Immune responses to outer membrane proteins and lipopolysaccharide of Salmonella Typhimurium and their relations to enteric reactive arthritis.

Abdal Razak H. Ahmed*, Mohammed Hadiand Jabbar Salman Hussan****

الخلاصة

يعد التهاب المفاصل التفاعلي من الامراض الشديدة والمعيقة التي تتطور اثناء الاصابة او بعد الاصابة في مكان اخر من الجسم ؛وخاصة التهابات المجاري البولية والتناسلية او التهابات الامعاء، ولايمكن عزل المسبب المرضي من المفصل المصاب.

هدفت الدراسة الى ايجاد افضل مستضد وافضل ضد يساعد في تشخيص المرض وعلاقة الاستجابة المناعية بشدة وتطور المرض.

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 .

قسمت عينة الدم من كل شخص مريض الى جزئين، الجزء الحاوي على الهيبارين استخدم لحساب معدل ترسيب كريات الدم الحمر .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 .

تم استخلاص مستضد بروتينات الغشاءالخارجي وايضا متعدد السكريد الشحمي الخام من جرثومة السالمونيلا، وقدرت كمية البروتين في بروتينات الغشاء الخارجي وايضا قدرت كمية متعدد السكريد الشحمي .

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 .

اظهرت الدراسة بواسطة فحص الامتزاز المناعي المرتبط ب الانزيم (الاليزا) ان الاستجابة المناعية لبروتينات الغشاءالخارجي وايضا متعدد السكريد الشحمي لجرثومة السالمونيلا في اغلب المرضى موجودة ،وتمخضت الدراسة على ان متعدد السكريد الشحمي يعتبر المحفز المناعي الاول مقارنة ب بروتينات الغشاء الخارجي ،وايضا اضهرت النتائج ان الغلوبيلين الممنع(أ) هو الضد الاعلى استجابة لمستضد (بروتين الغشاء الخارجي) وان الغلوبيلين الممنع (ج) هو الضد الاعلى استجابة لمستضد (متعدد السكريد الشحمي لجرثومةالسالمونيلا).وايضا وجد في هذة الدراسة ان لاعلاقة بين شدة وتطور المرض و الاستجابة المناعية.

ومن هنا نستنتج انة يمكن الاعتماد على (متعدد السكريد الشحمي) لتشخيص المرض و بمساعدة اميونوغلوبيلين (اي) و اميونوغلوبيلين (ج) لجرثومةالسالمونيلا.

*Assistant Professor, Department of Medical Microbiology, College of Medicine AL-Nahrain University.

** Consultant, Department of Rheumatology Medical City hospital, Baghdad

**MSc Microbiology, Department of Medical Microbiology, College of Medicine AL-Nahrain University

Abstract

Objectives: **In the present work, we analyzed the predominant *Salmonella* typhimurum component outer membrane proteins and lipopolysaccharide (OMPs and LPS) that triggered an immune response in 45 patients with enteric reactive arthritis by assessing the anti-OMPs and anti-LPS antibodies (including IgG IgM and IgA) by ELISA**

Methods: **Forty-five patients with reactive arthritis, they were 22(48.89%) males and 23(51.11%) females, the age range was 20 - 40 year with mean (33.6). All patients were outpatient visitor or hospitalized in city hospital in Baghdad over the period of study.They were diagnosed clinically by consultant rheumatologist, as well as 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 (1), to be included in this study , patients were classified according to disease activity in to three group severe, moderate and mild by using (DAREA score)(2),the majority of patients 19 (42.22%) presented with high disease activity (severe) and 15(33.33%) patients were moderate and the remainder were mild disease group consist of (11) patients (24.44%).Thirty age and sex matched apparently healthy individual, were considered in this study as a control group. Wells were coated with antigens (OMPs and LPS of Salmonella) in coating buffer and anti-OMPs and anti-LPS antibodies were assayed using ELISA technique.

Results: **The mean age of patients was (33.6) years and there 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 (severe) and 15 (33.33%) patients were moderate and the remainder were mild disease group.**

Three classes of antibodies to OMPs and LPS antigens were studied by ELISA. Positive responses to OMPs and LPS in serum of patients were detected and the major antigenic target in Salmonella –induced ReA was LPS and the main antibodies were IgG anti-LPS. Also there was no significant difference between severe, moderate and mild among ReA patients.

