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Evaluation of Some Genetic Factors in Rheumatoid Arthritis patients in Iraq

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Abstract: Rheumatoid arthritis (RA) is a chronic, destructive autoimmune disease affecting the joints. With more sophisticated and effective therapies becoming available and with the understanding that early intervention is crucial in preventing irreversible joint damage. The main purpose of this observational study was to evaluate and detects a good genetic factors may be used in early detection of RA. A total of 40 patients with RA who were fulfilled four or more of the 1987 American College of Rheumatology (ACR), 20 patients with joints problems (JP), 20 RA patient relatives (PR) and 10 apparently healthy control individuals were included in this study. Human leukocyte antigen (HLA) genotyping was performed using Mr. Spot SSO system. The distribution of HLA class II genotypes in 20 RA patients and 30 control groups were studied. HLA-DRB1*04 was significantly most common genotypes in the RA patients (70%) compared to control groups (23.3%). While, the frequency of HLA-DRB1*11 was very high among controls (53.3%) compared to RA patients (25.0%), but the difference was not statistically significant. RA susceptibility in most Iraqi patients was associated with the HLA-DRB1*04 genotype. The HLA-DRB1*04 allele contributed significantly to the development of RA .HLA- Therefore, HLA-DRB1*04 allele appears to play an important pathogenic role in all subsets of RA.

Key word: Rheumatoid arthritis, HLA.

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تقييم بعض العوامل الوراثية في مرضى التهاب المفاصل الرثوي في العراق

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الخلاصة: التهاب المفاصل الرثوي هو من أمراض المناعة الذاتية المزمنة والمدمرة التي تؤثر على المفاصل بالعلاجِ المتطوّرِ والأكثرِ فعّالية الذي أصبح متوفرا وبالفَهْم بأنّ التدخّلِ المبكّرِ حاسمُ في مَنْع الضررِ المشتركِ الغير قابل للعكس فان من المهم والمهم جدا تشخيص التهاب المفاصل الرثوي في مرحلة مبكرة جدا ولتسهيل التشخيص في المراحل المبكرة من المرض، في كثير من الأحيان عندما لم يكن كل الأعراض السريرية واضحة للعيان، إن الغرض الرئيسي لهذه الدراسةِ هذه هو الكشف عن اختبار للعوامل الوراثية يمكن استخدامه في الكشف المبكر عن التهاب المفاصل الرثوي . شملت هذه الدراسة أربعين مريض يعانون من التهاب المفاصل الرثوي لديهم أربعة أو أكثر من العلامات المحددة من قبل الجمعية الأمريكية للروماتزم، 20 مريض يعانون من مشاكل في المفاصل، 20 شخص من أقارب

المرضى الذين يعانون من التهاب المفاصل الرثوي و 10 أشخاص أصحاء كمجموعة سيطرة. كما تم استخدم تقنية مستر سبوت نظام SSO (التسلسل المتخصص أحادي النيوكليوتيدات) من أجل الكشف الوراثي عن مستضد كريات الدم البيض البشرية (HLA). تم دراسة التوزيع الوراثي لمستضد كريات الدم البيض البشرية (HLA) الصنف الثاني لعشرين من مرضى التهاب المفاصل الرثوي وثلاثين من مجاميع السيطرة، أثبتت هذه الدراسة أن HLA-DRB1 * 04 كان معنويا وأكثر شيوعا (70٪) بين المرضى بالمقارنة مع مجموعات السيطرة (23.3٪). في حين HLA-DRB1 * 11 كان موجودا في عدد صغير نسبيا من مرضى التهاب المفاصل الرثوي (25٪) بالمقارنة مع مجموعات السيطرة (53.3٪). ومع ذلك، لا توجد هنالك فروق معنوية. وجد ان الحساسية للإصابة بمرض التهاب المفاصل الرثوي في المرضى العراقيين مرتبط بوجود العامل الوراثي HLA-DRB1 04 * و أن أليل HLA-DRB1 * 04 ساهم بشكل كبير في تطوير التهاب المفاصل الرثوي، ولذلك، يبدو أن HLA-DRB1 * 04 أليل تلعب دورا هاما في التهاب المفاصل الرثوي.

