

## HIGH EXPRESSION OF P53 PROTEIN IN TOXOPLASMOSIS AMONG WOMEN WITH SPONTANEOUS MISCARRIAGE IN BASRAH.

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### ABSTRACT

Recurrent abortion is a worldwide problem, with undefined causes. Apoptosis could play a major role in the process. The Objective of the work to detect the expression of p53 protein at the materno-fetal interface in patients with recurrent pregnancy loss (RPL). Immunohistochemistry analysis of P53 protein using paraffin embedded sections of curate samples obtained from 40 women divided into three groups : 16 women with recurrent abortion as positive with toxoplasmosis,10 women with recurrent abortion negative of toxoplasmosis and14 women with no history of abortion as control group .

The mean value of the expression of P53 protein was  $(40.87 \pm 7.54)$ , which is significantly higher than that of the second group  $(28.2 \pm 4.89)$ , and the third group  $(13.07 \pm 4.49)$ . The high expression of p53 protein in women with RPL may have a role in accelerating placental apoptosis leading to failure of pregnancy.

### INTRODUCTION

Toxoplasmosis is caused by an obligate intracellular tissue protozoan parasite , which is able to infect humans as well as other warm blooded domestic and wild animals ( 1). Ingestion of cysts in infected meat and oocysts from soil, food, or water contaminated with cat feces are the two major routes of transmission (2,3). Rarely, blood transfusions or infected organ transplantations have been implicated (1,4).

In immunocompetent subjects,90% of *T.gondii* infections are asymptomatic(5). The importance of this parasite is mainly in pregnancy as it can cross the placental barrier to infect the foetal tissues and thereby cause congenital deformities(6). programmed cell death (PCD) or apoptosis is one of the critical processes during fetal development. It is plausible that the process may play a significant role in placental development and maturation with the evolution of pregnancy, and also may be related to placental aging and complicated pregnancy. Various kinds of significant substances, including certain

types of oncogene products, are known to be physiologically expressed in the placenta among these products are Bcl-2 and p53 proteins (7).

studies have revealed high levels of host cell apoptosis associated with several protozoan infections including *T.gondii*, particularly among immune cells (8). among other factors, apoptosis of T lymphocytes triggered by *T.gondii* may restrict the immune response to the parasites (9). Indeed high levels of apoptosis in spleenocytes have been associated with unrestricted parasite multiplication leading to high parasite burdens in various tissues of host (10). Lymphocytes apoptosis may also influence the local immune response after natural parasite transmission via gut, because oral infection *T.gondii* led to apoptosis in Payer's patch T cells (11). In addition to these reports apoptosis *in vitro* and *in vivo* was studied, this phenomenon was confirmed *in vitro* study, showing that *T.gondii* infection in J774A.1 mouse macrophage cells with interferon- $\gamma$  (IFN- $\gamma$ ) treatment activated inducible nitric oxide synthase, and consequently produce nitric oxide (NO) facilitated apoptosis. apoptosis in the peritoneal macrophages of parasite-infected mice was reported. such apoptosis observed in immune cells have been thought to be result in the state of immunosuppression, contributing parasite survival in the host (12)

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The aim of the study to evaluate p53 as an anti-apoptotic and pro-apoptotic proteins in the trophoblastic tissue of *Toxoplasma gondii* positive, negative aborted women by Immunohistochemistry(IHC).

### **MATERIALS AND METHODS:**

This study was conducted from November 2013 to July 2014. 16 women with recurrent abortion as positive with toxoplasmosis, 10 women with recurrent abortion negative of toxoplasmosis and 14 women with no history of abortion as control group. were included in the study from Basrah Maternity and Children Hospital, Iraq.

