Detection of Heat Shock Protein (Hsp70) in aborted women infected with Toxoplasma gondii

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الخلاصة

إنّ الدراسة الحالية تهدف الى التحقق من أنتشار طرح بروتين الصدمة الحرارية (Hsp70) من قبل Toxoplasma gondii بين ٩٠ مِنْ النِساء المُجهضاتِ، ٢٠ أصبن بالإجهاض المتكرّر بسبب عدوى toxoplasmosis و٢٠ لم تثبت الإصابة بtoxoplasmosis (١٠ نِساء مُجهضاتِ محموماتِ و١٠ نِساء مُجهضاتِ محموعة سيطرة.

تم قياس مستوى تركيز بروتين الصدمة الحرارية (Hsp70)في النِساء المُجهضاتِ باستخدام تقنية تقييم الإنزيم المناعي طبقاً لعدد من المؤشرات مثل (العمر وعدد مرات الإجهاض) إضافة إلى أجراء مقارنة بين اختبار التراصي باللاتكس (LAT)واختبار تقييم الإنزيم المناعي (.IgG ELA و.IgG ELA) مع مستوى تركيز بروتين الصدمة الحرارية (Hsp70)المطروح من قبل داء المقوسات.

العدد الكامل للحالات الموجبة كانت ٧٠ (100%) بواسطة LAT و ٢٠ (29.5%) بواسطة IgM و ٢٩ (98.5%) بواسطة IgM و ٦٩ (98.5%)

.. أظهرت نتائج هذه الدراسة بأن تركيز (Hsp 70) كان مرتفع في الحالات التي كان فيها الإجهاض ثلاث مرات(٤.٢٦ %) والحالات التي كان فيها الإجهاض أربعة مرات(٩.٤ %) وظهر أيضا أن هنالك ارتفاع في تركيز الإنزيم المناعي IgM ناتج عن الإصابة الحادة بداء المقوسات حيث كان في حالات الإجهاض ل ثلاث مرات (٢.٧٥ %) و (٢.٨٩ %) في حالات الإجهاض لأربعة مرات على التوالي في النساء المُجهضات المصابات ب Toxoplasmosis.

لاحظت الدراسة الحالية بأن التركيز العالي ل(Hsp70) في النساء المجهضات المصابات بداء المقوسات في المجموعات العمرية(٣٠-٣٥) و(٣٦-٤١) كانتْ(٤٠٣١ % و٨٠٠٨ %) على التوالي.

Abstract

The present study is conducted to detect the prevalence of expression of heat shock protein (Hsp 70) by infection with *Toxoplasma gondii* among 90 aborted women only 70 of them with recurrent abortion due to toxoplasmosis infection and 20 without toxoplasmosis (10 feverish aborted women and 10 healthy aborted women) as control group. The concentration level of Heat Shock Protein (Hsp70) in aborted women was measured by ELISA technique according to many parameters such as age and number of abortion. Comparison between Latex, IgG- ELISA and IgM- ELISA concentration with Hsp 70 concentration expressed by *T. gondii* was carried out.

The total number of studied cases of toxoplasmosis were 70 (100%) by LAT and 20 (29.5%) by IgM-ELA. and 69 (98.5%) by IgG-ELA. The present study revealed that the concentration of Hsp70 was elevated among those with 3 number of abortion 4.26% and 9.47% among those 4 number abortion.

Introduction:

Toxoplasma gondii is globally distributed pathogen for human. Most of the infected people were clinically asymptomatic however *T. gondii* causes significant morbidity and mortality in developing fetuses and in immunocompromised patients (1,2,3).

Heat shock proteins Were used firstly to describe Drosophila melanogaster proteins expressed at elevated temperatures (4). Various heat shock proteins(Alpha-B-crystallin ,hsp 10, hsp 25, hsp 47, hsp 60, hsp 70,hsp72& hsp90) have been found to act as intercellular signaling molecules, which merits their inclusion among hormones ,

Kufa Med.Journal 2012.VOL.15.No.1

cytokines and growth factors (5,6). *Toxoplasma gondii* heat shock protein 70(*T. gondii* Hsp70) induces deleterious effects on protective immunity via mediation inhibitory effect on host protective immunity so The 70-kDa parasite stress protein may be part of an important survival strategy by which virulent strains down-regulate host parasiticidal mechanisms(7). The aims of the current study is detection of infection by Latex & ELISA techniques and detection the heat shock protein (HSP 70) in aborted women by ELISA techniques in positive cases of toxoplasmosis according to tow parameters {age and number of abortion}.

