Evaluation of Heat Shock Proteins 70 (HSP70) and some Risk Factors in Sera of Rheumatoid Arthritis Patients in Thi-Qar Province

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Abstract

Heat shock proteins (HSPs)were synthesized under different kinds of stress conditions and act as molecular chaperones for protein molecules. These proteins were first found in cells 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, smoking, chronic infections, sex hormones, stress and HSP 70. In this present study the role of heat shock protein 70 was investigated in pathogenesis and immune response in rheumatoid arthritis (RA). This study was conducted onfifty samples of rheumatoid arthritis patients. In addition twenty samples of superficially healthy volunteers. All these samples were used for determining the concentration of HSP70. The results of study demonstrate the existence of high significant difference ($P \leq 0.001$), between patients and control group when the concentration of HSP70 was measured for all samples. This study also demonstrates slight statistical differences ($P \leq 0.05$), among studied groups when taken into consideration the gender, age groups and characteristics of smoking.

It is concluded that the concentration of HSP70 is increasing at the beginning of the disease and it is higher in RA patients compared with control, also it is higher in female than in male groups.

Keywords: immune response, heat shock protein 70, rheumatoid arthritis, smoking.

Introduction

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).

Several risk factors are involved in the pathogenesis of autoimmune rheumatic diseases, including genetic factors, smoking, chronic infections, sex hormones and stress (Lipsky, 2005). The majority of the studies demonstrates HSP70 induces immune system, a potential hypothesis is that some auto antibodies and T-cells exist that recognize epitopes shared by the HSP of both the infectious agent and host cells. This would facilitate cross-reactivity of lymphocytes with host cells, and triggering an immunological reaction. This is referred to as molecular Mimicry (Kaufmann, 1990; Burmester *et al.*, 1991; Li *et al*., 1992).

The complements activation by(HSPs) in vivo may significantly amplify the power of immune activation in sites of cell damage and necrosis, where free extracellular and membrane-bound chaperone molecules may messenger cell stress because **(**Asea *et al*., 2000), several endogenous TLR2 and TLR4 legends such as, HSP60 and HSP 70 have been identified. This is a molecular alarm signal that triggers an immune response when released (Curtin *et al*., 2009; Abdollahi-Roodsaz *et al*., 2011).

HSP 70 with other factors stimulate the monocytes to secret high level of IL-23, IL-17 and IL-6. It has been demonstrated that IL-17 is mainly produced by Th17 cells, and NK, RAGE + T cells are also considered as IL-17-secreting cells (Akirav*et al*., 2012; Wondimu *el al*., 2010).

As the same cytokine is produced by these different cells, IL-17 is a pivotal proinflammatory molecule in immune inflammation or autoimmune diseases with IL6 and IL23. (Gardella *et al*., 2002; Sims *et al*.,2010). It is speculated that the interaction and competitive binding to HSP70 of HSP40 versus HLA-SE might influence the efficiency of antigen presentation by HLA-SE (Auger and Roudier, 1997; Maier *et al*., 2002) leading to autoimmunity. However, more direct evidence is needed to affirm this speculation.

Moreover, recent studies have suggested that both bacterial and human HSP 70 might be suppressive in RA through the induction of regulatory T-cells and the deviation of the cytokine response to Th2 (Bahr *et al*., 1988; van*et al*., 1997; Prakken *et al*., 2004; Sheikhi *et al*.,2008).There is also some evidence that cigarette smoking increases likelihood that disease will be severe when it occurs (Eisenberg and Quinn, 2006).

Smoking has greater impact on RA individual being carries HLA-DRB1 SE alleles (Auger *et al*., 2002). Moreover, the interaction between smoking and HLA-DBR1 SE allele further confers a higher risk for RF - positive RA(Auger *et al*., 2002;Padyukuv*et al*., 2004) and ACPA-positive RA (Klareskog *et al*., 2006;Pederson *et al*., 2007;Van der Helm *at al*., 2007). Other studies also confirmed the presence of antibodies against HSP70 (Bodman-Smith *et al*., 2004; Mavropoulos *et al*., 2005), implying a role of this autoantibody in RA pathogenesis (Blass *et al*., 2001).

Theaim of this study is to determine the levels of concentration of HSP 70 in rheumatoid arthritis patients comparison with control and the effect of some environmental factors then establishing the HSP 70 profile in patients with rheumatoid arthritis.

Materials and Methods

The Patients group: The study included50 Iraqi RA patients, aged from 20-70years. Those patients were attending the consultant clinic for Rheumatology in Al- Husain Teaching Hospital from September 2012 to March 2013. The committee of rheumatologists performed the clinical examination under the supervision of staff in rheumatology unit.

