) (3). PCD mediated by either extrinsic or intrinsic death receptors can be inhibited, and host cell PCD signaling pathways can be interfered with at the very least increases pathogen survival and prolongs the host cell's vitality. It is true that mycobacteria and genetically modified malaria parasites that are unable to stop caspase-dependent PCD in their host cells are rapidly destroyed following infection. The pro-apoptotic Bcl-2 effector protein Bax is not as active in parasite-positive cells, which contributes to the significantly decreased release of cytochrome c from mitochondria into the host cell's

cytoplasm (4) and this is partially due to a decrease in the activation of the pro-apoptotic Bcl-2 effector protein Bax (5). Infected cells may be protected from caspase-dependent intrinsic cell death by upstream signaling pathways such as protein kinase B/Akt (6) and NF-κB activation. Using a cell-free in vitro caspase activation system, we have recently shown that T. gondii or excretory-secretory proteins produced by the parasite can block cytochrome c-triggered activation of caspase 3/7.

Materials and Methods

Sample Collection:

115 smples of placenta aborted women were collected from women whom attended to (Al – kut Hospital for Gyneclogy and obstetrics and pediatrics) from 15 October 2022to 15 June 2023 .

Isolation of *Toxoplasma* parasite

Toxoplasma parasite was isolated from placenta samples of aborted women according to the method of Al-kennany and Hassan, (2010) after confirming the presence of the parasite in the samples using the direct swab method (impression) and as follows:

1- The placenta was cut into small pieces and mixed with an equal amount of physiological solution, then mashed with a mortar and the sample was filtered with gauze to get rid of large particles.
2- The model was centrifuged at 3000 rpm for 10 minutes, after that the precipitatewas suspended with physiological salt and this process was repeated three times.
3- Finally, 1000 units of penicillin and 100 mg of streptomycin were added to prevent contamination A Haemocytometer was used to count the tissue sacs of the parasite found in the placenta, and the suspension was kept in the refrigerator in case the injection was not done on the same day.

A method of Immunohistochemistry to detect the stimuli of apoptosis, whether intrinsic or extrinsic

Method for the detection of expression intensity of caspase 8 & 9 is as follows :- (7,8) . and manufacturer instructions.

Calculate expression intensity of 8&3 caspases

The expression scores of Casp-8 and Casp-3 were determined using the score provided by McDonald (9) (Table 1).

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Table (1) : IHC score criteria of Casp-8 and Casp-3 expression.

Score	0	1+	2+	3+	4+
Positive cells	<10%	10-25%	25-50%	50-75%	>75%

Genotyping isolation DNA by RFLP-PCR

Primers used in this study Activation of a portion of the GRA6 gene (for Toxoplasma genotyping) were used according to (10) supplied by the Korean company Macrogen and manufacturer instructions.

Target gene		Sequence (5'-3')	Ta (°C)	Product size	Accession number
GRA6	F	GTAGCGTGCTTGTTGGCGAC	56	322 bp	NEB #B7025)
	R	TACAAGACATAGAGTGCCCC			

Statistical Analysis

Version 30 of the statistical package for the social sciences (SPSS) software was used to analyze the data.Frequency and percentage were used to display qualitative data. We employed the $\chi 2$-test to examine the frequency of genotypes and the intensity of caspase 9. P-values less than 0.05 were regarded as statistically significant (11).

Result and Discussion

Isolation of *Toxoplasma gondii* from women

Toxoplasma gondii parasite has been isolated from a placenta model of women infected with toxoplasmosis and who had abortions due to infection, the result showed the presence of the parasite in 32 out of 115 (27.9%) as shown in Table (2), the samples of placentas were collected in maternity halls of hospital in Waist Governorate for women whose ages ranged between 18-42 years , and it was examined microscopically by the impression method after staining it with Gimza stain, the *Toxoplasma gondii* parasite It contained both phases of tachyzoite and Bradyzoite, or one of them, as shown in Fig.1. the results of this study agree with a previous study conducted by Al-Mayahi. (12) in Wasit Governorate (38.99%). It is an approach to the study of Darweesh *et al* ., (13) in Diyala Governorate, where it recorded 44%. , and the findings are closer to 43% that's recorded by Ali *et al*.,(14) study. The worldwide prevalence of Toxoplasmosis varies depending on many factors such as sample count, age, socioeconomic status, eating habits, hygiene, climate, educational level, and geographic location.

