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HLA-B genotype and *Escherichia coli* association in Iraqi patients with reactive arthritis

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Abstract:

Reactive arthritis (ReA) has been as joint developing after infection, it belongs to spongylo arthritis (SpA). The etiology of this disease was multi factorial, the combination between genetic and environmental factors for triggering this disease. This study included 75 Iraqi Arab patients and 39 healthy control. Urine samples and blood were collected from each subject. The results showed that *Escherichia coli* bacteria (*E. coli*) was isolated from 32% of urine samples. HLA-B^{*27} allele frequencies was higher in ReA patients infected with *E. coli*. This lead to suggest that *E. coli* may be trigger factor in ReA patients with UTI which had HLA-B*$_{27}$ positive.

Keywords: Reactive arthritis, infectious agent, HLA.

العلاقة بين النمط الوراثي لمستضد التوافق النسيجي نوع ب وبكتيريا القولون في المرضى العراقيين المصابين بإلتهاب المفاصل التفاعلي

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الخلاصة:

يعد إلتهاب المفاصل التفاعلي من الأمراض التي تصيب المفاصل والتي تحدث نتيجة للإصابة، حيث يعود الى عائلة الأمراض المرتبطة بإلتهاب المفاصل. إمراضية المرض تشترك فيها عدة عوامل متمثلة بالأرتباط ما بين الوراثة والبيئة التي تحث على المرض. تضمنت الدراسة 75 مريض عراقي و39 شخص أصحاء أستخدموا كسيطرة. جمعت عينات الأدرار وعينات الدم من مجاميع الدراسة، وأشارت النتائج الى أن نسبة بكتيريا القولون 32% حيث عزلت من أدرار المرضى وكان الليل HLA–B*$_{27}$ أكثر ترددا في المرضى وكانت له علاقة في الأشخاص المصابين ببكتيريا القولون، لذا يمكن إعتبار بكتيريا القولون عامل محث لمرض إلتهاب المفاصل التفاعلي في الأشخاص المصابين بإلتهاب المجاري البولية.

Introduction

Reactive arthritis (ReA) is by definition a septic arthritis can be caused by various infections agents. It is inflammatory sterile arthritis belonging to the group of arthritis known as spondyloarthropatients (SpA) which may develop within 2 – 4 weeks of urinary tract infection (UTI) [1-3]. It was autoimmune condition with onset as a reaction against infection located anywhere in the body [4]. The classic bacteria associated with ReA are gram positive obligate or facultative intracellular aerobic bacteria with LPS containing outer membrane, the primary focus of infection is through the mucus membrane either in the gut or in the urogenital tract [5]. Genetic, especially the

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presence of human leukocyte antigen HLA-B_{27}, was found to have a mixed association with ReA [6, 7], ReA is precipitated by infection and a distant site. Genetic susceptibility is marked by possession and HLA-B_{27} gene, but the mechanism was unclear [8]. Disease susceptible gene in the HLA-B locus, such as HLA-B_{27}, are common in SpA, such as reactive arthritis and ankylosing spondylitis [9], ReA is an acute HLA-B_{27} associated inflammatory joint disease triggered by certain bacteria. LPS and nucleic acid from affected joint suggesting that bacteria antigen might have a direct role in pathogenesis of ReA [10], for that reason the aim of this study is to investigate the relationship between HLA-B genotype and bacterial infection in ReA.

Material and methods

- **Subject**

  The current study was carried out 75 (40 females and 33 males) Iraqi reactive arthritis patients aged from 20 – 60 years who were referred to the consultant clinical department of Rheumatology, Baghdad Teaching Hospital, Ministry of Health. The diagnosis was made by the consultant medical staff in the hospital, American organization criteria was used to aid diagnosis.

- **Sample collection**

  - **Blood Samples**

    Ten milliliter of venous blood was drawn from each subject. Three milliliter of each samples were transferred to test tube centrifuged at 2500 rpm for 10 minute and separated serum. CRP, RF were evaluated, other part of blood is placed in EDTA tube to prevent clotting for ESR test, the remaining blood was kept at -20 C^o for genotyping HLA class I by PCR SSO-inno-Lipa typing.

  - **Urine samples collection**

    The sample was taken from midstream, blood agar and MacConkey agar were used for culture urine, identification of bacteria was done by autolysis Vitek2 compact device.

Results and discussion

The present study showed that sero-negative rheumatoid factor of all patients of reactive arthritis, while CRP was positive for all patients as shown in Table-1 and ESR was elevated in patients in compared with control group.

Table 1- Frequency of c-reactive protein test (CRP) and rheumatoid factor (RF) antibodies in studied groups by Latex agglutination test (LAT).

