

# Evaluation of Matrix Metalloproteinase-2 (MMP2) in Aborted Women Infected with Toxoplasma gondii.

## إظهار قيم مصفوفة أنزيم الزنك 2 في النساء المجهضات والمخجمات بداء المقوسات

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### الخلاصة

**الخلفية:** داء المقوسات يعتبر واحد من مسببات الأمراض الحيوانية المنشأ الأكثر شيوعاً في جميع أنحاء العالم، دورة حياة الطفيلي ممكن أن تتأثر بواسطة أفران أنزيمات متخصصة منها مصفوفة أنزيم الزنك 2.

**الهدف:** تهدف الدراسة الى التحقق من التعبير الانتشاري لقيم مصفوفة أنزيم الزنك 2 من قبل داء المقوسات بين 96 من النساء المجهضات، 76 أصبن بالإجهاض المتكرر بسبب العدوى بداء المقوسات و20 لم تثبت الإصابة ب داء المقوسات كمجموعة ضابطة في محافظة النجف الأشرف.

**المنهجية:** دراسة الشاهد والحالة تم اختيار عينة غرضية (عشوائية) تتكون من ستة وتسعون من النساء المجهضات في مختبر الصحة المركزي، ومستشفى الزهراء للولادة للمدة من 1 كانون الثاني 2014 ولغاية 1 من كانون الثاني 2015 وجمعت البيانات بواسطة استبانة خاصة تم قياس مستوى تركيز قيم مصفوفة أنزيم الزنك 2 في النساء المجهضات باستخدام تقنية تقييم الإنزيم المناعي طبقاً لعدد من المؤشرات مثل (العمر والسكن والوظيفة وعدد مرات الإجهاض).

**النتائج:** اشارت نتائج الدراسة بأن تركيز مصفوفة أنزيم الزنك 2 كان مرتفع في الحالات التي كان فيها الإجهاض مرة واحدة والحالات التي كان فيها الإجهاض مرتين، التركيز العالي ل مصفوفة أنزيم الزنك 2 في النساء المجهضات المصابات بداء المقوسات في المجموعات العمرية (20-24) و(25-29). وكان هناك علاقة ذات دلالة إحصائية بين مجاميع الدراسة المعتمدة على المهنة وأن هناك اختلاف في مستويات تركيز مصفوفة أنزيم الزنك 2 بحسب الموقع الجغرافي وكان هذا التركيز العالي في نساء الريف المصابات ومنخفضة في نساء المدينة المصابات بداء المقوسات، تم تحليل النتائج باستخدام الوسائل الإحصائية (فحص ليفين وأنوفا واختبار t).

**الاستنتاج:** مصفوفة أنزيم الزنك 2 لعبت دوراً هاماً في المناعة ضد الإصابة بداء المقوسات.

**التوصيات:** ينصح إجراء المزيد من الدراسات لمعرفة تركيز مصفوفة أنزيم الزنك 2 لدى النساء الحوامل ليكون واحداً من الفحوصات المختبرية الهامة في تحريض الاستجابة المناعية ضد هذا النوع من الإصابات.

**الكلمات المفتاحية:** مصفوفة أنزيم الزنك 2، توكسوبلازما كوندوي، النساء المجهضات

### Abstract

**Background:** Toxoplasmosis is one of the most common zoonotic pathogens worldwide; Life cycle of this parasite was affected by synthesis of specific enzyme like Matrix metalloproteinase-2 (MMP2).

**Objectives:** The major objectives of this study detection of prevalence expression of Matrix metalloproteinase-2 (MMP2) among ninety-six aborted women were tested; seventy-six of them was with recurrent abortions due to Toxoplasmosis and twenty without Toxoplasmosis as control group in Najaf governorate.

**Methodology:** Descriptive case-control, purposive (probability) sample among ninety six aborted women were tested, attending in Central Health Laboratory, Al-Zahra Maternity & paedriatic Teaching Hospital, The data collected from January 2014 to January 2015. The concentration level of Matrix metalloproteinase-2 (MMP2) in aborted women was measured by ELISA technique according to many parameters such as age, occupation, residence and number of abortions.

