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THE INVESTIGATING OF HEAT SHOCK PROTEINS 70 (HSP70) IN SERA OF RHEUMATOID ARTHRITIS PATIENTS IN THI-QAR PROVINCE

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ABSTRACT

Heat shock protein or HSPs are being synthesized under different kind of stress conditions and act as molecular chaperones for protein molecules. because theses protein were first found in cell that were exposed to high temperature, they are called "heat shock protein " Several risk factors are involved in the pathogenesis of autoimmune rheumatic diseases, including genetic factors, smocking, chronic infections, sex hormones and stress.

Stress is now recognized as an important risk factor in the pathogenesis of rheumatoid arthritis by considering that the activation the stress response of immune system. In this study that were investigating about role of heat shock protein 70 in pathogenesis and immune response in RA. The sample of the study includes 145 patients with RA. They were achieved four or more of the criteria of the 2010 American College of Rheumatology, the sample of control in the study include 60 person apparently healthy volunteers were included in this study.

This study shown high statistically significant ($P<0.00$). Between studied group and control when we measured concentration of HSP70 on Samples, and shown slightly statistically significant between studied groups($P<0.05$)according the gender. and this study shown high significant difference between subgroup of patients such as smokers, non-smokers, stressful, non-stressful, low economic status … e.t.c.

Conclusions

Some of environmental factors such as smoking, stressful, others chronic infections, duration of disease, age and sex reactive with genetic factors to play main role in the determination this immunological marker in patients with RA.

KEYWORDS: Rheumatoid Arthritis, Heat Shock Protein 70, Smoking, Stress, Immune Response

INTRODUCTION

The heat shock protein 70 family is a set of highly conserved protein that are induced by a variety of biological stresses, the human HSP70 family members include: HSP70, a 70 Kda protein which is strongly induced in all organisms but which is also constitutively expressed in primate cells HSP72, a72 Kda protein which is induced expressed in protein exclusively under stress conditions (Ritossa F., 1962).

As newly synthesized proteins emerge from the ribosomes, the substrate binding domain of Hsp70 recognizes sequences of hydrophobic amino acid residues, and interacts with them. This spontaneous interaction is reversible, and in the ATP bound state Hsp70 may relatively freely bind and release peptides. However, the presence of a peptide in the binding domain stimulates the ATPase activity of Hsp70. These co chaperones dramatically increase the ATPase activity of Hsp70 in the presence of interacting peptides (Bahr *et al.*,1990). By binding tightly to partially synthesized peptide

sequences (incomplete proteins), Hsp70 prevents them from aggregating and being rendered nonfunctional. Once the entire protein is synthesized, a nucleotide exchange factor (BAG-1 and HspBP1 are among those which have been identified) stimulates the release of ADP and binding of fresh ATP, opening the binding pocket. The protein is then free to fold on its own, or to be transferred to other chaperones.

For further processing. HOP (the Hsp70/Hsp90 Organizing Protein) can bind to both Hsp70 and Hsp90 at the same time, and mediates the transfer of peptides from Hsp70 to Hsp90**.** Hsp70 also aids in trans membrane transport of proteins, by stabilizing them in a partially folded state.**(** Wegele *et al*., 2004).

Hsp70 proteins can act to protect cells from thermal or oxidative stress. These stresses normally act to damage proteins, causing partial unfolding and possible aggregation. By temporarily binding to hydrophobic residues exposed by stress, Hsp70 prevents these partially denatured proteins from aggregating, and allows them to refold.((Albani *et al*., 1995) Low ATP is characteristic of heat shock and sustained binding is seen as aggregation suppression, suggesting a second mode of binding regulation based on oxidative stress. Hsp70 seems to be able to participate in disposal of damaged or defective proteins (Satpute *at el* 2009).

Interaction with CHIP (*C*arboxyl-terminus of HSP 70 Interacting Protein) – an E3 ubiquitin ligase–allows Hsp70 to pass proteins to the cell's ubiquitination and proteolysis pathways. Finally, in addition to improving overall protein integrity, HSP 70 directly inhibits apoptosis (Beere, *et al*., 2000).

