Immune response to heat shock protein 60 and its relations to enteric reactive arthritis

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Abstracts

Background: according to the molecular mimicry hypothesis, heat shock protein 60 (hsp60) is among the most conserved proteins that have been implicated as triggering agents in the pathogenesis of enteric reactive arthritis (ReA).

Objective: In the present work, we aimed to determine the prevalence of anti-hsp60 antibodies in sera of patient with enteric reactive arthritis.

Methods: Forty-five patients with Reactive arthritis were enrolled in this study. They were 22(48.89%) males and 23(51.11%) females, the age rang was 20 - 40 year with mean (33.6 ± 10.6). All patients were outpatient visitor or hospitalized in the medical City hospital in Baghdad. They were diagnosed clinically by consultant rheumatologist with the aid of some laboratory tests such as RF, CRP, E.S.R.

The patients should be sero-negative (RF-negative) and fulfill Amor and European Criteria to be included in this study. Patients were classified according to disease activity into three groups: sever moderat and mild by using (DAREA score). Thirty age and sex matched apparently healthy individual, were considered in this study as a control group. ELISA was used to detect immune response against hsp60 in the sera of each patients and controls. Wells of the micro titter plates were coated with hsp60 in coating buffer and anti hsp60 antibodies were assayed.

Results: the mean age of patients was (33.6± 10.6) years and they were 23 females and 22 males with females to male ratio 1.05:1, the majority of patients 19(42.22%) present with high disease activity (sever) and 15(33.33%) patients were moderate and the remainder 11 (24.44%) were mild disease group.

The result of anti-hsp60 Abs detection showed that there was highly significant difference between ReA patients and control groups. Also there was significant difference between sever, moderate and mild among ReA patients.

Conclusion: we concluded that bacterial hsp60 seems to be a major target of T-cell response in enteric ReA. and cross reactivity against autologous hsp60 has been documented as a triggering of enteric ReA.

Key Words: Reactive arthritis, heat shock proteins, Development of a disease activity index for the assessment of reactive arthritis (DAREA).

الخلاصة

تمهيد: طبقا لفرضية التماثل الجزيئي فأن بروتين الصدمة الحرارية رقم ستون -وهو احد أكثر البروتينات محافظة- يعد احد البروتينات التي يشتبه بكونها تلعب دورا في امراضية التهاب المفاصل التفاعلي.

الهدف: هدفت الدراسة الى تحديد دور بروتين الصدمة الحرارية رقم ستون في تطور هذا المرض وعلاقة الاستجابة المناعية بشدة المرض.

المواد وطرق العمل: شملت الدراسة خمس واربعون مريض مصاب با التهاب المفاصل التفاعلي من المرضى المراجعين لعيادة امراض المفاصل في مستشفى مدينه الطب في بغداد بالاضافة الى ثلاثين فرد صحيين ظاهريا.اجري تشخيص المرضى من قبل الطبيب المعالج بالاعتماد على الفحص السريري وبمساعدة بعض الفحوصات المختبرية. قسمت عينة الدم من كل شخص مريض الى جزئين ,الجزء الحاوي على الهيبارين استخدم في تحديد معدل ترسيب كريات الدم الحمر وحساب كريات الدم البيض .بينما الدم غير الحاوي على الهيبارين ترك ليتخثر ,جمع المصل ووزع في عبوات صغيرة ,وحفظ في درجة 20 درجة مئوية تحت ا لصفر لحين وقت التحري.

النتائج: اشتمل المرضى المدروسين على اثنان وعشرون رجل و ثلاث وعشرون امراة مع عدم وجود فرق احصائي هام من حيث الجنس ,اكثر المجموعات العمرية تكرارا كانت مجموعة العقد الرابع ثم العقد الثالث. اغلب المرضى(اثنان واربعون بالمئة) حضروا في مراحل متقدمة او شديدة من المرض, العامل الروماتزمي غير موجود في كل المرضى مع وجود نسبة عالية من معدل ترسيب الخلايا الحمر .

اظهرت الدراسة بواسطة فحص الامتزاز المناعي المرتبط ب الانزيم (الاليزا) ان الاستجابة المناعية لبروتينات الصدمة الحرارية رقم ستون عالية في مرضى التهاب المفاصل التفاعلي مقارنة بالاشخاص الاصحاء ,وتمخضت الدراسة على وجود فرق احصائي هام بين الاستجابة المناعية لمجاميع المرضى عند تقسيمهم على اساس شدة المرض .