placental sample were obtained from all patient with recurrent abortion during curttage procedure and placental sample were obtained from control group during delivery. and placed in 10% formaldehyde. Two to three paraffin embedded blocks were prepared for each patient (13), And confirmed by a pathologist, and then subjected for immunohistochemistry technique using cytomation detection kit (DAKO Denmark). Detection of P53 done by immunohisto-chemistry, the procedure includes briefly; 5 $\mu$ m thickness tissue sections on positively charged slides were deparafinized in xylene then rehydrated in a series of ethanol concentrations. And then 2-3 drops of peroxidase block were applied onto the tissue sections a step which is followed by application of the primary antibody (anti-P53 protein) (DAKO Denmark), then the secondary antibody was added, followed by application of the horseradish peroxidase (HRP) conjugate, and then its substrate DAB chromogen. Sections were counterstained with hematoxyline, sections dehydrated and mounted to be finally examined under the microscope. The expression of P53 was measured by counting the number of positive decidual and trophoblastic cells, which gave a brown cytoplasmic staining under the light microscope (figure 1). The extent of the immunohistochemistry signal in the villi was determined in 10 fields (X400 magnification). In each field the total number of villi

were counted and the extent of nuclear staining of the cytotrophoblast and syncytiotrophoblast in a given villous was counted and simplified as percent, the percentage of positively stained villi was calculated for each case by taking the mean of the percentages of the positively stained villi in the 10 fields (14). The scorer was blinded to the clinical diagnosis of the tissues at the time of assessment, and tissues were independently assessed by two observers. Negative controls were obtained by omitting the monoclonal antibody (Anti-P53) and using phosphate buffer saline to verify the signal specificity.

#### **Statistical Analyses:**

Statistics : (ANOVA test )was used to determine the difference in the expression of p53 protein among the patients and control groups. Values of p (0.001) were considered as statistically significant.

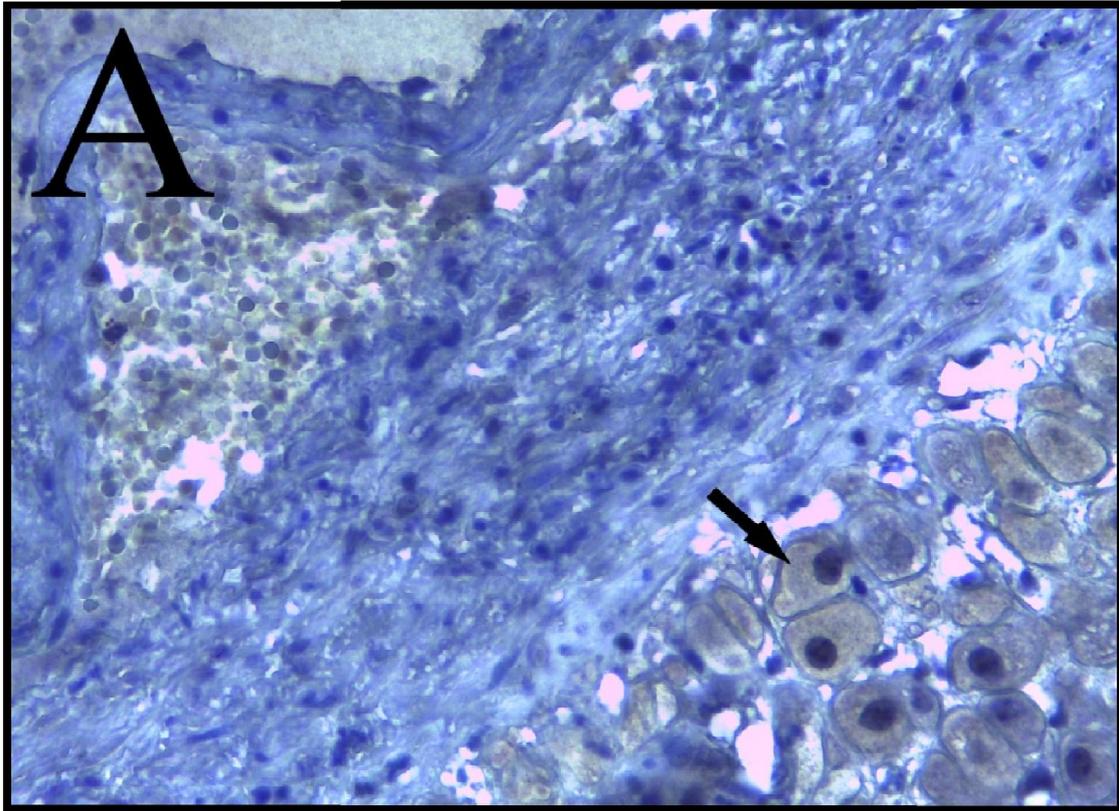
## **RESULT**

#### **Results of (IHC) detection of P53 protein among the study groups :**

The expression of p53 protein detected by immunohistochemistry (IHC) technique. Scoring system used to express the percentage of the expression of this protein.