There are a number of studies have confirmed the presence of antibodies against HSPs belonging to the HSP70 (Chukwuocha *et al*., 1999; Yoshida A., 2001), as well as other studies also confirmed the presence of antibodies against serpin (HSP47) (Hattori, 2000), against HSP60 (Tsoulfa *et al*.,1989) other studies also confirmed the presence of antibodies against HSP70 (Mavropoulos *et al*., 2005) previous researches were not limited on the member of the HSP family, even included the presence of auto-antibodies against HSP90, family have consistently been identified in sera from patients with RA. One of the target antigens for anti-HSP antibodies in RA is human GRP78 (BiP), which also serves as a T-cell target in this disease (Blass, Union *et al*., 2001; Mavropoulos, *et al*., 2005).

The majority of studies on HSP-induced T-cell activation in RA are focused on the HSPs family, including human HSP70 (Li *et al*.,1992;)and HSP70 with HSP90 (Huang *et al*., 2009) The mononuclear cells in the synovial fluid of RA patients frequently show higher responses after stimulation with HSP than those in the peripheral blood (Fischer, 1991). These results apparently offer support to the idea regarding the role of HSP-reactive T-cells in RA synovitis. Some investigators have identified specific epitopes of HSPs involved in T-cell activation in human RA, such as regions 1-170 (Celis *et al*., 1997), 241-255 (Li SG, *et al*.,1992) 303-540 (Celis *et al*., 1997), and 451-466 (Gaston *et al*., 1991) a peptide fragment of *Ecoli*HSP70 (Prakken *et al*., 2004). It has been proposed that bacterial HSP-induced T-cells may cause arthritis through cross-reactivity with the homologous human HSP (Li *et al*.,1992; Albani *et al*., 1995;Auger *et al*., 2002). A few studies have refuted any specific association between T- cells reactive against HSP70 and RA (Fischer, *et al*., 1991; Wendling., *et al*; Zou *et al*., 2002).

Aim of the study: The investigating of heat shock proteins 70 (HSP70) in sera of RA patients as the pathogenesis of autoimmune arthritis.

MATERIALS AND METHODS

Subjects

The study groups have been investigated, which include:

The Patients Group

The study included 145 Iraqi RA patients, aged from 5-75years. Those patients were attending the consultant clinic for Rheumatology in Al- Husain Teaching Hospital from September 2014 to September 2015. The committee of rheumatologists performed the clinical examination under the supervision of staff in rheumatology unite

The Control Group

Sixty person apparently healthy person included in this study as a healthy control group., who have no history or clinical evidence of RA or any other chronic disease, and no obvious abnormalities,

Blood Samples

Blood samples were collected from patients and controls (five milliliters of venous blood) were drawn by 22G disposable syringe under aseptic technique. each blood sample was divided into two parts:

- Three milliliters were put directly in a sterile tube containing EDTA for WBC total count, WBC deferential count and phagocytosis processes during 2 hour
- Two milliliters were placed in a sterile plane tube and allowed to clot, then serum was separated by centrifugation at 2500 r.p.m. for 15 minutes. The serum was stored at -10 C˚. These 200 sera (150 RA patients and 60 controls) were used for estimating The concentration of heat shock protein 70 (HSPs 70) by use (Human Heat Shock Protein 70 (HSP-70)ELISA Kit assay). From Human company, Germany.

RESULTS

Measuring concentration of HSP 70 in the samples which have been studied in the experiment. and depending in results which were detailed in table 4-8 shown a high significant difference between the patients group and the control group. HSP 70 concentration s we show significantly higher in male group Than of group female at 0.05 level (2-tailed) =.000, as shown in the table No. 2. Through statistical analysis of the table is blew. We found high significant difference between each of the group (21-40years) and (41-60) than in age group (1-20 years, and 60> years), age group (41-60 years) found significantly difference lower than the age group (21-40 years) and the group (1-20 years). Small differences between other age groups found to be non-significant