**Results:** The result indicated that the concentration of MMP2 was elevated in women with one or two abortions. The high concentration of MMP2 in infected aborted women with age group (20-24), (25-29). there was significant differences in study groups according to occupation and there were differences in MMP2 concentration levels according to residency. It was demonstrated that the high concentration of MMP2 was appear in rural infected aborted women and low in urban women with Toxoplasmosis, Results were analyzed by using statistical methods (Levene test, ANOVA test, t test).

**Conclusion:** Matrix metalloproteinase-2 (MMP2) play a significant role in immunity against infection with Toxoplasma gondii.

**Recommendation:** Further studies are recommended to know the concentration of MMP2 among pregnant women to be one of an important laboratory tests in the induction of immune response against this type of infection.

**Keywords:** Matrix metalloproteinase-2 (MMP2), Toxoplasma gondii, aborted women.

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## **INTRODUCTION:**

Toxoplasma gondii has a variety of distribution <sup>(1)</sup>, close relationship to other species of coccidian, and some similarities to malaria parasites <sup>(2)</sup>. Human has a chance for infection with T.gondii while cats considered the biggest source of injury<sup>(2)</sup>. T.gondii infection shows a broad range of clinical signs most of them asymptomatic especially in an immunocompetent patient<sup>(1)</sup>.

Matrix metalloproteinases (MMPs) are a type of enzymes located outside the cell<sup>(3)</sup>. Invertebrates MMPs family different in their tissue productions <sup>(4)</sup>.

MMPs are expressed in cells including macrophages, endothelial cells and vascular smooth muscle cells <sup>(4)</sup>. MMPs play a substantial role in the development of inflammatory, neurodegenerative, and malignant diseases <sup>(5)</sup>. In recent years, some studies have found that MMPs are associated with invasive diseases<sup>(5)</sup>. Increased expression of MMPs may be considered as an indication of generating placental abnormalities and delivery or abortion<sup>(6)</sup>. MMP2 was the most prevalent representative of this MMP family, and expressed in the endometrium <sup>(5)</sup>. It was important in various processes especially with that dealing with infection during pregnancy <sup>(6)</sup>.

MMP2 may be associated with the premature rupture of the fetal membrane (PROM) <sup>(7)</sup>. MMP2 functional gene polymorphisms might be associated with an increased risk of recurrent spontaneous abortion (RSA) in women <sup>(8)</sup>.

## **OBJECTIVE OF THE STUDY:**

The major objective of this study is to follow up the role of MMP2 in aborted women infected with Toxoplasmosis.

## **METHADODOLOGY:**

### **Study Sample**

Ninety six serum sample of suspected patient were involved, the women were 20-45 years of age. They were attending Al-Zahra Maternity and paediatric Teaching Hospital in Najaf governorate from January 2014 to January 2015. The age groups, number of abortions, occupation and address were depended in following of the study.

**Control:** Twenty apparently healthy women (non infected) were enrolled as a control group.

**Data Analyses:** the following statistical analysis approach by using social sciences (SPSS) version 20 in order to analyzed and assess the data of the study.

### **Matrix metalloproteinase-2 (MMP2).**

Human MMP-2 (ELISA), Executed according to manufacturer.

### **Statistical Methods:**

(t) Test and LSD was applied to find out the significant difference between the data. Differences were recorded as significant whenever the probability (P) was less than 0.05.

**RESULTS:**

**Table (1) Toxoplasmosis by using IgG& IgM ELISA technique.**

<b>Tests</b>	<b>IgG No. (%)</b>	<b>IgM No. (%)</b>
<b>Results</b>		
<b>Positive</b>	<b>76(79%)</b>	<b>42(44%)</b>
<b>Negative</b>	<b>20(21%)</b>	<b>54(56%)</b>
<b>Total</b>	<b>96</b>	<b>96</b>

Table 1 show that the positive percentage of IgG,IgM by ELISA technique were 76(79%), 42(44%) respectively.