Figure(1) and table (2 ) showed that there was highly significant increase in mean percentage of p53 in *Toxoplasma gondii* positive group ( $40.87 \pm 7.54$ ) compared to *Toxoplasma gondii* negative group ( $28.2 \pm 4.89$ ) and induced abortion group ( $13.07 \pm 4.49$ ).

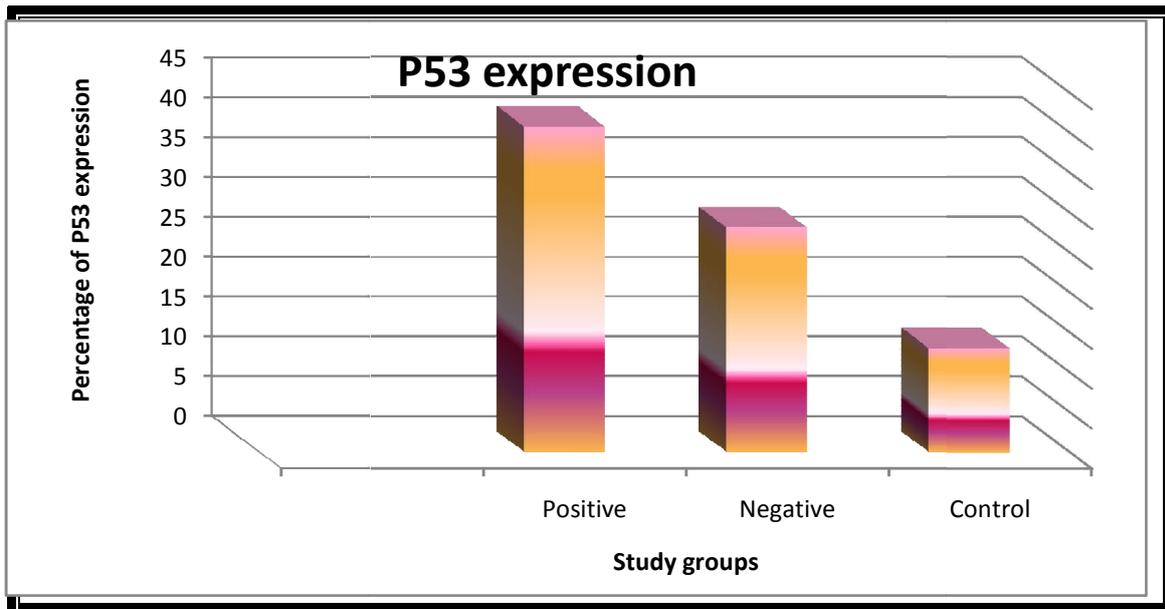
These differences was highly significant ( $P < 0.001$ ) as compared with the *Toxoplasma gondii* negative, and control groups.



**Figure: 1** Detection of P53 protein by immunohistochemistry in women with pregnancy loss. (A) in *Toxoplasma gondii* positive group .Staining of P53 protein by DAB chromogen (dark brown) counterstained with Mayer's Heamatoxylin . Magnification power (X400).

**Table 2:** The mean percentage results of immunohistochemistry of P53.

Marker	Grouping	No.	Mean	±SD	SE	P-Value
P53 Expression	Toxo Positive	16	40.87	7.54	0.502	P≤ 0.0 01 Highly significant
	Toxo negative	10	28.2	4.89	0.49	
	Control	14	13.07	4.49	0.32	



**Figure (2) the expression of p53 protein among the three groups**

## DISCUSSION

In the present study, we found a highly significant increase in the expression of p53 protein in the women with recurrent abortion. This is in agreement with other recent studies showed important regulatory effect of P53 protein on placental function and overexpression of p53 might cause a pathological effect and failure of the gestation(15, 16, and 17). This finding might be explained by the facts that fetal growth and development depend on intact placental function. Maintenance of placental structure and differentiation is essential for the provision of adequate gas, nutrient and waste exchange between the fetus and its mother .