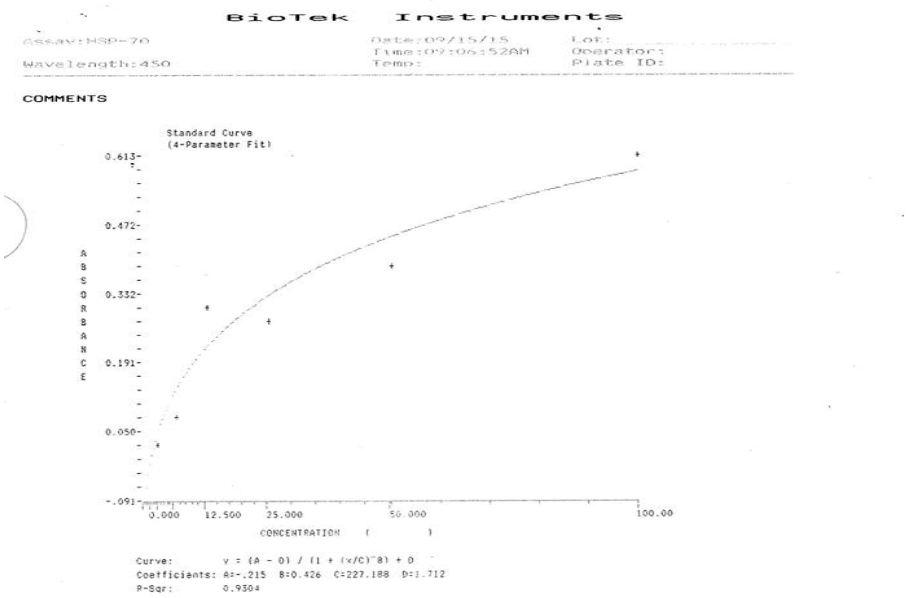

Figure 1: Curve of Concentration of HSP 70

Table1: Evaluation the Concentration of HSP70 (Pg/Ml) in Studied Group

Groups	No.	% of No.	Mean of HSP70 Concertation	T	Df	Sig.
Patients gruop	50	83.3%	1.022±.33	5.136	58	.000 HS
control	10	16.6%	0.47±.07			
male	33	66%	1.010±.32	-.343	48	.540 NS
female	17	34%	1.046±.36			

HS= High Significant difference (P<0.001).
NS= Non Significant difference (P>0.05).
df :Degree of freedom

According to the statistical analysis in tables blew 1 and 2, the results demonstrated that there were found out the concentration of HSP70 in the age group (41-60) was higher than others age groups by significant difference (P≤0.001).

Table 2: Shown Concentration of HSP70 (Ng/Ml) According the Age Groups

Groups	No.	% of No.	Mean±SD of HSP70 Levels
(1-20) group age	10	20%	.73 ±.30
(21-40) group age	20	40%	1.11±.36**
(41-60) group age	10	20%	1.20±.15**
>60 group age	10	20%	.95±.25

The mean difference is significant at the .05 level.**is significant at 0.001
NS mean non difference is significant (P>0.05).

Table3: Shown the Frequency of Concentration of HSP 70 in Patients Group with Mean of Age

Age Mean	No. of Cases	Mean*	Std. Deviation	Percentge**
25.00	3	1.015	.178	6.0%
26.00	3	.939	.425	6.0%
30.00	5	1.110	.399	10.0%
32.00	3	.893	.249	6.0%
35.00	2	1.318	.126	4.0%
38.00	3	.860	.356	6.0%
40.00	2	1.038	.187	4.0%
42.00	2	1.387	.168	4.0%
45.00	2	1.180	.132	4.0%
47.00	5	1.064	.340	10.0%
50.00	6	.883	.408	12.0%
60.00	2	.734	.523	4.0%
65.00	6	1.200	.236	12.0%
70.00	6	.823	.401	12.0%
Total	50	1.015	.337	100.0%

*mean of concentration of HSP70 in sera of RA. Patients.
** percentage of number of cases.

In this table No. 3, we show frequency of concentration of HSP 70 in treatment group with mean of age. Found that higher concentration in age 42 years than lower concentration in age 60 years and we show the concentration lower when the age increase >60 years.