Inflammatory marker	Patients	Control
CRP	**(+)**	**(-)**
RF	**(-)**	**(-)**
ESR	**70±2**	**23±1.8**

Currently study showed elevated of ESR in patients group comparing with control group. These agreed with other studies [11] they found that CRP positive and RF negative. CRP is elevated at the onset of disease, ESR is important in the diagnosis of the inflammatory condition and in the prognosis of the non-inflammatory condition [12].

Escherichia coli (*E. coli*) was isolated in 32 % of patients with ReA in present study, the higher percentage of *E. coli* in ReA patients with UTI, other study showed the high percentage of *E. coli* in UTI and had major public health implication [13] UTI is one of the commonest infection to affected human occur by uropathogenic bacteria usually *E. coli* [14]. Injection of bacterial DNA originated from *E. coli* into knee joint of mice was induce arthritis and this indicate an important pathogenic role for bacterial DNA in septic arthritis [15]. HLA-B*$_{27}$ allele was the etiological factor in Iraqi ReA patients as in Table-2, while HLA-B*$_{07}$ was protective factor. Other showed that HLA-B*$_{27}$ and ReA is illustrated by the fact that prevalence of disease in HLA-B*$_{27}$ positive individuals is five times greater than general population [16]. The mechanism that proved the role of HLA-B_{27} was genetically predisposed subject, HLA-B_{27} appears to lack the ability to eliminated infected macrophage normally in proving the intracellular survival of pathogen [17].

The results showed also the association between HLA-B alleles with *E. coli*, HLA-B*$_{27}$ allele frequencies were higher in *E. coli* 50 % than negative group 40%, while HLA-B_{44} recorded 25% in ReA patients with *E. coli* and HLA-B*$_{35}$ allele in group of patients negative to *E. coli* in their urine culture, as in Table-3.

Others showed spondyloarthropathies are associated by unknown mechanism with HLA-B_{27} and in certain bacteria HLA-B_{27} shares sequence with protein from enteric bacteria. HLA-B*$_{2705}$ sequence contain an auopeptide (LRRYLENGK) predicted to bind in the binding cleft B_{27} some nanopeptide from enteric organism that share sequence with this nanopeptide of B_{27} [18]. Presentation by host MHC class I molecule at such closely related peptides from HLA-B_{27} or inactivated cross reactive T cell response against from HLA-B_{27} or inactivate cross reactive anti-bacterial T cell inducing autoimmunity or presistant infection respectively [19]. HLA-B*$_{4402}$, HLA-B*$_{4403}$ and HLA-B*$_{4405}$ were expressed in *E. coli* [20]. From the present study we concluded that *E. coli* was etiological factor in UTI of ReA disease and HLA-B_{27} allele was the risk factor of ReA while HLA-B*$_{08}$ was protective factor for the disease and HLA-B*$_{27}$ allele was associated with *E. coli* in those patients.

Table 2- HLA-B alleles frequencies between studied groups.

Allels	No. of patients (39)		Percentage of patients %	No. of Control (39)		Percentage of Control %	ODD ratio	Fischer exact probability	EF	PF
	Positive	Nagative		Positive	Nagative					
B07	0	39	0.00	3	36	7.69	0.13	0.24		
B08	1	38	2.56	9	30	23.08	0.01	0.014		0.211
B09	1	38	2.56	1	38	2.56	1	1	0	
B13	4	35	10.26	4	35	10.26	1	1	0	
B14	1	38	2.56	4	35	10.26	0.23	0.358		0.079
B15	2	37	5.13	4	35	10.26	0.47	0.675		0.054
B18	6	33	15.38	2	37	5.13	3.36	0.263	0.108	
B27	16	23	41.03	2	37	5.13	13.45	0.00012	0.39	
B35	5	34	12.82	4	35	10.26	1.29	1	0.029	
B37	0	39	0.00	1	38	2.56				
B38	1	38	2.56	1	38	2.56	1	1	0	
B39	1	38	2.56	0	39	0.00	3.08	1		
B40	3	36	7.69	0	39	0.00	7.58	0.24		
B41	5	34	12.82	4	35	10.26	1.29	1	0.029	
B44	8	31	20.51	6	33	15.38	1.42	0.769	0.061	
B47	0	39	0.00	1	38	2.56	0.32	1		
B49	6	33	15.38	5	34	12.82	1.24	1	0.029	
B50	0	39	0.00	5	34	12.82	0.08	0.055		
B51	2	37	5.13	1	38	2.56	2.05	1	0.026	
B52	0	39	0.00	3	36	7.69				
B53	0	39	0.00	1	38	2.56				

Table 3- Frequency of alleles according to culture of bacteria

Bacteria culture result	alleles	Percentage (%)
E. coli	B_{27}	50
	B_{44}	25
Negative culture	B_{27}	40
	B_{35}	33

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