ALTERATION OF TROPHOBLASTIC TISSUE APOPTOSIS AND THEIR ROLE IN TOXOPLASMOSIS INDUCE MISCARRIAGE

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ABSTRACT:

Toxoplasma gondii (*T. gondii*) , a common protozoan parasite. *T. gondii* infection occurs worldwide and it is one of the most common infections in humans. The infection is mainly acquired by ingestion of undercooked or raw meat containing viable tissue cysts or by ingestion of food and water that is contaminated with oocysts shed by cats . In addition, it has been established that *T. gondii* can be transmitted from a recently infected mother to her fetus. During pregnancy the primary infection may lead to severe if not fatal complications for the fetus. These direct effects can lead to spontaneous miscarriage, stillbirth or congenital anomalies .apoptosis in villous trophoblast is increased in pregnancy complications such as Toxoplasmosis infection**.** From our study we found that *Toxoplasma gondii* downregulated apoptosis when caspase 3 and caspase 9 levels were investigated using immunohistochemistry technique **.**

Aims of Study: So the aims of this search are to investigate the levels of Caspase 3 and Caspase 9 expression within *T.gondii* infected trophoblastic cells and their association with infection.

Method: Fifty patients (aborted women), Their range age between (16 − 46) years, were included in this study. All patients sera were subjected to Enzyme Linked Immunosorbent Assay **(**ELISA) to detect specific *Toxoplasma gondii* IgM and IgG . In addition , trophoblastic tissues from the same patients were taken to confirm the infection of the *T.gondii* and to evaluate the expression of caspase 9 and caspase 3 using immunohistochemstry method.

Results : Immunehiostochamical technique is more sensitive than ELISA in diagnosis of *T. gondii* when 18 patients were positive for Toxoplasmosis by immunohistochemistry While in ELISA only 16 patients were positive for specific anti – *Toxoplasma* IgM. The levels of Caspase3 and caspase 9 were downregulated in infected group.

Conclusion : Caspase 9 expression and Caspase 3 expression are significantly highly decreased in women with spontaneous miscarriage in *T.gondii* positive group compared to patients control group indicating that *Toxoplasma gondii* downregulated apoptosis.

Keywords : *Toxoplasma gondii,* apoptosis, miscarriage

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INTRODUCTION

Toxoplasma gondii a common protozoan parasite responsible for both severe congenital birth defect and fatal toxoplasmic encephalitis in immunocompromised people . Congenital fetal toxoplasmosis may result in miscarriage, stillbirth, or severe mental retardation; infections in late pregnancy may be asymptomatic but present with retinal or neurological damage later in life (1). Infection by *Toxoplasma gondii* is mainly acquired by ingestion of food or water that is contaminated with oocysts shed by cats or by consuming contaminated meat containing tissue cysts . In addition , infection may be acquired by contact with cat feces containing oocysts (2). The human placenta is an important for the maintenance of pregnancy , and comprises both functionanal and mechanical interphase between the mother and the fetus . from the initiation of pregnancy , the placenta itself grows and matures until the end of pregnancy . As gestation progresses, the villi and lining trophoblast show evidence of maturation and differention , and the rate of placental growth is known to decline gradually after 34 to 36 weeks of gestation .Considering that programmed cell death or apoptosis is one of the critical processes during fetal development (3)**.** Since 1995 the number of publications investigating apoptosis in villous trophoblast has increased exponentially . This scientific interest is due to observations that is specialized form of cell death is increased in pregnancy complications such as pre-eclampsia and intra-uterine growth restriction and the infection with various pathogens (4). The infection of cells by *T.gondii* has been studies .The results indicated that *T.gondii* either inhibits the apoptosis of the cells infected by the parasite or on the other hand it induces apoptosis (5) . western analyses demonstrated that both cytotrophoblasts and syncytiotrophoblasts express the four pro- forms of the caspases (3,6,8,and 9) (6) which are the executioners of apoptosis (7).