Table 4: Shown HSP 70 Concentration between Studied Group According Smoking Factor

Name of Group	N	Mean*	Std. Deviation	Percentage **
Smoking patients	17	1.0199	0.3396	26.6%
Patients(non-smoking)	13	0.8327	0.3021	21.2%
Healthy (smoking)	14	0.4405	0.1012	25.5%
Healthy nonsmoking)	16	0.4306	0.1163	26.6%
Total	60	0.6753	.35015	100.0%

*mean of concentration of HSP70 in sera of RA. Patients. ** percentage of number of cases.

HSP 70 concentration which measured in samples showed a significantly higher concentration group 1 (smoking patients) with group 3-4 (mean= **1.019±0.339** pg/dl) in comparison with the normal average calculated for two health groups (**0.440** ± **0.1012**) - (**0.4306** ± **0.1163** pg/dl. t-test value =11.225, and 11.491 significance (2-tailed) =.000, 95% confidence interval from(0.6818) to (0.4769) and (0.6911 to .487)

The mean of HSP70 concentrations of patients (non-smoking) samples found significantly higher too than that of groups 3-4 samples (0.8327436 pg/dl) comparative normal value of healthy(smoking group) is 0.4405745 pg/dl. t- test value = 8.359, significance (2-tailed) = .000, mean difference is .39216912

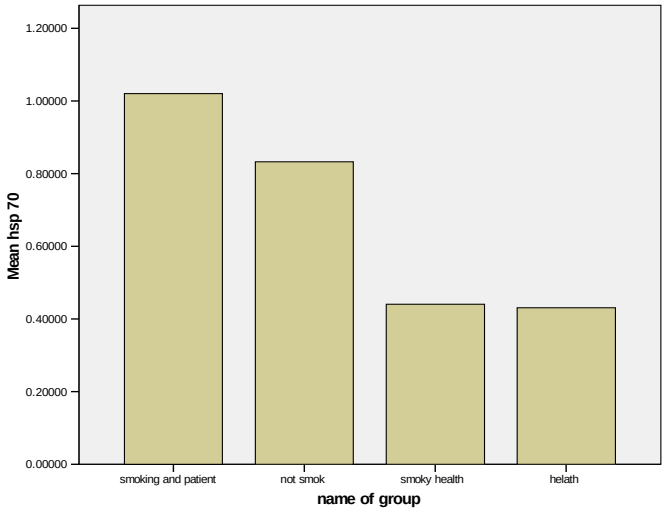

Figure 2: shown comparison of levels of concentration of HPS70 according smoking

In the figure above.This can be seen that the concentration of heat shock protein70 in the category of smokers more than other categories.

Table 5: HSP 70 Concentrations According the Duration of Disease

Duration Of Disease	No.	Mean Of HSP*	Std. Deviation	Percentage**
.50	2	1.335	.2206	4.0%
.70	2	1.3015	.0346	4.0%
.80	2	1.3185	.1265	4.0%
.90	3	.9396	.4255	6.0%
1.50	2	1.2210	.3903	4.0%
2.00	12	1.1264	.2449	24.0%
3.00	12	.9820	.3250	24.0%
4.00	3	1.1370	.2141	6.0%
5.00	3	.53266	.1404	6.0%
7.00	3	.90633	.3709	6.0%
10.00	6	.76016	.3993	12.0%
Total	50	1.01524	.3377	100.0%

*mean of concentration of HSP70 in sera of RA. Patients.
** percentage of number of cases.

Comparative mean between HSP 70 concentration with duration of disease we found higher level of HSP 70 in male group < one year than in female group in 2 year and the all-time of disease from 0.5 years to 10 years.

Risks affecting in RA and some environmental factors by form of a questionnaire which data are summarized in the table below, as follows.