Materials& Methods:

The Patients

The Hsp 70 study was conducted on 70 aborted women who gave positive toxoplasmosis reaction by Latex Agglutination Test (LAT), IgM-EIA and IgG-EIA out of many aborted cases attending AL-Zahraa Maternity and AL-Hakeem and AL-sadder teaching hospitals in Najaf governorate hospitals from December 2010 to April 2011. This study includes 20 of aborted women as control group.

Serological Tests

A-Latex Agglutination Test (LAT) according to(8).

B- Toxoplasma IgG and IgM Enzyme ImmunoAssay test kit According to (10).

C- Heat Shock Protein 70 (Hsp70) according to Bio Assay of T. gondii ELISA Kit. company(US Biological)

Principle of the Assay:

Samples and standards are added to wells coated with a monoclonal antibody specific for Hsp70. After incubation, the plate is washed, leaving only bound Hsp70 on the plate. A yellow solution of polyclonal antibody specific for Hsp70 is then added. This binds the Hsp70 captured on the plate. After incubation, the plate is washed to remove excess antibody. A blue solution of HRP conjugate is added to each well, binding to the antibody. The plate is again incubated. The plate is washed to remove excess HRP conjugate. TMB substrate solution is added. and the HRP-catalyzed reaction generates blue color in the solution. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450nm. The amount of signal is directly proportional to the level of Hsp70 in the sample.

Statistical Methods:

(t) test and LSD was applied to find out the significant difference between the data. Differences were recorded as significant when ever the probability (P) was less than 0.05 (11).

Results:

The study of heat shock protein 70 (Hsp 70)was curried out on 70 aborted women who give seropositive diagnosis of toxoplasmosis by Latex test, IgM ELA. Test and IgG ELA. Test and 20 women as a control group(10 feverish aborted women and 10 healthy aborted women according to clinical investigation). (Table-1)

Kufa Med.Journal 2012.VOL.15.No.1

Table -1: Distribution of toxoplasmosis in aborted women according to Latex test,	IgM and
IgG ELA.	

	LAT		IgM		IgG		Total number
	Number of cases	%	Number of cases	%	Number of cases	%	Number of cases
Aborted women with toxoplasmosis	70	100	20	29.5	69	98.5	70
Feverish aborted women without toxoplasmosis	10	0	10	0.7	10	0.6	10
Healthy aborted women without toxoplasmosis	10	0	10	0.4	10	0.5	10
Total number		•				•	90

A-Heat shock protein 70 and IgM.

1- a-Hsp 70 and IgM in feverish aborted women and healthy aborted women with out toxoplasmosis according to number of abortion.

The low and high concentration of Hsp 70 in feverish aborted women was 1.16 and 2.29 respectively While the low and high concentration of Hsp70 in healthy aborted women was 1.06 and 1.54 respectively.(Table-2)

Table -2: Distribution of feverish aborted women and healthy aborted women according to number of abortion in Hsp 70 and IgM.

	No. of	No. of	HSP70	Mean of IgN
feverish aborted women	abortion	cases	Pg/ml	Negative No.
without toxoplasmosis	1	3	1.16	0.6
	2	4	1.17	0.7
	3	2	1.54	0.7
	4	1	2.29	0.8
healthy aborted women	1	4	1.06	0.6
without toxoplasmosis	2	5	1.18	0.8
	3	1	1.54	0.6
Total No.	20			

b:-HSP70 and IgM in aborted women with toxoplasmosis according to number of abortion.

The concentration of Hsp 70 in women with 3 and 4 number of abortion was high (4.26 and 9.47 respectively) and low in women with 1 and 2 number of abortion (1.30 and 2.47 respectively). It was found that there are a significant differences (P < 0.05) between the number of abortion and Hsp70 and positive IgM cases. (Table -3)

Table -3: Hsp 70 and IgM in aborted women with toxoplasmosis according to number of abortion.

No. of abortions	No. of Cases	Hsp 70 Pg/ml	Mean of IgM Positive No.
1	21	1.30	1.34
2	22	2.47	1.79
3	19	4.26	2.75
4	8	9.47	2.89
Total No.	70		

2- a-Hsp 70 and IgM in feverish aborted women and healthy aborted women without toxoplasmosis according to age.