Materials & Methods

- Subjects and Selection of the patients:
Fivity women with spontaneous miscarriage who had curettage operation at the Obstetrics and Gynecology Department of Al-Kadhimyia Teaching Hospital in Baghdad between December 2009 and June 2010 were the subjected of this study ,their age ranged from 16- 46 years . The patients were divided into two groups:
Group 1 :- 16 positive for *Toxoplasma gondii* .
Group 2:- 34 negative for *Toxoplasma gondii* (patients control).
-Laboratory Methods:
1-Enzyme Linked Immunosorbent Assay for the detection of IgM and IgG antibodies for *Toxoplasma gondii* in serum :
The following kit was used (Bio Check , Inc . Foster City , CA, USA;2004). The kit was contained these materials : Microtiter plate :purified *Toxoplasma* antigen coated wells (12×8) wells , Enzyme conjugate reagent ,sample diluents ,Negative control ,cut -off calibrator ,positive control ,Wash buffer concentrate (20 X),TMB reagent and the Stop solution .the steps of reaction were down according to kit instruction.
2- Immunohistochemical analysis for the detection of *Toxoplasma* antigen , caspase 3 and caspase 9 proteins in paraffin embedded sections :
Trophoblastic tissue was collected from the evacuation of retained pieces during the procedure of curettage and placed in 10 % formaldehyde . Two to three paraffin embedded blocks were prepared for each patient(8). staining with haematoxyline and eosin was carried out to decide which block can be used in the study (only sections that contained trophoblastic tissue was included in this study) .
The following kit was used in the search (Immunohistochemistry detection kit Dakocytomation LSAB+ System – HRP* Code KO679 (Dakocytomation , USA)

,Monoclonal antibodies are as follow (Rabbit anti – *Toxoplasma gondii***,** United State Biological**,** Mouse anti – Human Caspase 9, United State Biological and Mouse anti – Human Caspase 3, United State Biological)**.** The procedures and results were done according to the kit instructions **.**

RESULTS:

● **Enzyme Linked Immunosorbant Assay (ELISA) results:**

Regarding the patients groups (spontaneous aborted women) 16 (32%) of samples out of the (50) serum samples were positive for anti- *Toxoplasma gondii* IgM and the rest 34 (68%) were negative for IgM (figure- 1).and 4 (8%) out of the (50) serum samples were positive for anti- *Toxoplasma gondii* IgG and the rest 46 (92%) were negative for IgG and these 4 positive for anti- *Toxoplasma gondii* IgG were positive for anti- *Toxoplasma gondii* IgM (figure- 2).

● **Results of IHC for detection of *T.*gondii antigen within the trophoblastic tissue among the study groups :**

The results showed that (18)of 50 women (36%) have *Toxoplasma* antigen within the trophoblastic tissue and (32)of 50 women (64%) were negative to *Toxoplasma* antigen by IHC method as shown in the (figure- 3)) and (figure 4).

● **Results of IHC of detection of caspase 3 within the trophoblastic tissue among the study groups:**

The expression of caspase 3 protein detected by Immunohistochemistry (IHC) technique. Scoring system used to express the percentage of the expression of this protein. Figure -5 and 6 showed that there was highly significant decrease in mean percentage of caspase 3 protein in Toxoplasma gondii positive group (7.088%) compared to control group (37.624%)(P<0.001).

● **Results of IHC of detection of caspase 9 within the trophoblastic tissue among the study groups .**

The expression of caspase 9 protein detected by Immunohistochemistry (IHC) technique. Scoring system used to express the percentage of the expression of this protein. Figure- 7 and Figure -8 showed that there was highly significant decrease in mean percentage of caspase 9 protein in *Toxoplasma gondii* positive group (13.975%) compared to control group (45.831%)(P<0.001).

DISCUSSION:

In this research ELISA method for detecting IgM and IgG anti-*Toxoplasma* antibodies in the serum and the immunohistochemistry method for detection of *Toxoplasma* antigen within the trophoblastic tissue were used depending on the fact that the diagnosis of toxoplasmosis in humans is made indirectly by serological methods and directly by a polymerase chain reaction (PCR), isolation of the organism, histology, or by some combination of the above (9). A point to be noted in this study, is the presence of two cases which were negative by ELISA test but were positive by IHC could be explained depending on the fact that IgM might be below the detection level of the kit used (very early in the disease or a period of switching from IgM to IgG) (10) or those cases might be false negative by ELISA method and indicate that the Immunohistochemistry technique is more sensitive than ELISA (11). In the present study, the relatively high frequency of toxoplasmosis in women with miscarriage could be due to the sample selection. The samples were collected from Al- Kadhimyia Teaching Hospital which is a reference hospital for the surrounding rural areas where they have habits in favor of acquiring toxoplasmosis by eating unwashed raw vegetables or unpadded fruits. In addition, in the rural areas there is close contact with cats and consequent exposure to sporulate oocysts by ingestion of these oocysts that contaminate soil during farming, or eating undercooked meat contaminated with cysts or due to deterioration of sewage system