Table 6: Suspected Major Risk Factors for RA Patients

Risk factor		Studeing group		df	Chi-Square	P-value
		No caese	% of Total N			
Stress		63	43.4%	1	2.490	.115 NS
Without Stress		82	56.6%			
smoking		50	34.5%	1	13.96	0.00 HS
No Smoking		95	65.5%			
Hard work		52	35.9%	1	11.59	.001
Easy work		93	64.1%			
Practice exercises		24	16.6%	1	64.89	0.00 HS
Do not practice exercises		121	83.4%			
Economic	Low	15	10.3%	2	150.71	0.00 HS
	Medium	118	81.4%			
	high	12	8.3%			
Other chronic diseases	Yes one	16	11.0%	2	93.77	0.00HS
	no	103	71.0%			
	More than one	26	17.9%			
Total		145	100.0%			

Table 7: HSP 70 Concentration According Risk Factors

Type of Studied Group		No of Cases	Mean of HSP Level	Std. Deviation	Percentage of No. Cases
Type of work	Hard work	27	1.031	.343	54.0%
	Easy work	23	.996	.337	46.0%
	Total	50	1.015	.337	100.0%
Sport-exercising patients	yes	11	1.016	.284	22.0%
	no	39	1.014	.354	78.0%
	Total	50	1.015	.337	100.0%
Economic Status	low	4	1.026	.458	8.0%
	med	43	1.019	.330	86.0%
	high	3	.939	.425	6.0%
	Total	50	1.015	.337	100.0%
Other Diseases	yes	12	1.118	.384	24.0%
	no	24	1.046	.284	48.0%
	lots	14	.872	.358	28.0%
	Total	50	1.015	.337	100.0%
Stressful Patients	stressful	33	1.048	.354	66.0%
	Non stressful	17	.997	.310	34.0%
	Total	50	1.015	.337	100.0%

In this table we can show high significant difference that said their work type were hard and between groups of patients whom said there economic status is low and med comparison with patients high living cost, and we found significant difference between patients with one or loss other diseases comparison with patients have more than one other disease. All of this statically analysis at level significant 0.005. P value =0.00

As well as the results shown no significant difference between patients whom said practicing exercises comparison with other group whom said not practicing sport.

DISCUSSIONS

In the table number one, the results showed no significant difference between the concentration of heat shock protein in the male category of female. But in contrast we can note the presence of a significant difference can be explained statistically significance in interpreting the results between patients group and the healthy group. According the statistical table No. 2 demonstrated that presence clear significant difference between the age groups and also demonstrated the 41-60 category highest concentration than the rest of the age groups also came in second place age group 21-40 also showed clear significant difference with the other categories, which goes with scientific opinion, which said that these is the age groups are frequently the disease.

The distribution of patients according to duration of disease explained in table (5) indicates that there is no significant difference between duration of RA by mean is (2.52±2.5 years) but we can found wide range between the Maximum 10 years and the minimum 6> month by range is (9.90). So we can show high frequency of duration of disease in 2-3 years and higher concentration of HSP 70 at 6< weeks this meant high concentration in acute phase of RA in initiate the inflammation.

There was high significant difference (P< 0.00) between the mean duration of RA and control group. This findings agreed with Jawaheer *et al*. (2006). It has noted that there was no significant difference of duration of disease between female group and male group

This study demonstrated that there is clear significant difference between the patients group and control group as clarified in the table **1**, this goes in correspondence with: 1- According to new hypotheses, extracellular heat shock proteins (Hsp70) may represent an ancestral danger signal of cellular death or lysis-activating innate immunity. Recent studies demonstrating a dual role for Hsp70 as both a chaperone and cytokine provided support for thehypothesis that extracellular Hsp70 is a messenger of stress (Zolta *at el*., 2002) 2- Certain aspects of the role of HSP-reactive T-cells in RA patients need clarification. First, what is the predominant phenotype of the HSP-reactive T-cells in RA based on the T-cell lineage surface markers and function? A few studies (Prakken *et al*., 2004; Zou *et al*., 2002) have addressed this issue, but no clear consensus has emerged. In this study demonstrated that there is clear significant difference in No of lymphocytes (P<0.005) between the patients group and control group as clarified in the table This was agreed with public hypotheses of role of heat shock protein 70 in pathogenesis and immune response in RA (Arend, 1997). Several risk factors are involved in the pathogenesis of autoimmune rheumatic diseases, including genetic factors, smocking, chronic infections, sex hormones, environments factors and stress. Stress is now recognized as an important risk factor in the pathogenesis of autoimmune rheumatic diseases (i.e. rheumatoid arthritis) The suspected major risk factors for RA patients were shown in (Tables 6 and 7).