**Table(2) Significant difference of MMP2 concentration in infected aborted women and non-infected aborted women.**

<b>Clinical group</b>	<b>Mean concentration ±SD MMP2 No.(Conc. Pg/ml)</b>	<b>t. Test value</b>	<b>df</b>	<b>P-value</b>
<b>Aborted women with Toxoplasmosis</b>	<b>5.260±5.499</b>	<b>2.735*</b>	<b>94</b>	<b>0.001</b>
<b>Aborted women without Toxoplasmosis</b>	<b>1.871±0.907</b>	<b>5.113*</b>	<b>88</b>	<b>0.003</b>

Table 2 revealed that a highest of serum concentration of MMP2 was recorded in aborted women with Toxoplasmosis (5.260±5.499) when compared with aborted women without Toxoplasmosis (1.871±0.907); there was significant difference (p<0.005) between serum level of MMP2 in both positive cases and control groups

**Table (3) Mean concentration of MMP2± SD according to age groups.**

<b>Clinical groups</b>	<b>Mean concentration ±SD MMP2 No.(Conc. Pg/ml)</b>	
	<b>Aborted women with Toxoplasmosis</b>	<b>Aborted women without Toxoplasmosis</b>
<b>Age group(year)</b>		
20-24	9.693±8.229	1.792±1.087
25-29	4.405±1.448	2.727±0.749
30-34	3.502±1.718	1.349±0.653
35-39	3.532±1.530	1.629±0.382

	≥40	2.246±1.863	1.830±1.060
	Total	5.260±5.499	1.871±0.907
*Levene test=82.935 P- value=0.001		**ANOVA test=7.283	**ANOVA test =1.390

Table 3 shows that the high concentration of MMP2 in aborted women with age 20-24 years and 25-29 years was 9.693±8.229, 4.405±1.448 respectively and the low level in aborted women with age (≥40) years was 2.246±1.863. It was found that there were statistical differences (P<0.05) of serum concentration of MMP2 in the cases of age groups.

**Table (4) Mean concentration of MMP2± SD according to residence.**

Clinical groups		Mean concentration ±SD MMP2 No.(Conc. Pg/ml)	
		Aborted women with Toxoplasmosis	Aborted women without Toxoplasmosis
Residency	Urban	3.179±1.810	2.166±1.037
	Rural	6.107±6.242	1.577±0.685
	Total	5.260±5.499	1.871±0.907
*Levene test=12.209 P- value=0.03		**ANOVA test =4.676	**ANOVA test =2.236

Table 4 revealed that the highest concentration appear in rural area was 6.107±6.242 .It was found that there were statistical differences (P<0.05) of serum concentration of MMP2 according to residency.

**Table (5) Mean concentration of MMP2± SD according to occupation.**

Clinical groups		Mean concentration ±SD MMP2 No.(Conc. Pg/ml)	
		Aborted women with Toxoplasmosis	Aborted women without Toxoplasmosis
Occupation	Housewife	6.993±7.151	2.098±0.980
	Official	3.713±1.152	1.038±0.174
	Student	3.281±1.697	1.829±0.759
	Total	5.260±5.499	1.871±0.907
*Levene test=26.809 P- value=0.02		**ANOVA test =4.365	**ANOVA test =1.776

Table 5 shows that the high concentration appear in housewife was  $6.993 \pm 7.151$ . It was found that there were statistical differences ( $P < 0.05$ ) of serum concentration of MMP2 according to occupation.

**Table (6) Mean concentration of MMP2± SD according to numbers of abortion.**

Clinical groups	Mean concentration ±SD MMP2 No.(Conc. Pg/ml)	
	Aborted women with Toxoplasmosis	Aborted women without Toxoplasmosis
1	$7.496 \pm 7.505$	$2.109 \pm 1.084$
2	$5.679 \pm 1.386$	$1.551 \pm 0.750$
3	$3.068 \pm 1.663$	$1.899 \pm 0.211$
≥4	$2.946 \pm 1.905$	$1.723 \pm 1.477$
Total	$5.260 \pm 5.499$	$1.871 \pm 0.907$
*Levene test=28.937 P- value=0.001	**ANOVA test=3.837	**ANOVA test =1.430

Table 6 the result of this table shows that the concentration of MMP2 in aborted women with one and two abortion was  $7.496 \pm 7.505$ ,  $5.679 \pm 1.386$  respectively. It was found that there were statistical differences ( $P$ -value=0.001) of concentration MMP2 accordingly number of abortions.