The low and high concentration of Hsp 70 in feverish aborted women was 1.02 and 1.75 respectively. While the low and high concentration of Hsp70 in healthy aborted women was 1.04 and 1.62 respectively .(Table-4)

 Table -4: Distribution of feverish aborted women and healthy aborted women according to age in Hsp 70 and IgM

	Age (years)	No. of cases	Hsp70	Mean of IgM
feverish aborted women			Pg/ml	Negative No.
without toxoplasmosis	18-23	2	1.02	0.6
	24-29	5	0.83	0.5
	30-35	2	1.55	0.7
	36-40	1	1.75	0.7
healthy aborted women	18-23	2	1.04	0.3
without toxoplasmosis	24-29	3	1.08	0.6
	30-35	3	1.52	0.5
	36-40	2	1.62	0.7
Total No.	20			

b:-Hsp70 and IgM in aborted women with toxoplasmosis according to age.

The concentration of Hsp 70 in women with age (30-35)and (36-40) was high (4.31 and 8.08 respectively) and low in women with age 18 and 23 years 1.65. It was found that there are a significant differences (P < 0.05) between the age and HSp70 and positive IgM cases.(Table -5)

Table-5: Hsp 70 and IgM in aborted women with toxoplasmosis according to age (years).

Age (years)	No. of cases	Hsp 70	Mean of IgM
		Pg/ml	Positive No.
18-23	20	1.65	1.6
24-29	33	2.92	1.09
30-35	10	4.31	1.21
36-40	7	8.08	1.83
Total No.	70		

B-Heat shock protein 70 and IgG.

1-a-Hsp 70 and IgG in feverish aborted women and healthy aborted women without toxoplasmosis according to number of abortion.

The low and high concentration of Hsp 70 in feverish aborted women was 1.16 and 2.29 respectively. While the low and high concentration of Hsp70 in healthy aborted women was 1.06 and 1.54 respectively. (Table-6).

Table-6: Distribution of feverish aborted women and healthy aborted women according to number of abortion in Hsp 70 and IgG.

			No. of abortion	No. of	Hsp70	Mean of IgG
feverish aborted	women	without		cases	Pg/ml	Negative No.
toxoplasmosis			1	3	1.16	0.7
			2	4	1.17	0.6
			3	2	1.54	0.8
			4	1	2.29	0.4
healthy aborted	women	without	1	4	1.06	0.5
toxoplasmosis			2	5	1.18	0.6
			3	1	1.54	0.8
			4	-	-	-
Total No.			20			

b:-Hsp70 and IgG in aborted women with toxoplasmosis according to number of abortion.

The concentration of Hsp 70 in women with 3 and 4 number of abortion was high (4.26 and 9.47 respectively) and low in women with 1 and 2 number of abortion (1.30 and 2.47 respectively). It was found that there are a significant differences (P < 0.05) between the number of abortion and Hsp70 and positive IgG cases. (Table-7).

able -7: Hsp 70 and IgG in aborted women with toxoplasmosis according to number of abortion.

No. of abortion	No. of Cases	Hsp 70 Pg/ml	Mean of IgG Positive No.
1	21	1.30	1.22
2	22	2.47	1.56
3	19	4.26	1.63
4	8	9.47	2.32
Total No.	70		•

2-a-Hsp 70 and IgG in feverish aborted women and healthy aborted women without toxoplasmosis according to age.

The low and high concentration of Hsp 70 in feverish aborted women was 1.02 and 1.75 respectively .While the low and high concentration of Hsp70 in healthy aborted women was 1.04 and 1.62 respectively. (Table -8)

	Age (years)	No. of cases	Hsp70	Mean of IgG
feverish aborted women			Pg/ml	Negative No.
without toxoplasmosis	18-23	2	1.02	0.8
	24-29	5	0.83	0.7
	30-35	2	1.55	0.6
	36-40	1	1.75	0.5
healthy aborted women	18-23	2	1.04	0.5
without toxoplasmosis	24-29	3	1.08	0.6
	30-35	3	1.52	0.7
	36-40	2	1.62	0.6
Total No.	20			

Table-8: Distribution of feverish aborted women and healthy aborted women according to age in Hsp 70 and IgG.

b:-Hsp70 and IgG in aborted women with toxoplasmosis according to age .

The concentration of Hsp 70 in women with age (30-35) and (36-40) was high (4.31) and 8.08 respectively) and low in women with age 18 and 23 years 1.65. It was found that there are a significant differences (P<0.05) between the age and Hsp70 and positive IgG cases. (Table-9)

Table-9: Hsp 70 and IgG in aborted women with toxoplasmosis according to age (years).