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Table (2) . the present of *Toxoplasma gondii* in women

Number ofsamples	The positive samples	The negative samples
115	2 (27.9%)	83(72.1%)

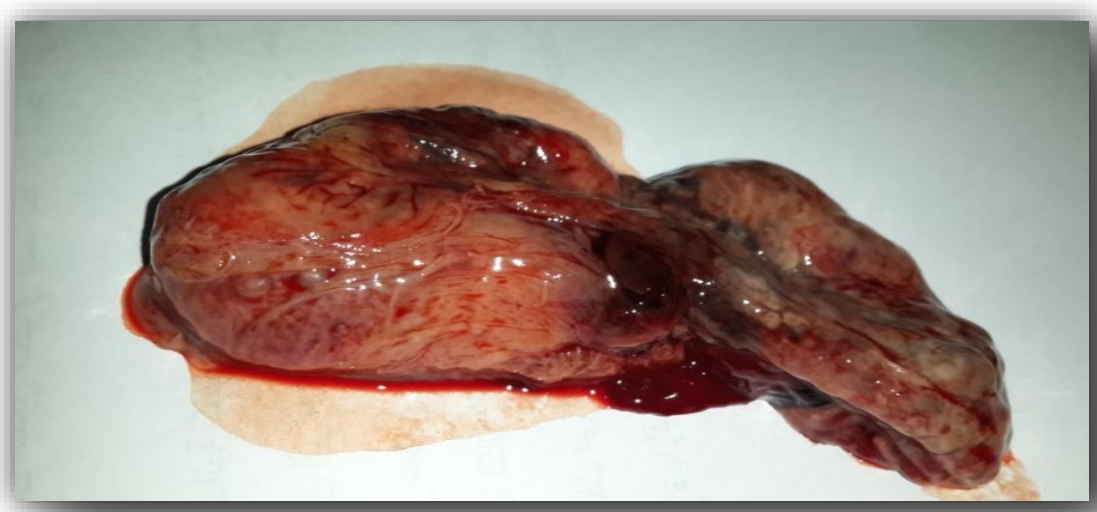

Fig. 1. Placenta infected with *Toxoplasma gondii*.

Detected type of *Toxoplasma gondii* by GRA6 gen

To detect type Toxoplasma gondii, the GRA6 gene was amplified using the RFLP-PCR method on 32 positive placenta samples. The results indicated that the GRA6 enzyme, MseI, could distinguish between three types based on the length of DNA fragments: As can be seen from type I and type III as shown in figure 2, Table (3), genotype I was cut into two bands, 544 bp and 194 bp, while genotype II was cut into two bands, 700 bp and 100 bp, and genotype III could cut it into two fragments, 600 bp and 100 bp. (3). The result is agree with Maryam and Hamed, (15) Study in Iran revealed that all positive isolates belong to Type III of GRA6 allele of T. gondii. and another study by Rufash (16), 56 percent were classified as genotype I and 43 percent as Type III. For women infected with Toxoplasmosis, there are no comprehensive studies dedicated to the genotyping of T. gondii in Iraq that use GRA6gene by the RFLP- PCR . Grigg et al. ,(17) discovered a high prevalence of Type I strain in another study of immunocompromised patients, indicating an increase in the prevalence of Type I in humans .

Table (3) . The Genotype of *Toxoplasma gondii in women*

Toxoplasma gondii	Genotype I	Genotype II	Genotype III
32	19 (59.3%)	12(37.5%)	1(3.1%)
X 2	19.04		
P value	**0***		