Introduction

Rheumatoid arthritis (RA) is a common autoimmune disease characterized by chronic inflammation of the synovial joints. In most cases, this chronic inflammation will induce the formation of pannus tissue, ultimately leading to joint destruction. RA is one of the most common autoimmune diseases, affecting 0.5-1.0% of the population. This systemic disease eventually leads to disability of the patient (1).

Also Rheumatoid arthritis development is associated with the class II major histocompatibility complex (MHC), in particular, the human leukocyte antigen-D (HLA-D) region. Strong links have been continuously publicized with the HLA-DR4 epitope, (2). Much research has been conducted to date on the role of genetics in RA, with the "shared epitope" theory a popular suggestion (3). It is clear from the research that there is a significant risk to individuals possessing certain gene epitopes or regions. The exact region or sequence is still being investigated and may still only be the cause in some cases or populations. Other possible causes need to still be considered.

Therefore, This study was planned to find methods for early diagnosis of RA by Evaluation of the role of some genetic factor in patients with Rheumatoid arthritis such as HLA-DRB1 alleles by using Histo Spot SSO system.

Study Design

This study was performed on 90 person who were attended to Al Sadder Medical City, in the period from May, 2010 to May, 2011. Four study groups were investigated which included:

-First group: Forty patients with Rheumatoid arthritis (RA)
fulfilling the 1987 ACR criteria for RA (4).

-Second group: Twenty relatives of patients with Rheumatoid
arthritis.

-Third group: Twenty Patients with joint problem but not RA as a
disease control.

-Fourth group: Ten healthy control group who had no history or
clinical evidence of RA or any chronic disease.

Information from all patients was taken to fill the study protocol sheet

Collection of Samples

Ten milliliters (ml) of venous blood were collected from patients as well as controls by venipuncture. A volume of 10 ml of blood were drawn aseptically using the following procedure: gloves

were worn and extraction sites scrubbed in an expanding circular motion first with 70%
isopropyl alcohol which was allowed to dry, then with iodine tincture (iodine in alcohol), allowed to dry before needle insertion (5). Blood was divided into aliquots was added to EDTA tubes for HLA genotyping.

Molecular Test

HISTO SPOT SSO system

1. Product description

The HISTO SPOT SSO system is an In -vitro diagnostic test for tissue typing of HLA alleles on a molecular genetic basis and provides low to medium resolution typing results. It consists of the HISTO SPOT typing kits, the HISTO SPOT reagent kit, the MR.SPOT processor and the HISTO MATCH interpretation software. The HISTO SPOT typing kits contain all components required for the PCR reaction and test wells with immobilized sequence-specific oligonucleotide probes for the detection of the PCR products. The HISTO SPOT reagent kit contains the reagents for the hybridization and detection and can be used in combination with all HISTO SPOT typing kits. The MR.SPOT processor is specifically designed to be used with the HISTO SPOT kits in order to process between 1 and 96 samples, automating the process from hybridization, detection through to result interpretation. The HISTO MATCH software is required to interpret the results.

2. Test principle

The test includes four basic steps:
- DNA isolation
- PCR amplification
- hybridization and detection
- data interpretation

DNA isolation was performed on the clinical sample, using the DNA isolation method established in the laboratory or using commercial kits. Then the DNA was amplified in a locus specific PCR reaction using the
mastermix and the MgCl 2 solution provided with the kit. The specificity of the amplification was governed by a set of biotinylated primers that have been designed to uniquely amplify the chosen HLA locus. After the PCR amplification process, the PCR plate containing biotin labeled amplicon was transferred to the MR.SPOT processor.

Statistical Analysis

All the statistical analysis was done by using Pentium-4 computer through the SPSS (statistical package for social science) program (version-14) and Excel application.

Results

The demographical distribution of studied group in Figure (1) reveals that the majority of RA patients were females, 34/40 (85.0 %) with female: male ratio 5.6:1. The female: male ratio among RA patient, relative patients with joints problems and healthy groups were 1.85:1,
1.5:1and 2.33:1 respectively. Moreover, the mean age of the RA patients, patients relatives, patients with joint problems and healthy groups were (45.43 ± 14.25), (36.90 ± 13.81), (30.4 ± 16.47) and (40.2 ± 12.74) respectively.