Age(years)	No. of cases	Hsp 70 Pg/ml	Mean of IgG Positive
			No.
18-23	20	1.65	2.12
24-29	33	2.92	2.64
30-35	10	4.31	2.74
36-40	7	8.08	3.02
Total No.	70		

Discussion:

This study applied the following serological tests LAT, IgG-EIA and IgM-EIA for detection of toxoplasmosis in aborted women who were confirmed having no another types of infection .The present study showed that 70 cases (100%) were positive by LAT among suspected serum samples. This percentage was more than that reported by (11) which was 55.4% in pregnant Nepalese women, while (12) pointed out that 41.75% of pregnant women in India were positive by LAT. In Iraq this percentage was more than the percentage recorded by (13) who studied 320 persons in Duhok Province and found 134 (41.8%) positive by LAT and (14) reported that seropositivity was 39.33% by LAT in Mosul Province. (15) showed that out of 64 patients was 19.7% positive by LAT in Najaf Province, (16) said that 49.65% were positive by LAT in different professional categories in Diwanyia Province and These results was inagreement with the present study. Regarding to table-1, it was observed that the present study demonstrated the prevalence of anti-Toxoplasma IgG and IgM antibodies , the positive cases were 69 (98.5%), 20 (28.5%) respectively. In Iraq (1) recorded that, anti-

Toxoplasma IgG antibody was (93.1%) and IgM was (31%). (15) showed that, the percentage of toxoplasmosis by IgM-EIA was 13.95%. In the present study the percentage of IgG and IgM-EIA was compatible with the result recorded by (17) in Duhok who recorded 3 women (1%) out of the 310 patients were positive by IgM- EIA while (18) recorded 4.25% was positive by IgM-EIA in Najaf Province. (19) pointed out that, the percentage of infection was 7% by IgG-EIA in Basrah. It was found that there were no any study of Hsp 70 in patient with toxoplasmosis in Iraq, this is the first study that deal with Hsp70 concentration among aborted women who infected with toxoplasmosis. The relationship between Hsp 70 and latex test, IgM ELISA & IgG ELISA was found that the concentration of Hsp70 was positively correlated with number of abortion(4.26%, 9.47% for 3 and 4 number of abortion respectively). It was found that there were a significant difference (P < 0.05) between these results and the results that recorded in feverish and health aborted women without toxoplasmosis. (20) showed that there was a significant increase of lymphocyte Hsp70 levels in early pregnant women with Adverse pregnancy outcomes(APOs) compared with the wellmatched controls. Adverse Pregnancy Outcomes (APOs) are a group of common obstetric diseases, including abortions, dead fetus, abnormalities(21).(22) mentioned that Hsp-70 may have an important role in T. gondii adaptation during differentiation organ event.(23)showed that bradyzoite induction is associated with increased T gondii Hsp70 levels. The present study appear to be consistent with published data by(24) who suggested that serum Hsp 70 levels were particularly high in preterm delivery cases.

In the present study it was observed that the high concentration of Hsp 70 in aborted women with toxoplasmosis with age group (30-35)and (36-40) were 4.31% and 8.08% respectively and it was found that there are a significant difference between them. The Heat Shock Protein 70 in feverish aborted women without toxoplasmosis was 1.55% and 1.75% in age group (30-35) and (36-40) respectively and also the recorded Hsp70 in healthy aborted women without toxoplasmosis in age group (30-35) and (36-40) was(1.52% 1.62% respectively). The result in current study was incompatible with the interpretation of (25) who proved that the evidence regarding the age-related differences in circulating Hsp70 levels by comparing 190 elderly persons stratified for clinical profile with 100 healthy young controls and his results showed an age-related decrease in the serum levels of Hsp70.(26) reported that there was An age-related decrease in Hsp70 levels for particularly the age-group 25-29 years. Consequent, the age is a very important factor to investigate the role of Hsp70 as a biomarker in the evaluation of diseases and stress status. The current data showed that there was an increased between Hsp70 levels and age factor. There are other reports of aged-related changes in Hsp70 in human lymphocytes(27).(28) showed that lymphocyte Hsp70 was inversely related to the age of their subjects (aged between 20 and 80 years), but they did not elaborate on this finding.

The justification of the correlation between serum concentration of Hsp70 and age of our aborted women under normal and stressed conditions seems warranted. There is an increase in the incidence of infections and in general morbidity in the elderly and common replacement for investigating the biomedical significance of many genes and proteins in some environmental diseases and stresses. **References:-**

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