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РОЛЬ TNFα И IL-10 ПРИ РЕВМАТОИДНОМ АРТРИТЕ И АССОЦИАЦИЯ С НЕКОТОРЫМИ АЛЛЕЛЯМИ HLA-11 DR И DQ

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Резюме. Ревматоидный артрит (РА) – системное заболевание, приводящее к прогрессирующему повреждению суставов и инвалидности. При этом пораженные ткани характеризуются выраженной инфильтрацией воспалительными мононуклеарными клетками, такими как Т-клетки и макрофаги, и пролиферацией синовиальных фибробластов. Провоспалительные цитокины, продуцируемые в основном макрофагами, в том числе фактор некроза опухолей (TNF) и IL-6, играют центральную роль в развитии синовиита. Например, показано, что TNF непосредственно индуцирует пролиферацию синовиальных фибробластов, что ведет образованию воспалительного очага. TNF также критически важен для экспрессии воспалительных хемокинов и адгезии, что в совокупности облегчает дальнейшее привлечение лейкоцитов и продолжение воспалительной реакции.

Помимо средовых факторов, генетическая конституция организма может играть ключевую роль в возникновении и развитии болезни. Данное исследование проводилось для изучения ассоциации между HLA II класса (DR, DQ) и заболеваемостью РА путем генотипирования пациентов в Ираке, а также для сбора данных о генотипах, связанных с предрасположенностью или резистентностью к заболеванию. Целью исследования было установление роли, интенсивности и характера иммунного ответа у пациентов с РА путем определения уровней TNFα и IL-10 по сравнению с группой здоровых лиц и идентификация роли конкретных аллелей в выраженности заболевания.

Для этого исследования 5 мл венозной крови были взяты от 30 пациентов с подтвержденным диагнозом ревматоидного артрита, из них – 19 женщин и 11 мужчин, а также 30 образцов контрольной группы. Во всех пробах определяли уровни TNFα и IL-10 методом ИФА (сыворотку извлекали из 3 мл крови). Из оставшихся 2 мл выделяли ДНК, и затем проводили генотипирования HLA II класса с посредством сиквенс-специфической ПЦР (PCR-SSO).

Показана высокодостоверная разница уровней TNFα, и IL-10, между пациентами с РА и группой здорового контроля ($p < 0,001$). Не выявлено существенных половых различий по частоте РА ($p = 0,119$). Генотипирование HLA II класса у пациентов с РА и в контроле показало значительные различия между группами по ряду аллелей. Некоторые аллели DR оказались информативными, в частности, DR*0403 был более частым в контрольной группе (35% по сравнению с 6,67% в группе РА, $p = 0,02$). Аллель DR*701 встречался чаще у пациентов с РА – в 9 случаях (30%, $p = 0,007$). При генотипировании локуса DQ не было выявлено значимых изменений частоты аллелей. Хотя аллель *0202 выявлена у 40% больных и 15% контрольной группы, это различие не является статистически достоверным ($p > 0,05$).

Ключевые слова: ревматоидный артрит, TNFα, IL-10, HLA, генотипирование, частота аллелей

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ROLE OF TNFα AND IL-10 IN RHEUMATOID ARTHRITIS AND ASSOCIATION WITH SOME HLA II DR AND DQ ALLELES