الاستنتاجات: ومن هنا نستنتج انة يمكن اعتبار بروتينات الصدمة الحرارية رقم ستون تلعب دور فاعل في تطور التهاب المفاصل التفاعلي .

Introduction

Reactive arthritis (ReA) is a synovitis developing after a distant infection usually in the genitourinary or gastrointestinal tract which suggest a contribution from bacterial product [1], but the organism can not be isolated or cultured from the joint [2], Many Gram negative bacteria including *Chlamydia trachomatis, Shigella, Salmonella, Yersinia and Campylobacter* have been implicated in the underlying pathogenesis of ReA [3] ReA affect male and females with same frequency [2]. However, it was previously claimed to be more common in males, and most patients are aged between 20-40 years [3] and the exact etiology of ReA is unknown. However genetic factors play a role in susceptibility to the disease and 65-80% of patient are positive for HLA –B27, and many infections may be implicated in the etiopathogenesis of ReA [2]. At the time of arthritis, stool cultures are usually negative, and the background of ReA has usually been confirmed by serological method [4].

There are two hypothesis explain develops of ReA in HLA-B27 positive subjects [5] The first is the arthritogenic peptide hypothesis: these suggest that the arthritis is triggered by a T-cell response to specific antigenic peptides derived from the triggering bacteria and The other hypothesis is molecular mimicry hypothesis: this theory postulates that an autoimmune process can ensue after an infection if there is some degree of cross-reactivity in host and microbial antigens [6].

Heat shock proteins (HSPs) are highly conserved intracellular proteins expressed in all pro- and eukaryotic cells, both constitutively and under stress conditions [7]. Cross reactivity against outologous hsp60 has been postulated as triggering of ReA [8].

In the present work, we aimed to determine the prevalence of anti-hsp60 antibodies in sera of patient with enteric reactive arthritis.

Patients and Methods

This study included forty-five patients with Reactive arthritis, they were 22(48.89%) males and 23(51.11%) females, the age rang was 20 - 40 year with mean (33.6± 10.6) .All patients were outpatient visitor or hospitalized in medical city hospital in Baghdad our the period of study. The patients were diagnosed clinically by consultant rheumatologist, and some

laboratory tests such as RF, CRP, and E.S.R.

The patients should be sero-negative (RF-negative) and fulfill Amor and European Criteria [9] to be included in this study. Moreover, the patients were classified according to disease activity into three group sever, moderat and mild by using (DAREA score)[10]. The patients with definite history of diarrhea were grouped as enteric reactive arthritis. (3.5 ml) blood were aspirated and transferred into a plan tube allowed to clot at room temp., then centrifuged for15 minutes approx. 500 rpm to obtain unheamolyzed cell –free serum. Serum sample were divided in aliquots and stored at -20Ċ to avoided freezing and thawing in each steps of this study.

Thirty age and sex matched apparently healthy individual, were considered in this study as a control group, all control persons had no history of diarrhea since at least 3 months

The procedure of ELISA was done according to the Hunter, et al 1986 [11] as following:

Wells were coated over night at 4Ċ with 100µ of 1/40 diluted hsp60 in coating buffer ,Next day the plates were emptied and washed three times with washing buffer .then uncoated sites were blocked with 100µ/ well blocking buffer for one hour at 37Ċ incubation was carried out in a shaker incubator. Then, plates were emptied and 100µ of 1/2 diluted sera in dilution buffer were added to each well and the plates incubated for 1hr. at 37Ċ. Then the excess non –reacted sera removed through three cycle of washing with washing buffer while the reacted sera detected by adding to each well 100µ of 1/1000 diluted conjugate and plates incubated for 1hr.at 37Ċ. After incubation the plates were washed with washing buffer and 100µ of substrate solution (OPD) were added to each well and incubated in dark place for 30 minutes at 37Ċ and the reaction was stopped by addition of 100µ stopping solution and the absorbance was determined with an ELISA reader at 550 nm. An optical density (OD) value of more cut off value (mean plus two standard deviations x̄ standard errors of normal control) was considered as positive.