The control group consisted of twenty persons apparently healthy who were included in this study as a healthy control group, who have showed no history or clinical evidence of RA or any other chronic disease, and no obvious abnormalities,

Blood samples collection:- Blood samples were collected from patients and controls (two milliliters of venous blood) were drawn by 22G disposable syringe under aseptic technique. Each two milliliters of blood sample 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 70 sera (50 RA patients and 20 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, number .CSB–E 04638h). From Human co. Germany, the procedure was carried out according to the producing company instruction that was attached with the Kit, and according to Lequin, (2005).

Statistical analysis

Statistical analysis was conducted using ANOVA multiple test for the comparison of means (Daniel, 2005)

Results

The results of the present study demonstrate high significant difference which is about ($P<0.001$) between patients group and control group (Table (1)). The same table displays the results of the study also demonstrated the existence of high significant difference ($P<0.001$) between female and male groups whereby female groups were higher than male. According to the statistical analysis, it was found that the concentration of HSP70 in the age range (41-60) was higher than those of other age groups.

Table (1)
Evaluating the concentration of HSP70 (pg/ml) between studied groups.

Groups	*No.*	*% of No.*	*mean±SD*	*df*
Patients gruop	50	83.3	1.022±0.33**	68
Control	20	16.6	0.47±.07	
Total	70	100	0.746±0.51	
Males withRA	33	66	0.99±.32	48
Females with RA	17	34	1.046±.36**	
Total	50	100	1.022±0.33	
(1-20) age group	10	20	0.73 ±.30	3
(21-40) age group	20	40	1.11±.36*	3
(41-60) age group	10	20	1.20±.15**	3
>60 age group	10	20%	0.95±.25	3

***** = significant difference at the level 0.001***
**** = significant difference at level 0.05***
df= degrees of freedom

According to table number 2, there was a high significant difference among groups. This depends on the existence of characteristic of smoking since they have higher concentration of HSP70, compared with other groups.

Also the results demonstrated that non-smoker patients were higher than healthy smokers and non-smoking healthy individuals respectively with elevation causing a significant difference ($P \leq 0.05$).

Table (2)
HSP 70 concentration (pg/ml)between studied group according smoking factor.

name of group	*No.*	*% of No.*	*Mean*	*Std. Deviation*
Smoking patients	**13**	**26.0**	**1.0199****	**0.3396**
Patients nonsmoking	**12**	**24.0**	**0.8327***	**0.3021**
Healthy (smoking)	**12**	**24.0**	**0.4405**	**0.1012**
Healthy, nonsmoking	**13**	**26.0**	**0.4306**	**0.1163**
Total	**50**	**100.0**	**0.6753**	**0.35015**

***** significant difference at level 0.001*** **** significant difference at level 0.05***

Table (3)
HSP 70 concentration (pg/ml) according the duration of disease.

Duration of disease	*No.*	*% of No.*	*Mean*	*Std. Deviation*
5 months	**2**	**4.0**	**1.335**	**.2206**
7 months	**2**	**4.0**	**1.3015**	**.0346**
8 months	**2**	**4.0**	**1.3185**	**.1265**
9 months	**3**	**6.0**	**.9396**	**.4255**
1year &.5 months	**2**	**4.0**	**1.2210**	**.3903**
2years	**12**	**24.0**	**1.1264**	**.2449**
3.years	**12**	**24.0**	**.9820**	**.3250**
4. years	**3**	**6.0**	**1.1370**	**.2141**
5. years	**3**	**6.0**	**.53266**	**.1404**
7. years	**3**	**6.0**	**.90633**	**.3709**

10. years	**6**	**12.0**	**.76016**	**.3993**
Total	**50**	**100.0%**	**1.01524**	**.3377**

Comparison between HSP 70 concentration and duration of disease demonstrated higher level of HSP 70 in the period 8 months and low level of HSP 70 in 5 years. Results also showed higher rate if patients suffering from RA for the period of disease at the time in 2-3 years.

Table (4)
Frequency of concentration of HSP 70 (pg/ml) in patients group with mean of age.

age	*No.of cases*	*Percentageof N0*	*Mean*	*Std. Deviation*
25.00	**3**	**6.0**	**1.015**	**.178**
26.00	**3**	**6.0**	**.939**	**.425**
30.00	**5**	**10.0**	**1.110**	**.399**
32.00	**3**	**6.0**	**.893**	**.249**
35.00	**2**	**4.0**	**1.318**	**.126**
38.00	**3**	**6.0**	**.860**	**.356**
40.00	**2**	**4.0**	**1.038**	**.187**
42.00	**2**	**4.0**	**1.387**	**.168**
45.00	**2**	**4.0**	**1.180**	**.132**
47.00	**5**	**10.0**	**1.064**	**.340**
50.00	**6**	**12.0**	**.883**	**.408**
60.00	**2**	**4.0**	**.734**	**.523**
65.00	**6**	**12.0**	**1.200**	**.236**
70.00	**6**	**12.0**	**.823**	**.401**
Total	**50**	**100.0**	**1.015**	**.337**

In this table we show frequency concentration of HSP 70 in treatment group according to the age. The result demonstrated that highest concentration in age 42 years than lower concentration in age 60 years, and also show that the lowest concentration in age 60 years.