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Abstract. Rheumatoid arthritis (RA) is a systemic disease that causes progressive joint damage and disability. The affected tissues are histologicaly characterized by prominent infiltration with inflammatory mononuclear cells, such as T cells and macrophages, and proliferation of synovial fibroblasts. Inflammatory cytokines, including tumor necrosis factor (TNF), and IL-6, which are mainly produced by macrophages, play a central role in the development of synoviitis. For example, TNF is shown to directly induce synovial fibroblast proliferation, which leads to the pannus formation. TNF is also critical for the expression of inflammatory chemokines and adhesion molecules, which, in combination, facilitate further leukocyte attraction and perpetuation of inflammatory responses. In addition to environmental factors, genetic constitution of host organism seems to play a crucial role in acquiring and development of the disease. The present study was carried out to investigate the association of HLA-class 11 (DR, DQ) with RA disease by genotyping in Iraqi patients, as well as to provide information about genotypes that confer susceptibility or resistance to this disease. Aim of the study was to assess the role, strength and profile of immune response in patients with rheumatoid arthritis by estimation of TNFα, IL-10 and levels, as compared to healthy control group, and to identify a role for certain alleles in occurrence of the disease. The 5-ml samples of venous blood were taken from 30 patients suffering from confirmed rheumatoid arthritis, 19 patients were females and 11 males, as well 30 healthy control samples were enrolled in this study. All the samples were subjected to ELISA test, in order to estimate TNFα, and IL-10 levels in serum from 3 ml of blood. DNA was extracted from 2 ml of blood, and HLA-Class Il genotyping was performed by polymerase chain reaction-sequence specific oligonucleotide probes (PCR-SSO). A highly statistical significant variation, both in TNFα, and IL-10 levels between RA patients group and healthy control group was observed ($p < 0.001$). No statically significant differences between males and females in frequency of the RA ($p = 0.119$). HLA-class II genotyping of RA patients in comparison with healthy control showed significant differences in some alleles between the both groups. Some DR alleles proved to be informative, e.g., the DR*0403 allele showed a significantly increased frequency in control group with 35%, compared with 6.67% in RA group ($p = 0.02$). The DR*701 allele showed increased frequency in the patients with 9 cases (30%, $p = 0.007$). Genotyping of DQ alleles did not any no significant differences. Although *0202 allele occurred in 40% of patients group *versus* 15% in control groups, it was not significant ($p > 0.05$).

Keywords: rheumatoid arthritis, TNFα, IL-10, HLA genotyping, allele frequency

Introduction

RA is one of the most common autoimmune diseases worldwide and is characterized by the inflammation of synovial tissues and the formation of rheumatoid pannus, which is capable of eroding adjacent cartilage and bone and cause subsequent joint destruction [3]. RA occurs as a result of complex interaction between numerous genetic and environmental factors [5].

Rheumatoid arthritis (RA) is a systemic disease that causes progressive joint damage and disability. Rheumatoid synovium is histologically characterized by prominent infiltration of inflammatory mononuclear cells, such as T cells and macrophages; it is well known that many different inflammatory cells such as T cells, B cells, fibroblast-like synoviocytes, and antigen-presenting cells and the massive produced proinflammatory mediators, such as TNF and IL-1, are implicated. Histopathologic features of RA synovial tissue encompass infiltration by macrophages and T cells, synovial lining hyperplasia, neoangiogenesis, and pannus formation [2, 4].

Many evidence refer to an autoimmune component in RA; mainly the recognition of HLA-DR subtypes, which are associated with RA indicate the involvement of antigen-presenting cells, such as dendritic cells and macrophages, as well as T cells [7, 10]. Also, RA is associated with the production of autoantibodies such as the rheumatoid factor and antibodies against cyclic citrullinated peptide [9, 12] the disease is a result of a complicated interaction between immunologic and genetic factors of the host. Therefore, populations were categorized into susceptible and resistant to probably the most effective genes in the HLA genomic region which is known as high dense and polymorphic

genes [11]. The most important determinants of genetic susceptibility to RA located on the short arm of chromosome 6; it is a kind of genetic marker of human beings [6]. Numerous studies in Iraq reported associations of HLA and RA diseases [1].

Materials and methods

Blood samples

Five ml of venous blood were obtained from each subject, from which 2 ml were kept in EDTA tubes for DNA extraction, and the other 3 ml in plane tubes from which serum was obtained and kept at -20 C until use. The patients related to the medical city in Baghdad- orthopedic unit, during the period from February to august 2017, in addition to thirty healthy control group enrolled in this study.