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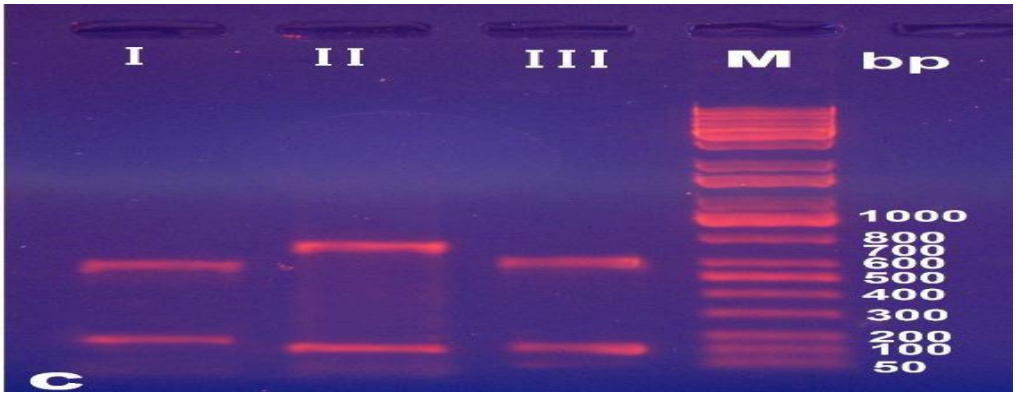

Fig. 2: RFLP-PCR examination of genotype strains of Toxoplasma gondii on agarose gels stained with ethidium bromide.(Grape digestion using the MseI enzyme, which identified three different strain types.

Inhibiting cytochrome c-induced caspase (8,3) activation

The method of immunohistochemistry was used for the purpose of identifying the expression of caspase-8 and caspase-3. From these results, we can obtain important information about programmed cell death (PCD) whether it is an inhibitor or increase. And when caspase-8 was applied to all samples, 32 tissue sections of infected placenta, the results for all types of *Toxoplasma* genotype showed a weak expression of caspase 8 intensity that cannot be measured. Therefore, the programmed death was not caused by an external stimulus. The method IHC was used again, and Caspase-3 was applied to all tissue sections. The results showed that there was a different positive expression in intensity, and this proves that apoptosis is present and its signal was initiated by an internal stimulus. as shown in the figures(3),(4) and (5) .

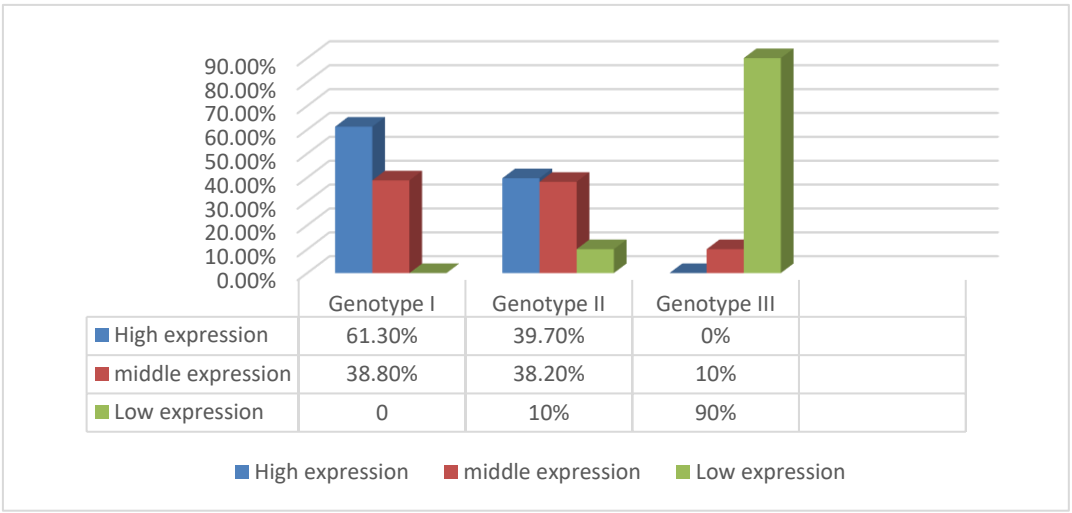

Fig.3 showing the expression intensity of caspase 3 for each genotype of *Toxoplasma* isolates