and general hygiene . Moreover, the low level of education in the women about the risk factors for toxoplasmosis may play an important role in the high rate of infection (12).

Our results indicated a lesser percent of caspase 3 and caspase 9 expression in *Toxoplasma gondii* positive group when detected by (IHC) technique , The results showed that there was highly significant decrease in mean percentage of caspase 3 and caspases9 protein in *Toxoplasma gondii* positive group .the activation of the caspase 9/caspase 3 pathway during apoptosis involves release of mitochondrial cytochrome c into the cytoplasm, which then leads to dATP-dependent formation of an Apaf1/caspase 9-complex and to activation of caspase 9 (13). On the single cell level, the presence of intracellular parasites negatively correlated with a mitochondrial distribution of cytochrome c and the absence of DNA strand breaks as a characteristic feature of apoptosis. the anti-apoptotic activity of *T. gondii* was accompanied by interference with mitochondrial cytochrome c release and subsequent downregulation of caspase activation. Furthermore, the protein level of Poly(ADP-ribose) polymerase (PARP) was prominently downregulated by the parasite. This suggests that *T. gondii* has evolved different mechanisms that may contribute to the inhibition of host cell apoptosis (14). This confirms and extends previous findings that protection of cells from apoptosis requires the presence of viable, but not necessarily replicating, intracellular parasites (15). Thus, intracellular *T. gondii* might excrete a parasitic factor that mediates inhibition of mitochondrial cytochrome c release, leading to decreased activation of the caspase 9/caspase 3 pathway. Alternatively, active invasion by the parasite may irreversible modify the host cell physiology, which results in protection against induction of apoptosis(16).