Placental trophoblast and placental reorganization are ongoing processes during pregnancy. Therefore, apoptosis and cell proliferation is frequently observed during pregnancy in maternal blood vessel cells , and trophoblasts of the placenta .

Imbalances in these highly regulated processes of tissue or cell differentiation caused by an increased number of cells arrested at the G1 checkpoint, might to some extent cause inadequate supply of nutrients , gases or waste exchange between mother and fetus, leading to preterm abortion(15, 18,19).

During early pregnancy , the shape of the fetus is spherical, and maternal blood supply is abundant. The process of fetal growth continues until a critical time, specific to each species , when the conceptus reaches a maximum spherical radius and the uterine tissue is stretched.

Tension is so great that it creates ischemia, resulting in circulatory stasis , which is detrimental to maternal blood flow . the conversion of embryo shape from a sphere to a cylinder , which requires only a few hours , causes a release of uterine tissue tension and reestablishment of the maternal blood supply throughout the uterus (20) . Notably , late gestation is accompanied by rapid

growth of the fetus, and it also is marked by a second period of mechanical stretch, which ends at parturition. It is a reasonable hypothesis that uterine conversion can cause transient ischemia in a stretched myometrium that can lead to a hypoxic response in this tissue and activation of the intrinsic apoptotic-pathway (21).

To have more support of our finding, recently, it was shown that p53 is a potential mediator of pregnancy by estrogen and progesterone activation (22).

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### الخلاصة

اجريت الدراسة الحالية للكشف وتحليل وجود بروتينات موت الخلايا المبرمج التي تؤدي إلى نهاية الحمل والإجهاض (P53) باستخدام تقنيه الفحص الكيميائي المناعي النسيجي (IHC) (لأول مرة في محافظة البصرة) لتشخيص وفحص داء المقوسات .

قسمت الدراسة المرضى إلى ثلاث مجاميع :-

المجموعة (A) :- وهي مجموعه الاسقاط المتكرر موجبة لمستضدات طفيلي داء المقوسات المجموعة (B) :- وهي مجموعه الاسقاط المتكرر سالبة لمستضدات طفيلي داء المقوسات المجموعة (C) :- مجموعه خاليه من الاسقاط اعتبرت مجموعة السيطرة .

تم فحص نماذج النسيج المغذي للجنين (التروفوبلاست) المطمورة بالشمع لكل النساء في هذه الدراسة بواسطة اختبار الكيميائي المناعي النسيجي (IHC) للكشف وتحليل وجود بروتينات موت الخلايا المبرمج التي تؤدي إلى نهاية الحمل والإجهاض (P53).

تم العثور على أعلى نسبة من التعبير عن بروتين P53 في العينات التي كانت موجبة لوجود المستضد الخاص بداء المقوسات في النسيج المغذي للجنين (  $7.5 \pm 40.8$  )، وكانت العينات السالبة لوجود المستضد الخاص بداء المقوسات ومجموعة السيطرة بواسطة الفحص النسيجي المناعي الكيميائي ( IHC ) أقل نسبة (  $4.89 \pm 28.2$  ) و (  $9 \pm 4.4 \pm 13.07$  ) على التوالي . كان هناك فرق إحصائي (  $P \geq 0.001$  ) في نسبة متوسط البروتين p53. هذه المستويات العاليه من البروتين P53 وجدت في عينات إيجابية لعدوى التوكسوبلازما قد أشار على أهمية دور هذا البروتين في موت الخلايا

واستحثات موت الخلايا المبرمج التي تؤدي إلى نهاية الحمل مع الإجهاض . وهذا يؤكد مدى خصوصية وحساسية الفحص الكيميائي المناعي النسيجي في فحص داء المقوسات .

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