DNA extraction and genotyping

DNA was extracted from whole blood using ready kit (KIAGEN, Germany) according to the manufacturer's instructions. Sequence-specific oligonucleotide primed PCR (PCR-SSO) method was used for the amplification of HLA-DRBI and HLA-DQ using ready kit (Lipa HLA DRB, Innogenetics. Murex Biotech Limited, Dartford, UK). Molecular typing of HLA alleles was performed using a reverse hybridization Automatic Line probe assay (Auto-Lipa) supplied by the same company, in which typing tests were based on the reverse dot blot hybridization. Positive probes on each strip were recognized by typing table (provided with the kit).

Serum levels of TNFα, IL-10

Commercial kits were utilized for estimation of serum levels of IL-10, TNFα (Demeditec Diagnostic, Germany) and using automated ELISA apparatus (Diagnostic Automation Inc., USA) and following the manual protocol supplied with each kit.

Statistical analysis

The Statistical Package for the Social sciences (SPSS, version 14) was used for statistical analysis. The association between different alleles and the development of RA was calculated through adjusted odd ratio and 95% confidence intervals using Chi-square test. Serum levels of cytokines were quantitative variables, but were non-normally distributed as shown by Shapiro-Wilk test. These variables are better to be analyzed by nonparametric test, and median but not mean was calculated. The Mann–Whitney test was used to further explore the significance of difference in median between each pair of study groups. The P value < 0.05 was considered statistically significant.

Results

Regarding statistical analysis of serum cytokines levels: a significant elevation was noticed in the median serum level of Th2-cells related cytokines (TNFα, and IL-10) in patients with RA when compared with healthy control group.

The current study revealed positive relation between serum TNFα level,IL-10 and the progression of the disease. And revealed no significant role for gender in the occurrence of RA. Since no statistical difference between male and female patients as shown in the (Figure 1, 2, 3).

HLA-class II genotyping of RA patients in comparison with healthy control evoked significant differences in some alleles between both groups. Among DR alleles there were some alleles showed higher frequency in control group; DR*0403 allele showed increase frequency in control groups with