Conclusion: **We concluded that LPS were the main bacterial antigens that triggered enteric ReA in this study, and determining the triggering bacterial components can help elucidate the precise causes of ReA and will contribute to the designing of a specific serological diagnostic method for this arthritis**

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

Introductions

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 (3), but the organism can not be isolated or cultured from the joint (4), Many Gram negative bacteria including *Chlamydia trachomatis, Shigella, Salmonella, Yersinia and Campylobacter* have been implicated in the underlying pathogenesis of ReA (6) ReA affects male and females with same frequency (5).However, it was previously claimed to be more common in males, and most patients are aged between 20-40 years (6) 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 (5). At the time of arthritis, stool cultures are usually negative, and the background of ReA has usually been confirmed by serological method (7).

There are two hypothesis explain develops of ReA in HLA-B27 positive subjects (8).

The first is the arthritogenic peptide hypothesis: which suggest that the arthritis is triggered by a T-cell response to specific antigenic peptides derived from the triggering bacteria. 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 (9).

Salmonella typhimurum is a major cause of food –borne illness (4).is the most frequently detected causes of outbreak of human Salmonellosis(10) ,and a high frequency of ReA has been observed after infection with this bacteria (11)

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) .All patients were outpatient visitor or hospitalized in medical city hospital in Baghdad over the period of study They were diagnosed clinically by a consultant rheumatologist, as well as 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 (1) to be included in this study. The

patients with definite history of diarrhea were grouped as enteric reactive arthritis. (3.5 ml) blood were collected and transferred into a plan tube allowed to clot at room temp .Then centrifuged 15 minutes approx. 500 rpm to obtain unheamolyzed cell –free serum. Serum sample were divided in aliquots 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.

Lipopolysaccharide (LPS) extraction was obtained by LPS extraction kit which is designed for rapid and convenient extraction of LPS from bacterial cell the extraction procedure is take only 60 minute and give high yields of LPS, While (OMPs) extraction process making according to (Murphy, *et al*., 1983 and Hunter, *et al*., 1986)(12,10)

The protein concentrations of the (OMPs) were determined Biuret method, the concentration of protein in OMPs of Salmonella was 0.9 mg/ml by using (BSA) standard curve, and the estimation of LPS concentration (Ketodeoxyoctinate) by using Thiobarbituric acid assay(13) the concentration of LPS in Salmonella it was 112 microgram /ml by using LPS standard curve.

Procedure of ELISA was making according to the Hunter, et al 1986 (10) as following:

Wells were coated over night at 4Ċ with 100µ of 1/40 diluted antigens 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. (The conjugated used were):

-Horse raddish peroxidase-anti human IgG conjugate
- Horse raddish peroxidase-anti human IgM conjugate
- Horse raddish peroxidase-anti human IgA conjugate

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 than mean plus two standard deviations of normal control was considered as positive.

Results

The study included 45 patients with enteric ReA. And 30 age and sex matched apparently healthy control. The mean age of the patient was (33.6) years with range from 20-40 years the highest incidence of ReA was in 4th decade followed 3rd decade. Figure (1)

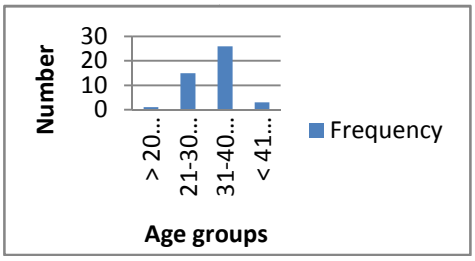

Figure (1): Age frequency among ReA patients

There were 23 females and 22 males with females to male ratio 1.05:1 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 .Although; there was a slight inclination for an association with female sex.

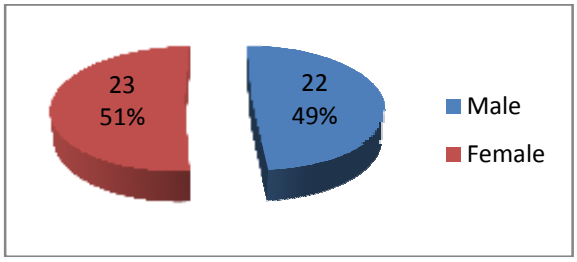

Figure (2): Gender distribution of ReA patients

When all class antibodies to OMPs and LPS of Salmonella were studied by ELISA, we detected positive response to OMPs and LPS in serum of most patients, the highest antibody response were the response against LPS, in addition a higher Salmonella- specific IgA response was detected against OMPs compare with anti-OMPs IgM and anti-OMPs IgG, In contrast we found that the IgG antibody response was stronger to LPS compare with anti-LPS IgM and anti-LPS IgA.

Table1: Comparison between reactive arthritis patients group regarding immune response against OMPs and LPS.