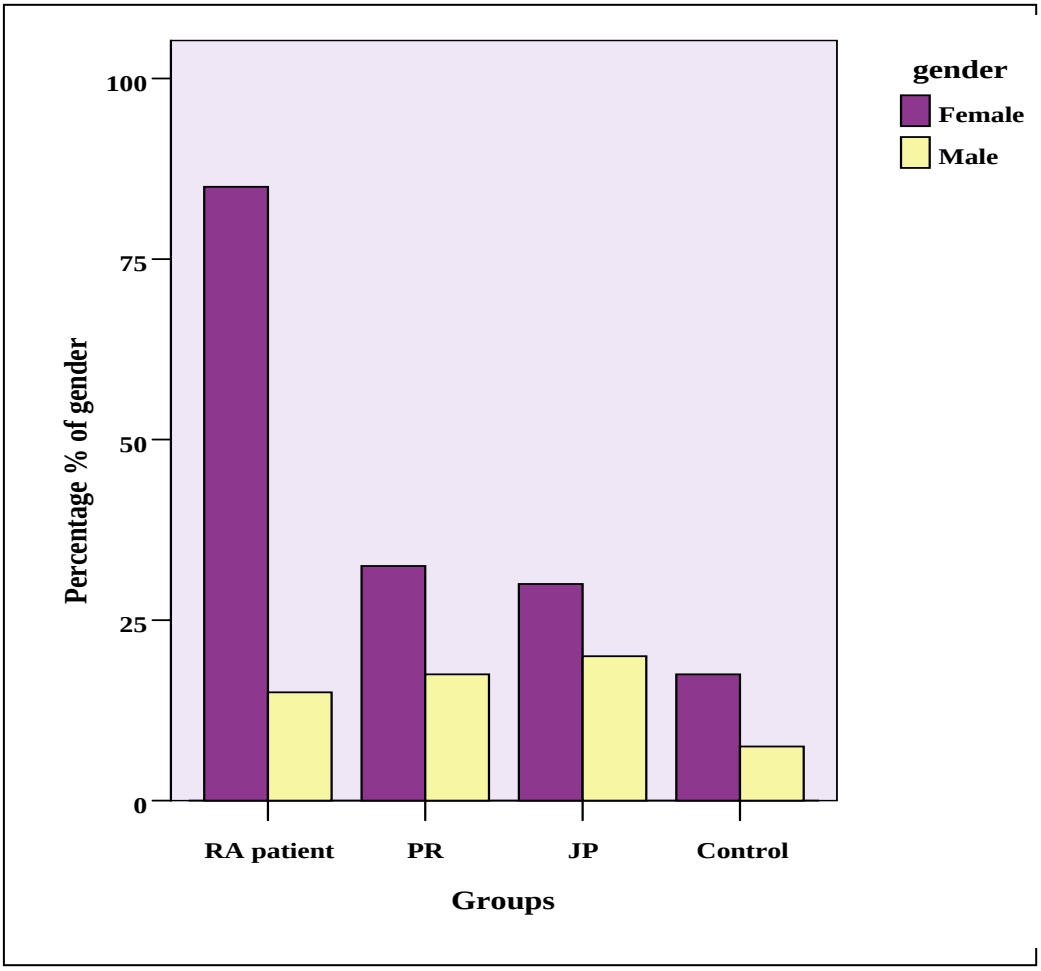

Figure (1): Distribution of studied groups according to the gender

One of the RA markers was the presence of RF in the patients' sera. It has been detected by using screening test and then quantitative determination for RF isotypes has been applied for samples. Table (1) showed the frequency of RF screening, among RA patients and other studied group beside RF-isotypes. The RF screening value in the current study showed that 70% RA patients were positive compared with other studied groups. these differences were highly significant ($P \leq 0.01$).