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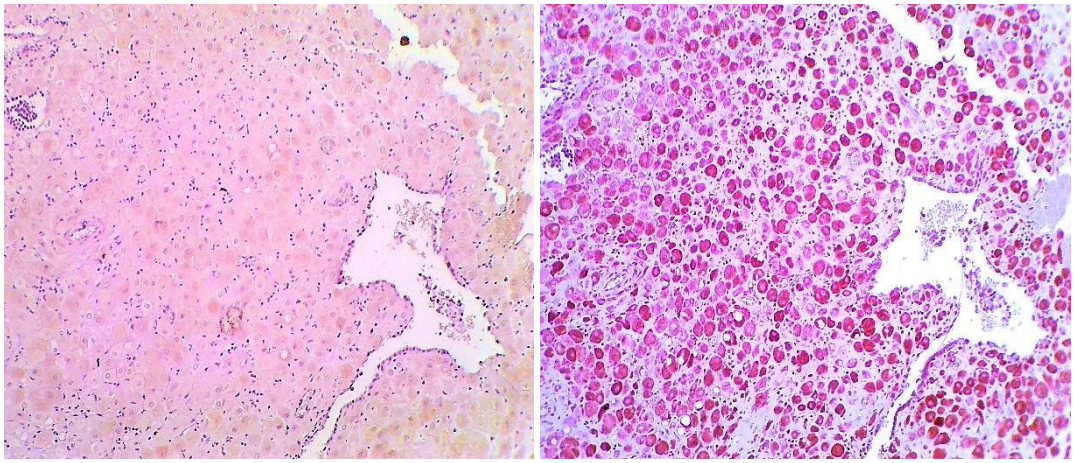
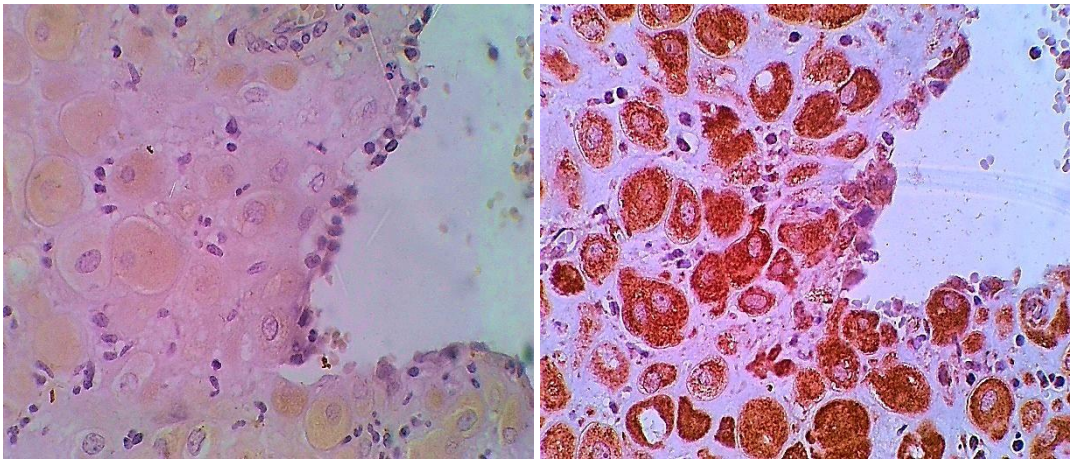
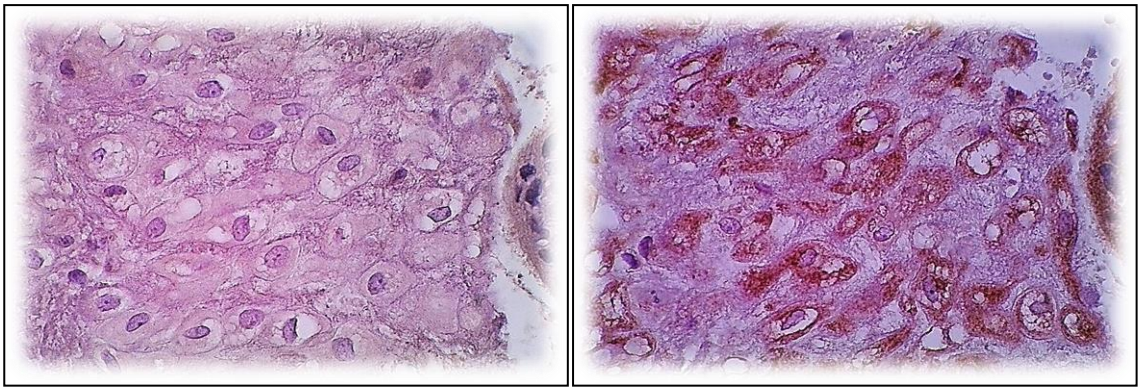


A. B.



C. D.

Fig.4: Caspase 3 and 8 in a photomicrograph of a placenta infected with Toxoplasma gondii (Type I).A&C: The decidua region of the placenta has weak expression of caspase 3 (arrow). B&D/Caspase 9 overexpression (arrow) in the placenta's decidua region. DAB and H&E. 10x for A and B and 40x for C and D.