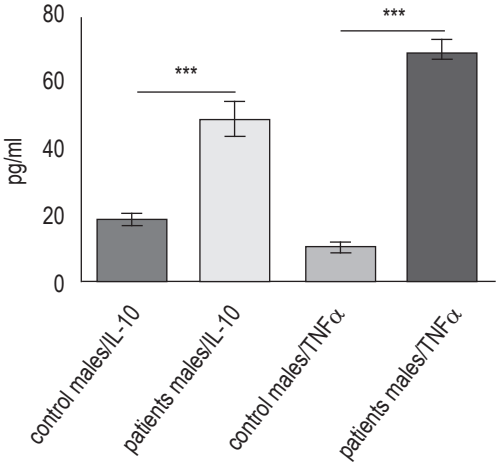

Figure 1. Effect of rheumatoid on blood IL-10 and TNFα

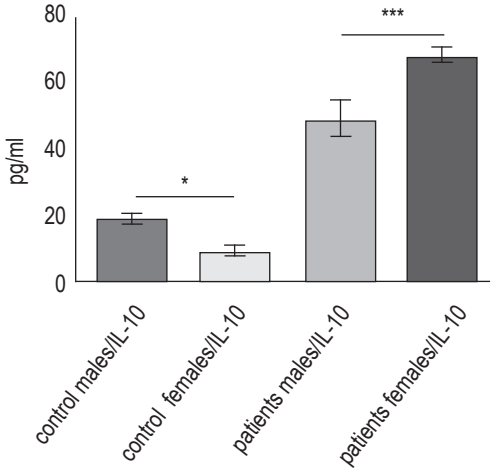

Figure 2. Effect of gender on blood IL-10 in healthy and patient individuals

TABLE 1. HLA-DR GENOTYPING IN RA PATIENTS IN COMPARISON TO HEALTHY CONTROL

HLA-DR allele	RA	%	Control	%	OR	IOR	EF	PF	P value
*0203	1	3.33%	0	0.00%	2.085	0.480	0.52	-1.08	NS
*0204	0	0.00%	1	5.00%	0.213	4.692	0.00	0.00	NS
*0302	0	0.00%	1	5.00%	0.213	4.692	0.00	0.00	NS
*0308	4	13.33%	5	25.00%	0.479	2.090	-4.36	0.81	NS
*0309	1	3.33%	0	0.00%	2.085	0.480	0.52	-1.08	NS
*0318	1	3.33%	0	0.00%	2.085	0.480	0.52	-1.08	NS
*0319	2	6.67%	0	0.00%	3.596	0.278	1.44	3.25	NS
*0329	1	3.33%	0	0.00%	2.085	0.480	0.52	-1.08	NS
*0402	0	0.00%	1	5.00%	0.213	4.692	0.00	0.00	NS
*0405	1	3.33%	0	0.00%	2.085	0.480	0.52	1.08	NS
*0415	0	0.00%	1	5.00%	0.213	4.692	0.00	0.00%	NS
*0435	1	3.33%	1	5.00%	0.661	1.513	-0.51	0.34	NS
*0440	1	3.33%	0	0.00%	2.085	0.480	0.52	-1.08	NS
*0442	1	3.33%	0	0.00%	2.085	0.480	0.52	-1.08	NS
*0446	1	3.33%	0	0.00%	2.085	0.480	0.52	-1.08	NS
*0456	2	6.67%	2	10.00%	0.649	1.541	-1.08	0.52	NS
*0459	1	3.33%	4	20.00%	0.186	5.364	-4.36	0.81	NS
*0603	0	0.00%	1	5.00%	0.213	4.692	0.00	0.00	NS
*0701	9	30.00%	0	0.00%	18.116	0.055	8.50	1.13	.007
*0707	1	3.33%	0	0.00%	2.085	0.480	0.52	-1.08	NS
*0713	1	3.33%	0	0.00%	2.085	0.480	0.52	-1.08	NS
*0716	0	0.00%	1	5.00%	0.213	4.692	0.00	0.00	NS
*0717	4	13.33%	3	15.00%	0.849	1.178	-0.71	0.42	NS
*1001	1	3.33%	0	0.00%	2.085	0.480	0.52	-1.08	NS
*1101	1	3.33%	1	5.00%	0.661	1.513	-0.51	0.34	NS
*1107	3	10.00%	0	0.00%	5.218	0.192	2.43	1.70	NS
*1109	2	6.67%	2	10.00%	0.649	1.541	-1.08	0.52	NS
*1112	1	3.33%	0	0.00%	2.085	0.480	0.52	1.08	NS
*1122	2	6.67%	0	0.00%	3.596	0.278	1.44	3.25	NS
*1137	2	6.67%	0	0.00%	3.596	0.278	1.44	3.25	NS
*1152	1	3.33%	0	0.00%	2.085	0.480	0.52	-1.08	NS
*1156	0	0.00%	1	5.00%	0.213	4.692	0.00	0.00	NS
*1165	1	3.33%	1	5.00%	0.661	1.513	-0.51	0.34	NS
*1301	0	0.00%	1	5.00%	0.213	4.692	0.00	0.00	NS
*1302	2	6.67%	0	0.00%	3.596	0.278	1.44	3.25	NS
*1359	1	3.33%	0	0.00%	2.085	0.480	0.52	-1.08	NS
*1360	1	3.33%	0	0.00%	2.085	0.480	0.52 -	1.08	NS
*1370	0	0.00%	1	5.00%	0.213	4.692	0.00	0.00	NS
*1374	0	0.00%	3	15.00%	0.082	12.200	0.00	0.00	NS
*1401	2	6.67%	0	0.00%	3.596	0.278	1.44	3.25	NS
*1525	1	3.33%	0	0.00%	2.085	0.480	0.52	-1.08	NS
*1601	1	0.00%	1	5.00%	0.213	4.692	0.00	0.00	NS
*1605	0	3.33%	0	0.00%	2.085	0.480	0.52	-1.08	NS
*1607	1	3.33%	0	0.00%	2.085	0.480	0.52	-1.08	NS
*1613	0	0.00%	1	5.00%	0.213	4.692	0.00	0.00	NS
*6389	1	3.33%	0	0.00%	2.085	0.480	0.52	-1.08	NS
*9045	1	3.33%	0	0.00%	2.085	0.480	0.52	-1.08	NS
*0403	2	6.67%	7	35.00%	0.158	6.333	-10.67	0.91	0.020