Table (1): Frequency of RF screening and its isotypes among the sera of the studied groups

Studied group	No.	Immunological parameters (%) of positivity				P -value
		Screening RF	RF-IgA	RF -IgG	RF-IgM	
RA Patient	40	70	52.5	70	62.5	
PR	20	10	5	55	25	P ≤ 0.01
PJ	20	0	20	60	30	
Control	10	0	0	0	0	
Total	90					

The newly diagnostic marker for RA is anti-CCP3 antibody that has been detected in the sera of the studied groups.

Regarding the results shown in Table (2) the present study found that anti-CCP3 was elevated significantly in the sera of RA patients (136.67 ± 221.097 IU/ml) in comparison with low levels of these antibodies among the control groups that included PR (40.054 ± 53.757 IU/ml), patients with JP (40.054 ± 49.25 IU/ml) and healthy control cases (5.800 ± 2.107 IU/ml) (P ≤ 0.01).

Table (2): Mean distribution of Anti-CCP3 level (IU/ml) among studied groups

Studied group	No.	Mean	Standard deviation	ANOVA test	
				P -value	Sig.
RA patient	40	136.67	221.097	0.018	Highly Sig. (P ≤ 0.01)
PR	20	40.054	53.757		
PJ	20	40.054	49.25		
Healthy control	10	5.800	2.107		
Total	90				

This study demonstrated that the HLA-DRB1*04 was significantly (p= 0.04) more common among patients (70%) than the control groups (23.3%). While, HLA-DRB1*11 was present in a relatively small number of RA patients (25%) as compared to the control groups (53.3%). However, this distribution was not found to be statistically significant (p= 0.19) as shown in Table (3) .

Table (3): Distribution of HLA-DRB1 alleles in rheumatoid arthritis patients versus the other study groups

Type of HLA-DRB1 allele	N0. (%)	N0. (%)	P-value
	RA patients (n=20)	Other groups (n=30)	
*01	5(25)	6(20)	0.73-NS
*02	0(0)	0(0)	1 .00-NS
*03	4(20)	7(23.3)	0.82 -NS
*04	14(70)	7(23.3)	0.04-S
*05	0(0)	0(0)	1.00-NS
*06	0(0)	0(0)	1.00-NS
*07	6(30)	7(23.3)	0.68-NS
*08	0(0)	1(3.3)	0.41-NS
*09	0(0)	0(0)	1.00-NS
*10	4(20)	4(13.3)	0.59-NS
*11	5(25)	16(53.3)	0.19-NS
*12	0(0)	0(0)	1.00-NS
*13	1(5)	4(13.3)	0.38-NS
*14	0(0)	1(3.3)	0.41-NS
*15	1(5)	6(20)	0.18-NS
*16	1(5)	1(3.3)	0.77-NS