FIGURES AND TABLES

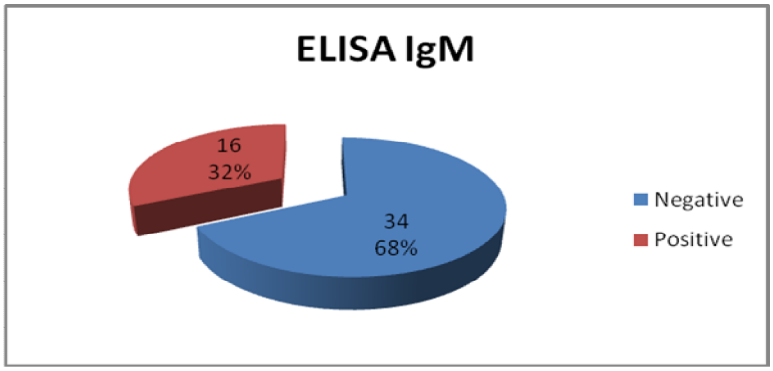


Figure 1: Percent of positive and negative cases for IgM against *T.gondii*

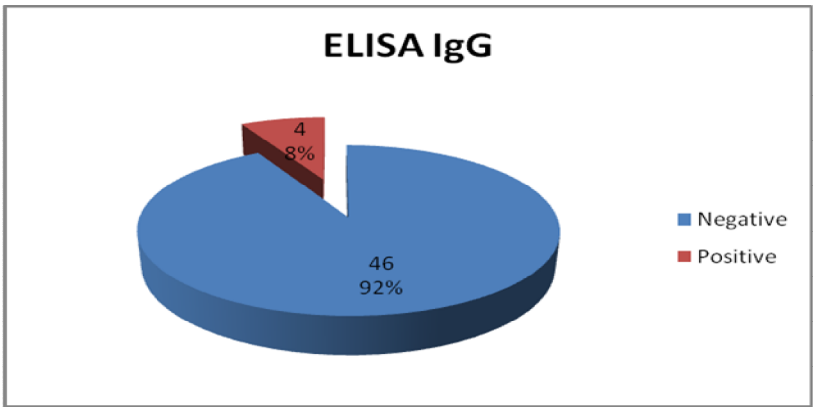


Figure 2: Percent of positive and negative cases for IgG against *T.gondii*

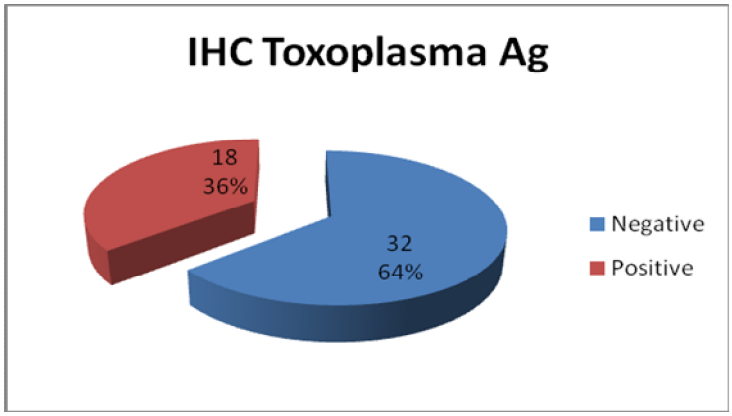


Figure 3:Percent of positive and negative cases of *T.gondii antigen* by IHC

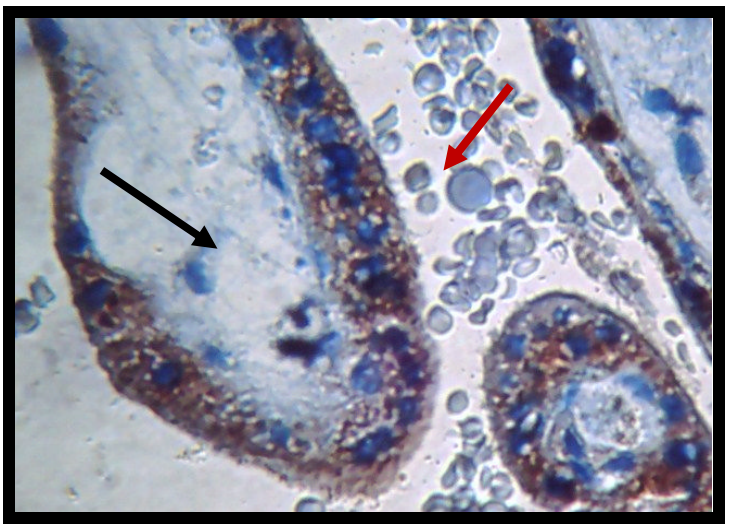


Figure (4) Immunohistochemical staining of T.gondii antigen in trophoblastic tissue in patients with aboprtion. staining by DAB chromogen (dark brown) counterstained with Mayer's Hematoxylin .Magnification (X 400).