The results demonstrated that stress factor including major life events (e.g. death of a spouse, severe long-term illness of a spouse… e.t.c. And miner life events, such as the progress of pain and fatigue in rheumatoid arthritis and the overall relation with problem mood (depression/anxiety), psychosocial resources (social support, self-efficacy) and burden (social distress, problematic social supports) as well as the overlapping with other risk factor example as economic situation and living cost and habits like smoking, coffee. as result we can show patients with stress (43.4%) which reprecent high rate becouse others factor and long time of dieaes role play in pathogesis of RA. Also we can shown rate of smokers is (34.5%), and the patient said their work is hard (35.9%) so high rate (the patients who do not exercise) (83.4%)

and the living cost to (81.4%) is medaim, and we found 103 pateints (71.0%) have RA diseaseonly, with out other diseases so we acording this group in measure all immunolgical marcker in this study. by considering that the activation of the stress response system influences the close relationships existing between the hypothalamic-pituitary-adrenal axis, the sympathetic nervous system and the immune system.

The reported changes of the hypothalamic-pituitary-adrenal (HPA) axis, the sympathetic nervous system (SNS) and the immune system (Straub and Cutolo., 2006), can lead to pro inflammatory reactions during minor stress in RA. It has been shown in RA that interpersonal stressors few days prior to the visit were related to increased numbers of circulating CD3+ T cells and increased serum levels of soluble IL-2 receptors (Zautra *et al*., 1997). In addition, during the cold pressure test, an enhanced IL-6 production by peripheral blood cells was observed (Hattor *et al*., 2000) and RA patients demonstrated enhanced IL-6 levels during mental stress before surgery(Blass S, Union A, *et al*.,2001; Mavropoulos, *et al*., 2005). Prior short-term cortisol infusion increased stimulated levels of interleukinIL-6 and tumor necrosis factor (TNF-α) in humans in vivo (Bahr *et al*.,1991).By considering norepinephrine and the SNS. We can conclusion that stressors induction the immune response in RA patients. The reported activation of the innate immune system by HSPs, as described above, has been hailed as an important new function of HSPs with broad biological significance. The induction of pro inflammatory cytokines by Hsp60 and Hsp70 may contribute to the pathogenesis of autoimmune diseases and chronic inflammation. Hsp70 frequently co localizes with human Hsp70 in macrophages of atherosclerotic plaques (Gaston *et al*., 2002). Induction of proinflammatory cytokine release from macrophages by Hsp70 would provide a potential mechanism by which Chlamydia infections may promote athero genesis and precipitate acute ischemic events(Bahr *et al*., 1990 ; Gaston *et al*., 2002). Likewise, the activation and maturation of dendritic cells by HSP 70 may be responsible for the HS P 70 -induced tumor immunity by inducing both the innate and adaptive immune responses.

CONCLUSIONS

Thus it has been proposed that through their cytokine function, HSPs may serve as a “danger signal” to the innate immune system at the site of tissue injury and that HSPs could be the endogenous ligands for theTLR2 and TLR4 In fact, HSPs are considered to be the prototype of endogenous ligands for Toll-like receptors. There is considerable interest to further explore then implications and therapeutic potential of these HSP cytokine effects.

Several risk factors are involved in the pathogenesis of autoimmune rheumatic diseases, including genetic factors, smocking, chronic infections, sex hormones and stress. Stress is now recognized as an important risk factor in the pathogenesis of autoimmune rheumatic diseases (i.e. rheumatoid arthritis) by considering that the activation of the stress response system influences the close relationships existing between the hypothalamic-pituitary-adrenal axis, the sympathetic nervous system and the immune system. At last the result of this study goes with a few studies have refuted any specific information such as Fischer, *et al*., 1991; Li SG, *et al*.,1992; Albani S, *et al*., 1995; Auger., *et al*. 2002; Zou, *et al*., 2002).

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