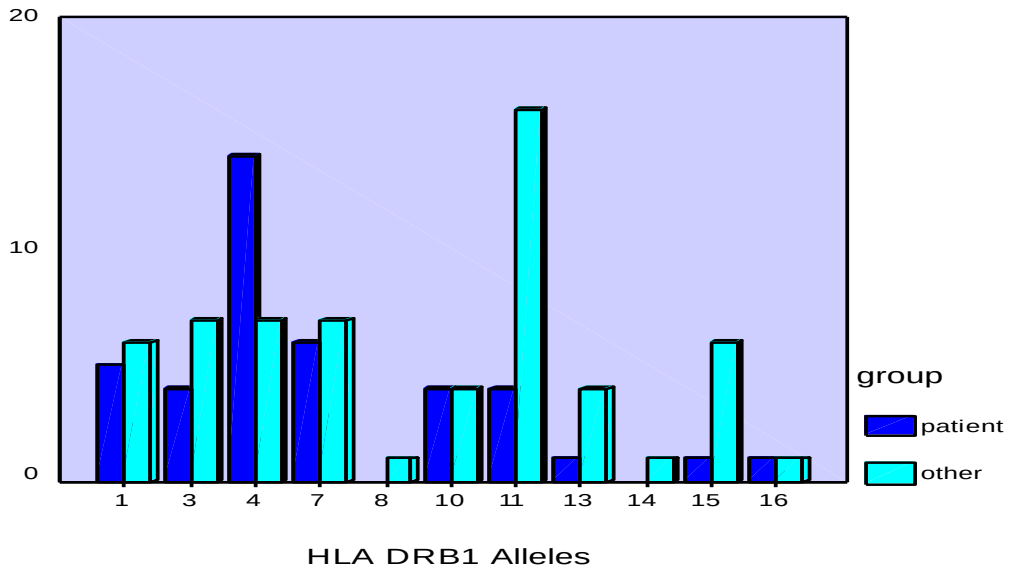

Figure (2) : The frequency of HLA-DRB1 alleles in studied groups

Discussion

Many studies performed in Iraq about immunological aspects of RA (6, 7, 8, 9), but no study was performed yet on new immunological diagnosis for detection of RA and HLA genotyping. So results of the present study were compared with studies done in other countries. In the present study, the distribution of studied group according to the gender showed that the majority of RA patients were females (85 %) with female: male' ratio 5.6:1. The incidence of RA in women was higher than in men, with a varying sex-ratio. Although representative studies regarding this issue were sparse, these observations already suggest a possible influence of reproductive and hormonal-depending factors on the occurrence of RA. The female to male ratio among RA patients in this study, which was higher than that for local previous studies reported in Iraq (2.9: 1and 3.3: 1) (6, 7). However, it was nearly comparable to that of the others (3.9:1 and 4:1) (8, 9). These variations may be due to sample size in addition to different circumstances and times of sample collection besides different populations. Also this result was suported by others researchers who found females were two to three times more likely to develop RA compared to males (10, 11, 12, 13).

The current study showed that the highest level of RF screening at the RA patients were 132.6±164.5 in comparison with RA patient relatives 1.60 ± 4.92, patients with joints problems (0.0) and apparently healthy control group (0.0) ($P < 0.01$). According to these findings, patients with a high titer of RF develop a more severe and eroding arthritis than do RF negative patients. Majorities of RA patients have increased levels of RF

auto antibodies directed against the Fc portion of Ig and serum concentration of RF has been found to correlate with disease severity.

In the present study regarding to the level of anti-CCP3 result was considered positive if the concentration of anti-CCP3 (≥ 25 IU/ml) .in this study the patient relative, patient with joint problem have the mean level (40.054 ± 53.757, 40.054 ± 49.25 respectively) that indicated to the positive Anti-CCP3 results in some of Patient relative, Patient with joint problem. these finding was agreed with those who reported by(14) who found that the presence of antibodies to anti-CCP predicts the development of RA in apparently healthy individuals in addition to (15) who revealed that anti-CCP also predicts the development of RA in patients with undifferentiated arthritis.

Results of present study indicated that HLA-DRB1*04 allele was present in 14 out of 20 RA patients and no HLA-DRB1*04 allele was detected in 10 healthy controls; the frequencies of the allele were 70% and 0% respectively. Present data showed significantly increased frequency of HLA-DRB1*04 in RA patients of Iraqi origin in comparison with control group. While, the frequency of HLA-DRB1*11 in control group was higher (16, 53.3%) than the RA patients (5, 25%), but no significant difference (P= 0.19) was observed. Whereas, DRB1*02, DRB1*05, DRB1*09, and DRB1*12 were conspicuously absent in Najaf population. It was estimated that about one-third to one-half of the total genetic contribution of RA could be attributed to genes in the HLA complex. This result was more compatible to the result reported by (16) who found that, the majority of RA patients in certain South Asian countries, including India had HLA-DR4. Consistent with present study, Kochi et al. (2004) (17) found that particular RA susceptibility alleles (subtypes of DRB1 alleles) have been associated with different ethnic groups (eg, the DRB1*0401 allele in Caucasians and the *0405 allele in Asian populations). Whereas, this result was contrary with the result reported by (18) who reported that human leukocyte antigen-DR10 was most commonly associated with RA in Saudi population and contrary with (19) who reported that HLADR4 frequency was not significantly different between the control and patient groups in their study.

Conclusion

RA susceptibility in most Iraqi patients was associated with the HLA-DRB1*04 genotype. The HLA-DRB1*04 allele contributed significantly to the development of RA .HLA- Therefore, HLA-DRB1*04 allele appears to play an important pathogenic role in all subsets of RA.

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