Positive cell for T.gondii antigen

Negative cell for T.gondii antigen

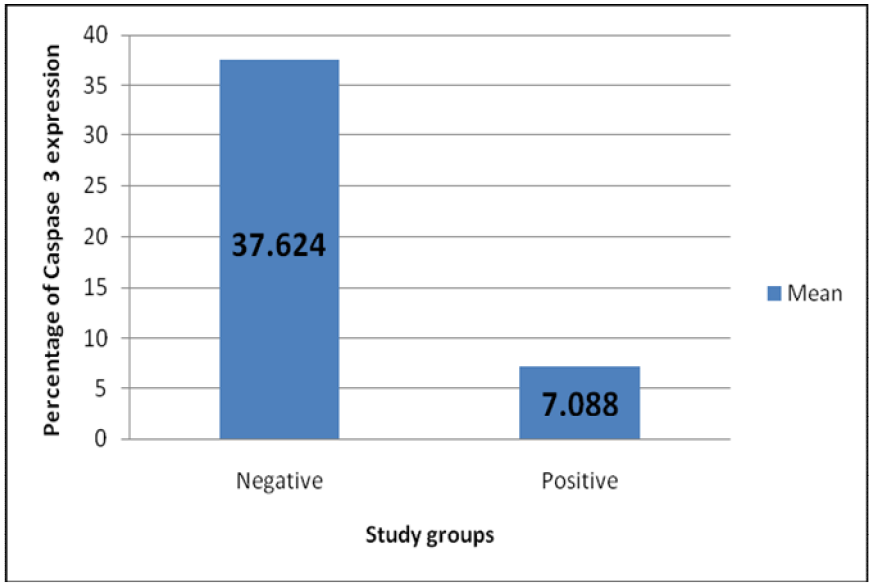
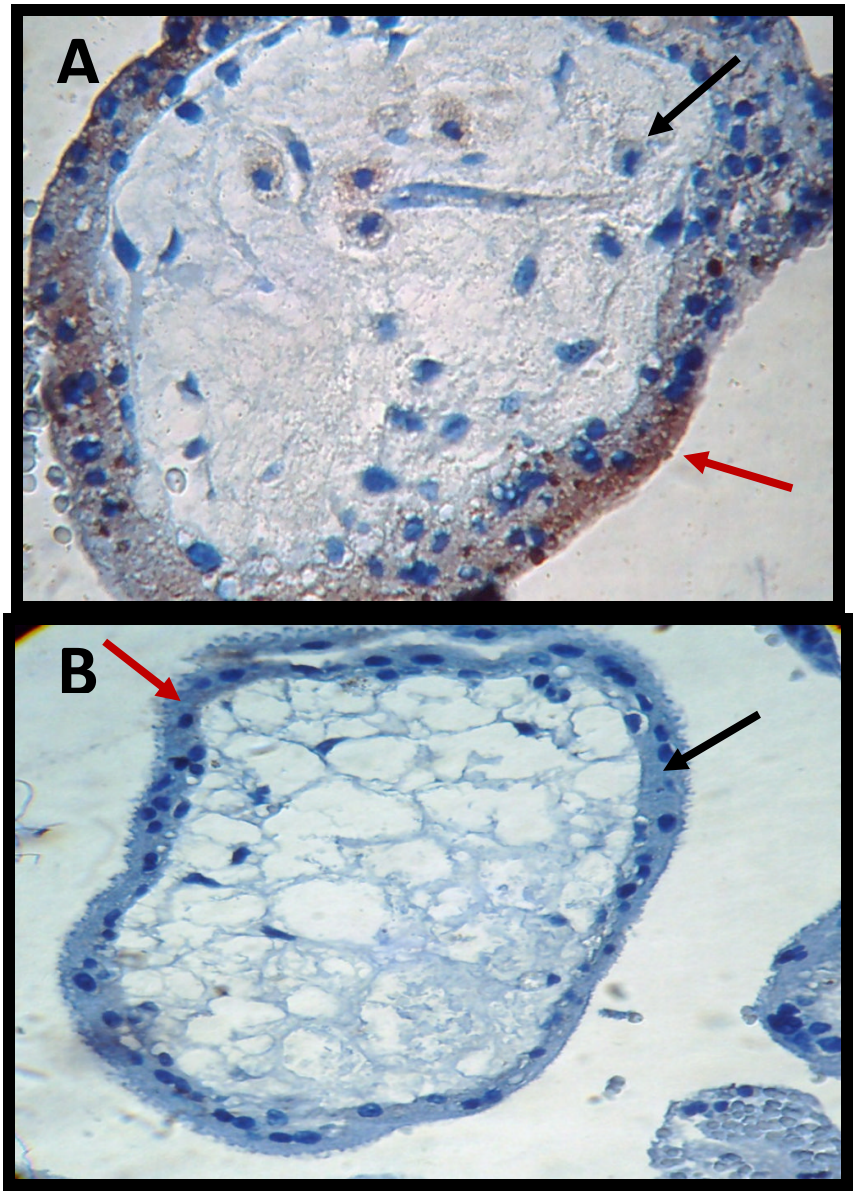


Figure (5) Caspase 3 protein expression in the two study groups.



Figure(6) Immunohistochemical (**IHC**) staining of Caspase 3 protein in the two groups under study .(A) In *Toxoplasma* negative "control" group .(B) In *Toxoplasma* positive group . staining by DAB chromogen (dark brown) counterstained with Mayer's Hematoxylin .Magnification (X 400).