Results

This study included 45 patients with enteric ReA, in addition to 30, age and sex matched apparently healthy control. The mean age of the patient was (33.6± 10.6) years with range from 20-40 years. The highest incidence of ReA was found in 4th decade followed 3rd decade and as shown in Figure (1)
There were 23 females and 22 males with females to male ratio 1.05:1. Although; there was a slight inclination for an association with female sex, however, Chi –square revealed no statistically significant difference in the frequency of patients between both sex (p=0.763) this mean there was no significant sex effect .
According to student t test, in regarding serum levels of anti-hp60 antibodies, we detected high significant difference between ReA patients and control group (figure 3)
Furthermore, there was significantly higher levels in sever than that of moderate and mild ReA patients, indicating that this response may reflect the disease activity (Table 1)

Discussion

Reactive arthritis is a potentially severe and crippling disease triggered by infections at a distant mucosal site by *Salmonella, Shigella, Yersinia, Campylobacter* and *Chlamydia* and the most common form of ReA are

urogential ReA and entero ReA, but its course and progressive rate show pronounced variation among individuals[4].

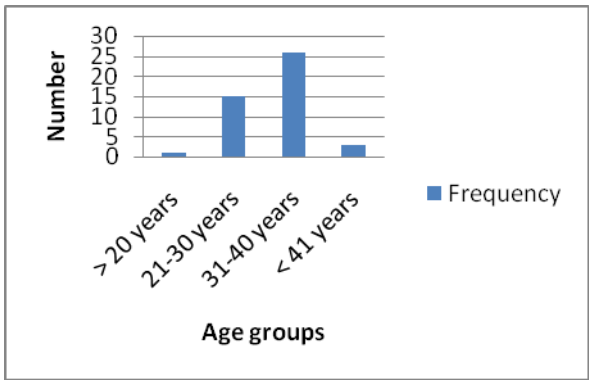

Figure 1. Age frequency among ReA patients

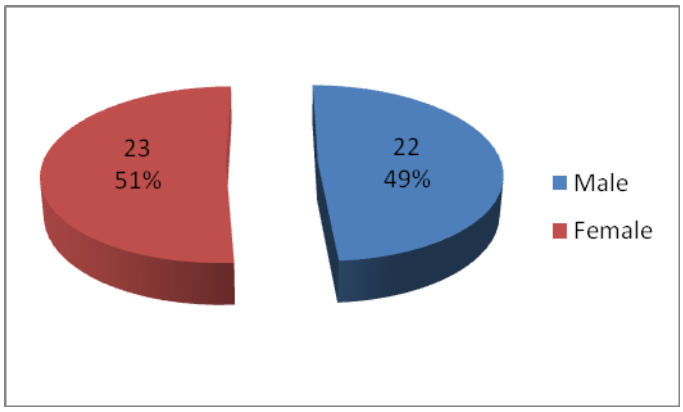

Figure 2. Gender distribution of ReA patients

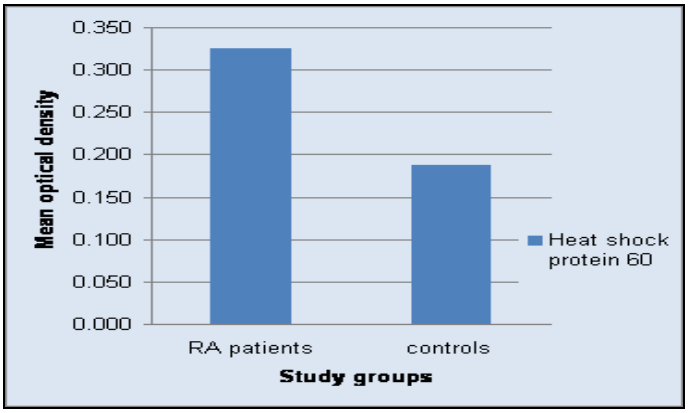

Figure 3. comparison of hsp60 levels between ReA patients and control group

Table 1. Estimation and comparison of hsp60 among ReA patients

	heat shock protein 60		Total	
	Negative	Positive		
Mild	5	6	11	0.805
Moderate	8	7	15	
Severe	8	11	19	
Total	21	24	45	
	heat shock protein 60		Total	

Bacterial hsp60 seems to be a major target of T-cell response in Reactive arthritis, and cross –reactivity against autologous hsp60 has been documented as a triggering of ReA disease [12]. However, immune recognition of hsp60 is also very common in infection, particularly where intracellular organisms are concerned [13].

In this study the results showed that anti-hsp60 antibodies in sera of patients with ReA, was higher than control group (mean 0.325 and 0.166 respectively).

Furthermore, with ReA patients grouped according to disease activity, our results showed a highly significant difference between active and mild disease activity groups (P=0.805). Our results corresponded data mentioned by (Kaufmann, *et al*., 1995; Koji, *et al*., 2000) [14, 15].

The results of our work could contribute to elucidating the important role of hsp60 as causes of ReA and to designing of a specific serological diagnostic method for this arthritis.

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