Immunoglobulin type	Negative	Positive
IgM anti- OMP	22	23
IgA anti- OMP	5	40
IgG anti- OMP	12	33
IgM anti- LPS	8	37
IgA anti- LPS	21	24
IgG anti- LPS	3	42

In this study there is no significant difference between immune response to OMPs and LPS regarding disease activity group

Table 2:Comparison among ReA patients according to humoral immune response regarding the disease activity.

			mild	moderate	sever	total	
Salmonella	IgM anti-OMP	Negative	5	8	9	22	0.384
		positive	6	7	10	23	
	IgG anti-OMP	Negative	5	3	4	12	0.268
		Positive	6	12	15	33	
	IgA anti-OMP	Negative	2	1	2	5	0.649
		Positive	9	14	17	40	
	IgM anti-LPS	Negative	0	3	5	8	0.185
		Positive	11	12	14	37	
	IgA anti-LPS	Negative	5	8	8	21	0.383
		Positive	6	7	11	24	
	IgG anti-LPS	Negative	0	2	1	3	0.834
		Positive	11	13	18	42	

Discussion

Three classes of antibodies (IgG, IgM and IgA) to LPS and OMPs of Salmonella typhimurum were studies by ELISA, We detected positive response to OMPs and LPS for Salmonella compared with healthy control group.

In addition, the highest antibodies were response against LPS of Salmonella. Indicating that they were the major antigenic target in Salmonella induced ReA disease in our studies compare with OMPs.

Also we found higher Salmonella specific Immunoglobulins was IgA against OMPs antigen (IgA anti-OMPs of Salmonella).

That suggests continuous antigen stimulation following Salmonella infection this quite accord with abroad studies, (Sukumar, *et al.*, 2000; Hannu, *et al*., 2004).

In addition the highest *Salmonella* specific Immunoglobulins was IgG against LPS antigen (IgG anti-LPS of Salmonella). This disagreed with other previous studies done by Maria and his Co-worker (2007) who found that OMPs is the major antigenic target in Salmonella induced ReA, while the present results come in agreement with those data reported by (Maki, *et al*., 1991) (14)

This study observed that there was no correlation between the immune response to the (LPS and OMPs) and disease activity. And that may be due to the disease activity rate influenced by many factors like genetic of host, resistant to antibiotic; in addition the patients came in late state.

References

1. Dougados M., S.vander linden, R.Juklin, B.Amor (1991): The European spondyloarthropathy study group preliminary criteria for classification of spondyloarthropathies. Arthritis rheumatism J.; 34:1218-1227
2. Eberl G., A. Studnicka- Benke, H.Hitzelhammer,F.Gschnait andJ.S.Smolen (2000): development a disease activity index for assessment a ReA.Rheumatology J; 39:148-155.
3. Maria Gabriela Lacoste,Diego Estebar,Hector Tamashiro (2007): Study of predominant Bacterial antigen triggering antibody response in Salmonella ReA . J. Infect.Disease; 60:220-224
4. Sukumar Mukherjee, Madhuchanda kar (2000): Reactive arthritis: Current perspective study .J.Indian Academy Medicine; 1:NO.3 October

5. Wright V.,Moll JMH.,perry M.B and Jann,K.(2008):Seronegative polyarthritis.Amsterdam North Holland publishing.

6. Granfors K, Marker-Hermann E, derkeyser F (2002) the cutting edage of spondloarthropathies research. Arthritis rheum; 46:21-30

7. Gaston JSH (1990): How do HLA-B27 Confer susceptibility to arthritis.Clin.Exp.Immunol. ; 82:1-2

8. Lopez-Larrea C, Gonzales S, Martinez-Borra J (1998): the role of HLA-B27 polymorphism and molecular mimicry in spondyloarthropathy. Mol.Med.today J.; 4:540-549

9. Bukau,B.E.Deuerling,C.Fund EA.Varela-Hernandel.(2000) Getting newly synthesized proteins in to Shape.J.cell; 101:119-120.

10. Hunter,S.B.Bibb,W.F,Shin, Kaufmann,A,F,Mitchell (1986):Enzyme linked Immunosorbent assay with major outer membrane protein of Brucella mellitensis to measure Immune response to Brucella Species.J.Clinical Microbiology,24:566-572.

11. Hannu T, Ward M, Brucke, J. Perry,M.B and Bundle D. (2004): summary of the 2003 annual research and education Meating .J Rheumatol; 33:978- 982.

12. Murphy, T.F; pudask.C, Mylottes&Apieetlam A (1983): A sub typing system for non typable Haemophilus influenzae based on outer membrane protein .J.infect.disease; 147(5).

13. Hanson,R.S. and Philip,J.A. Naylor S. Gally DS .(1981):Manual method for general bacteriology .American Society for microbiology , Washington :378-394

14. Miyake k (2004): Innate recognition of lipopolysaccaride by Toll-like receptor 4-MD-2, trends microbial; 12:186-192.