## DISCUSSION:

A high level of serum concentration of MMP2 was recorded among patients with ELISA seropositive with Toxoplasmosis ( $5.260 \pm 5.499$  pg/ml), and there was a significant difference between this level and level in control groups. Through researches and studies that show the significant role of MMP2 and its influence on the immune response through the direct association with others factors <sup>(9)</sup>.

MMP2 have a role in reducing the mechanism of parasitic invasion through the placenta<sup>(10)</sup>.

The present study finding was discordant with the research that concluded MMP2 have an inhibitory effect on the induction immune response resulting from parasitic and bacterial infections <sup>(11)</sup>.

The current result reveals that the concentration of MMP2 was elevated according to study groups. These findings matching to other reported result that referred to increase of MMP2 serum level index at the beginning of mechanical defenses caused by some parasitic infections <sup>(10)</sup>.

It is nearly identical to result that found high in the concentration of MMP2 resulting from the disintegration of tissue <sup>(12)</sup>.

The present study was observed high concentration of MMP2 in infected aborted women in age group (20-24) years and (25-29) and there was a significant difference of MMP2 according to age groups. The result was incompatible with other interpretation which proved the evidence regarding the age-related differences in circulating MMP2 levels <sup>(13)</sup>. It was contravention with that founding by <sup>(14)</sup> whose, mentioned that MMP2

increased in concentration during aging and age consider important marker on this highest concentration of MMP2.

The result in the current study was compatible with that reported by <sup>(15)</sup>who said that progress stages of age enhance the low levels of MMP2 concentration.

Justification for this inverse relationship between age and decrease in the concentration of MMP2 related to the components of these proteins <sup>(14)</sup>.

The present study results demonstrated that there was increased in concentration of MMP2 levels in aborted housewife women with significant differences with other study groups. It was the present study was nearly similar to the result that mentioned by <sup>(6)</sup>.

The current study showed a variation in MMP2 concentration levels among individuals of residence in which the concentration was high in aborted rural toxoplasmic women and low in aborted urban toxoplasmic women. This result was not agree with others who recorded elevation in serum concentration in urban area more than the rural <sup>(16)</sup>, While agree with other <sup>(17)</sup>.

This may be attributed to variable levels of exposure of environmental pollution factor, which affect directly on physical activities and basal metabolism, and these factors deal with the impact infection with Toxoplasmosis and these variations may contribute to differential disease susceptibility and level of stress tolerance.

The results show statistical differences of concentration MMP2 in one and two abortion .and this come no agree with others <sup>(18)</sup>. Moreover, agree with other who fined statistical differences between MMP2 and numbers of abortion <sup>(8)</sup>. Explanation of variance in concentration of MMP2 according to aborted women with Toxoplasmosis may be due to the occurrence of many problems in women's health including endometriosis.

## **CONCLUSION:**

From the results of this study, it is concluded that:

1. Matrix metalloproteinase-2(MMP2) play a significant role in immunity against infection with *Toxoplasma gondii*.
2. There is an association between elevated concentration of MMP2 levels in aborted women with Toxoplasmosis.
3. MMP2 concentration is elevated at young age's groups.
4. MMP2 concentration is elevated at one and two number of abortions women groups.

## **RECOMMENDATION:**

The following points are recommended:

1. Further experimental studies are recommended to know the concentration of MMP2 among pregnant women to be one of an important laboratory test in the induction of immune response against this type of infection.
2. Further studies to know the exact mechanisms by which MMP2 are excreted in serum.
3. Study the relationship between MMP2 with other pathogens or systemic disease that are causing abortion.

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