→ Positive cell for Caspase 3 expression

→ Negative cell for Caspase 3 expression

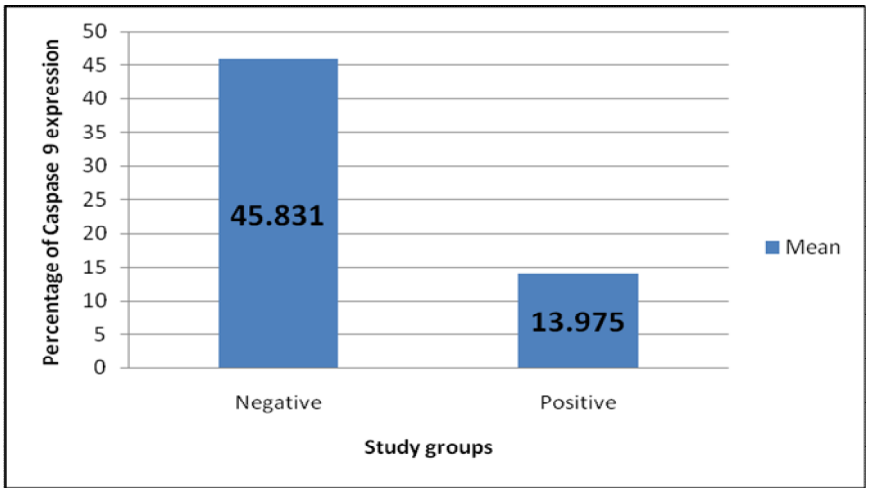
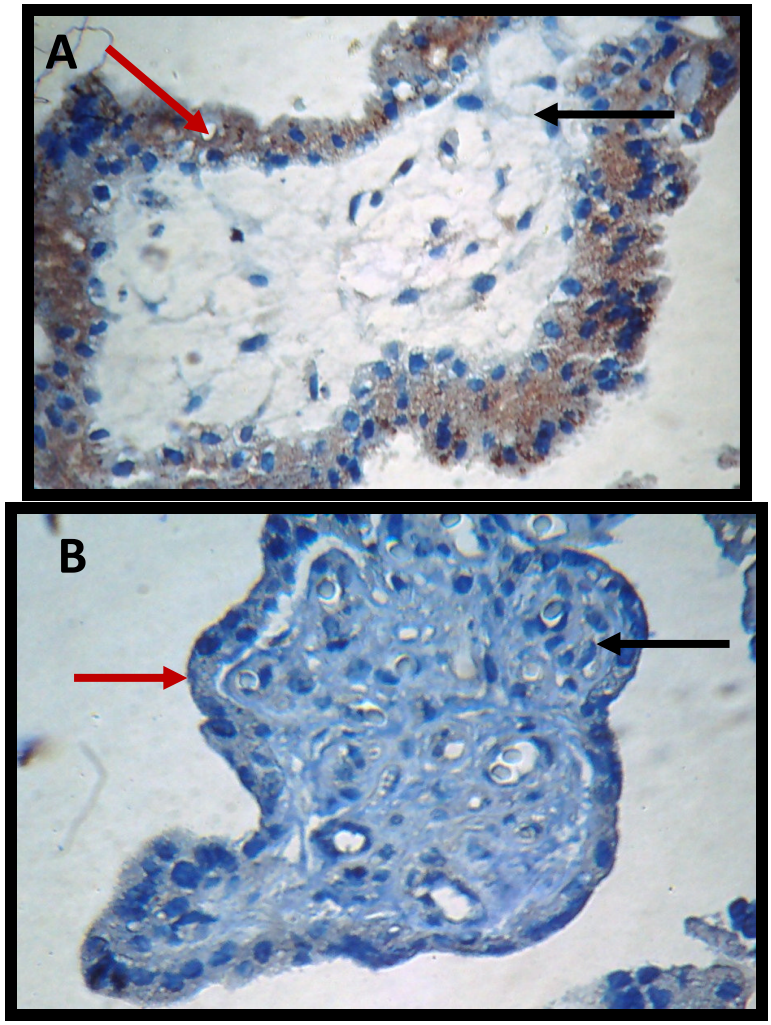


Figure (7) Caspase 9 protein expression in the two study groups.