A B

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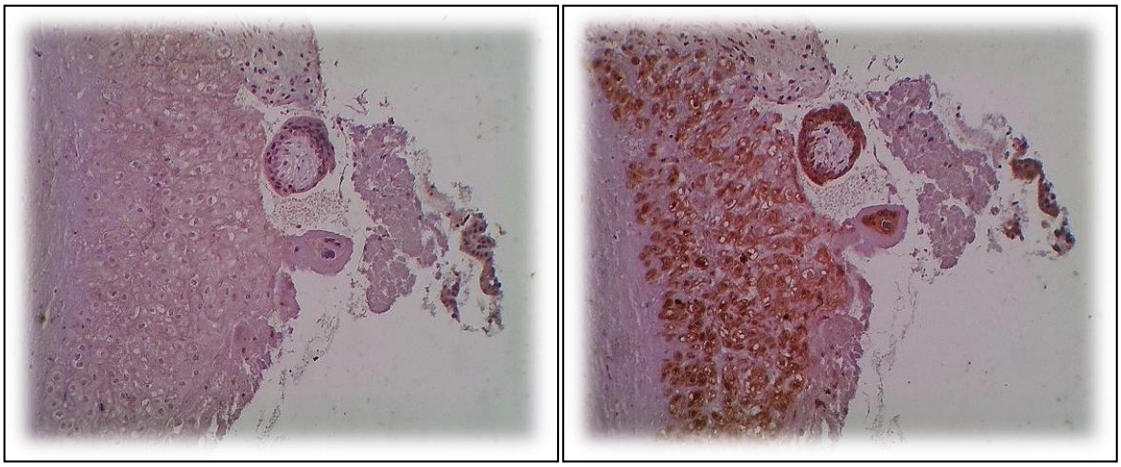

C. D.

Fig. 5: Negative expression of caspase 8 in placenta/Photomicrograph of caspase 3 and caspase 8 in Toxoplasma gondii-infected placenta Type III A&C. B&D/Caspase 3 overexpression (arrow) was seen in placental epithelial cells during the apoptotic process. DAB and H&E. 10x for A and B and 40x for C and D.

The result agree with Ameena and Shaimma (18)in Erbil confirmed in their study an increase in regulated cell death , but through an extrinsi effect by increasing the expression of caspases-8. The study of Ghazi (19) confirmed increased cell death by Women with RPL may have higher levels of p53 protein expression, which could accelerate placental apoptosis and cause pregnancy loss. While research conducted by (20) Germany suggests that T. gondii's unique anti-apoptotic activity is important for its capacity to inhibit the infected cells' caspase-dependent intrinsic apoptotic pathway. The study's Jurkat E6.1 human-derived leukemic T cells were grown in RPMI 1640 with 10% heat-inactivated fetal calf serum added as a supplement.On the other hand, Ji-Young Kim et al.'s (21) Korean study found that apoptosis in T. gondii-infected mice spleen cells was not elevated in comparison to uninfected controls.In the USA, human cells were investigated.(22) Verified a novel tactic for manipulating host cells: T. gondii infection prolongs primary human neutrophil survival by delaying apoptosis. Aged peripheral blood neutrophils undergo spontaneous apoptosis and move to the spleen, liver, and bone marrow, where macrophages eliminate them (23). A normal adult human's bone marrow produces approximately 100 billion neutrophils each day, which are created in an orderly balance between neutrophil development, mobilization from the bone marrow, migration to peripheral tissues, cell senescence, and death (24). The primary mechanism of cell death in neutrophils, which are terminally differentiated cells with a brief lifespan, is spontaneous apoptosis. The capacity of T. gondii to effectively control the host's immune system in order to create a fruitful infection and preserve an ideal reproductive niche is

well recognized (25, 26). In fact, T. gondii types I and II have the ability to prevent many mouse and human cells from going through apoptosis, which may aid the parasite in keeping intracellular cells alive (27).

Conclusion

The study determined that the programmed cell death was by intrinsic effect In samples of the placenta of aborted women by using the immunohistochemistry method.

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