Note. OR, odds ratio; NS, non-significant; IOR, inverse odd ratio; EF, etiological factor; PF, preventive factor.

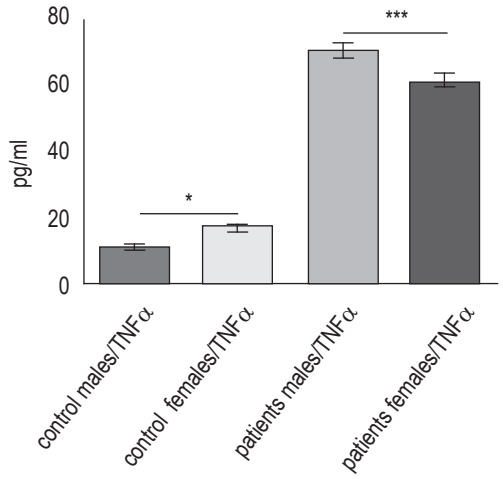

Figure 3. Effect of gender on blood TNFα in healthy and patient individuals

35% compared with 6.67% in patients group., and the P value was 0.020, which is considered as statistically significant. Another DR*701 allele showed increase frequency in patients groups with 9 cases 30% and the P value was 0.007. Table 3-1; show the frequency of various alleles in DR region in both patients and control groups with their P value and EF. Concerning DQ allele's genotyping no significant allele's frequency was noticed. Although *0202 allele occurred in 40% of patients group and 15% in control groups it was not significant statistically as the P value was more than 0.05 (Table 1).

Discussion

This study aimed to investigate the association of different HLA class II alleles with the incidence of Rheumatoid arthritis among Iraqi patients. Two alleles appeared to have significant effect on the resistance to RA. The first one, HLA-DR*0403, was a protective allele (OR = 0.158) which implies that carriers of this allele are 6.329-fold less likely to be infected with RA (protective allele) compared to non-carriers under the same circumstances. The other allele was HLA-DR*0701 which associated with increased susceptibility to RA (susceptibility allele) (OR = 18.116). That means carriers of this allele are 18.116-fold more likely to be infected with RA compared to non-carriers under the same circumstances. To explain the significant association of the two alleles (HLA-DR*0403 and HLA-DR*0701) with the resistance and susceptibility to RA. T cell receptors (TCRs) are designed to recognize antigens displayed by cell surface HLA molecules. Allelic variation of HLA gene will affect the efficiency by which HLA molecule could interact with TCR and subsequent activation of the T-cells particularly, the genetic alteration in loci encoding for side-chain binding pockets has the greatest effect on such interaction. That is because this pocket determines which peptide sequences can accommodating in the biding site [8].

Conclusion

This study aimed to investigate the association of different HLA class II alleles with the incidence of Rheumatoid arthritis among Iraqi patients the constitutional resistance may be depend upon a potential immunogenic predisposition with a potential HLA association .The presence of different HLA antigens among different studies of other societies and present study may be due to ethnic differences among world population and/or could be due to small sample of patients taken in this study, or could be due to interaction among ethnic groups of Iraqi society from very previous generations. This study concluded that HLA-class ll DR *0403 allele may might indicate resistance to disease among patients, while presence of HLA-DR*0701confer increase susceptibility. No significant alleles in regards of DQ region.

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