Figure(8) Immunohistochemical (**IHC**) staining of Caspase 9 protein in the two groups under study .(A) In Toxoplasma negative "control" group .(B) In Toxoplasma positive group . staining by DAB chromogen (dark brown) counterstained with Mayer's Hematoxylin .Magnification (X 400).

→ Positive cell for Caspase 9 expression

→ Negative cell for Caspase 9 expression

Table (1): Percent of positive and negative cases for IgM and IgG against *T.gondii*

		ELISA IgM			
		Negative		Positive	
		Count	Percent	Count	Percent
ELISA IgG	Negative	34	100.0%	12	75.0%
	Positive	0	0.0%	4	25.0%
	Total	34	100.0%	16	100.0%

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تغييرات موت الخلايا الفسلجي في النسيج المغذي للجنين (التروفوبلاست) ودوره في حث الإجهاض في داء المقوسات الكوندية

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خلاصة البحث

المقوسات الكوندية هي طفيليات وحيدة الخلية شائعة . وتحدث الإصابة بها في كل أنحاء العالم . وهي واحدة من اهم الإصابات الشائعة في الإنسان . وتحدث الإصابة بها عادة بهضم اللحم النيئ او المطبوخ بصورة غير كافية والحاوي على كيس النسيج الحي اوبهضم الغذاء او الماء الملوث بالبيوض المطروحة من قبل القطط .بالإضافة الى ذلك داء المقوسات الكوندية ممكن انتقاله من الام المصابة حاليا الى جنينها .خلال الحمل الاصابة ممكن إن تسبب مضاعفات شديدة إن لم تكن مميتة الى الجنين . هذه التأثيرات المباشرة تقود الى الإجهاض التلقائي ، ولادة جنين ميت وتشوهات خلقية . موت الخلية الفسلجي في النسيج المغذي للجنين يزيد من مضاعفات الحمل مثل الاصابة بالمقوسات الكوندية .من دراستنا وجدنا انه المقوسات الكوندية تؤدي الى تقليل موت الخلايا الفسلجي عندما تم قياس مستويات انزيم قاطع متسلسلة حامض الاسبارتيك الثالث والتاسع في النسيج المغذي للجنين (التروفوبلاست) باستخدام الفحص الكيميائي النسيجي .

أهداف البحث :-

لملاحظة مستويات تعبير إنزيم قاطع متسلسلة حامض الاسبارتيك الثالث والتاسع ضمن خلايا التروفوبلاست المصابة بالمقوسات الكوندية وعلاقتهم بالإصابة .

طرق البحث :-

تضمن الدراسة خمسون مريضة (امرأة مجهضة إجهاض تلقائي) أعمارهم ما بين ١٦-٤٦ سنة . أخضعت مصل المريضات الى فحص الاليزا للكشف عنIgM و IgG الخاص بالمقوسات الكوندية بنفس الوقت النسيج المغذي للجنين اخذ من نفس المريضات لتأكيد الإصابة بالمقوسات الكوندية ولقياس تعبير إنزيم قاطع متسلسلة حامض الاسبارتيك الثالث والتاسع باستخدام الفحص الكيميائي النسيجي .

النتائج:-

الفحص الكيميائي النسيجي أكثر حساسية من الاليزا في فحص المقوسات الكوندية . عندما وجد ١٨ مريضة موجبة لمستضدات المقوسات الكوندية باستخدام الفحص الكيميائي النسيجي بينما في فحص الاليزا وجد ١٦ مريضة فقط موجبة للمضاد المناعي نوع (م). وان مستويات إنزيم قاطع متسلسلة حامض الاسبارتيك الثالث والتاسع قد قلت في المجموعة المصابة .

الاستنتاج :-

مستويات تعبير إنزيم قاطع متسلسلة حامض الاسبارتيك الثالث والتاسع هناك فرق معنوي عالي بين المجموعة الموجبة للمقوسات الكوندية مقارنة بمجموعة السيطرة مما يدل على إن المقوسات الكوندية قد قللت الموت الفسلجي للخلايا.

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