Discussion

This study demonstrate that there is a significant differences between the patients group and control group as clarified in the Table (1) the results goes in correspondence with 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 the hypothesis that extracellular Hsp70 is a messenger of stress. (Klareskog*et al*., 2006). There was high significant difference ($P \leq 0.001$) between the mean duration of RA and control group. This finding agree with Jawaheer*et al*, (2006). It has been noted that there was a high significant difference of HSP70 concentration between females group which are found higher than males group as shown in Table (1).

The distribution of patients according to duration of disease explained in Table (3), indicates that there is no significant difference between duration of RA mean (2.52±2.5) but we can find wide range between the maximum 10 years and the minimum 6> month by range is (9.90). We can also show a high frequency of duration of disease in 2-3 years and higher concentration of HSP 70 at 6< weeks. This meansa high concentration in acute phase of RA in initiate the inflammation. This finding is in agreement with Al-Rawi*et al*, (1997).

Several risk factors are involved in the pathogenesis of autoimmune rheumatic diseases, including genetic factors, smoking, chronic infections, stress, sex hormones, and environments factors. In this study, we take smoking as a risk factor to determine if it represents a pathogenesis and risk factor. We can show in Table (2) which demonstrate a high significant difference, and it is mean (1.0199±0.3396) this resultsgoes in correspondence with Zou, (2002).

Smoking may increase the risk of developing Rheumatoid arthritis, to date; smoking is the only firmly verified environmental risk factor for RA (Klareskog *et al*., 2006). A recent study demonstrates that smoking over a long period of time (> 20 years) can significantly increase the disease risk. There is also some evidence that cigarette smoking increases likelihood that disease will be severe when it occurs (Eisenberg and Quinn, 2006).

Conclusions

Some of environmental factors such as smoking, stressful, others chronic infections, duration of disease, age and sex, reactive with genetic factors play a main role in the determination of this immunological marker (HSP 70) in patients with RA.

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الخلاصة

تُصنع بروتينات الصدمة الحرارية (HSPs) تحت أنواع مختلفة من ظروف الإجهاد ويكون بمثابة المرافقات الجزيئية للبروتينات. وجدتهذه البروتينات لأول مرة في الخلية عند تعرضها لدرجات الحرارة العالية، مما يطلق عليه بروتين الصدمة الحرارية، و تشارك العديد من عوامل الخطورة في التسبب في حدوث الإمراض الروماتيزمية وأمراض المناعة الذاتية، بما في ذلك العوامل الوراثية، التدخين، والالتهابات المزمنة، والهرمونات الجنسية والإجهاد.

وتتحرى هذه الدراسة عن دور بروتين الصدمة الحرارة كعامل خطورة لمرض التهاب المفاصل الرثياني الاستجابة المناعية له. اجريت هذه الدراسة على عينات من خمسين مريض بالتهاب المفاصل الرثياني، وقد شملت عيّنة السيطرة 20 شخصًا من الأصحاء ظاهريا. استعملت العينات للكشف عن مستوى تركيز HSP 70 في مصلِ مجموعة المرضى و مجموعة السيطرة في محافظةِ ذي قار للفترة مِنْ بِداية مِنْ سبتمبر/أيلولِ 2012 إلى النهايةِ مِنْ مارس/آذارِ 2013.

أظهرت النتائج مستوى عالي من القيمة الإحصائية ($P \leq 0.00$) بين المجموعةِ المدروسةِ والضابطة عندما تم قياس تركيزَ HSP 70 في العيناتِ، وأظهرت النتائج أيضا وجود دلالة إحصائية طفيفة بين المجموعاتِ المدروسةِ ($P \leq 0.05$) اعتمادا على الجنسَ، مجاميع الأعمار وعادة التدخينً. ونستنتج أن تركيز بروتين الصدمة الحرارية يكون مرتفع جدا في بداية المرض ويكون تركيزه أيضا مرتفع في مرضى التهاب المفاصل الرثياني مقارنة بالمجموعة الضابطة. كما ظهر ان التركيز في مجموعة الإناث أعلى من مجموعة الذكور.