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ROLE OF TNF- α AND IL-10 IN RHEUMATOID ARTHRITIS DISEASE AND THE ASSOCIATION WITH SOME HLA -11 DR AND DQ ALLELES

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a systemic disease that causes progressive joint damage and disability. Inflammatory cytokines, including tumor necrosis factor (TNF), and IL-6, which are mainly produced by macrophages, play a central role in the development of synovitis. For example, TNF play major role in the expression of adhesion molecules and inflammatory chemokines which, in combination, facilitate increase inflammatory leukocytes and severe inflammatory responses.

In addition to environmental factors, genetic constitution of hosts seems to play a crucial role in acquiring the disease and its development. The current study was carried out for the detection of any association of HLA-class 11 (DR, DQ) with RA disease by genotyping in Iraqi patients, as well as to provide information about genotypes that may confer susceptibility or resistance to the development of the disease.

Aim of the study: to assess the role, strength and profile of immune response in patients with rheumatoid arthritis by estimation of TNF- α, IL-10 and levels in compare to healthy control group. And to identify any role for certain alleles in exposure to the disease.

Material and Method: Five ml of venous blood samples withdrawn from 30 patients suffering from confirmed Rheumatoid arthritis disease, 19 patients were females and 11 males in addition to 30 healthy control samples were enrolled in this study all samples were subjected for (ELISA test) (Enzyme Linked Immunosorbent assay) to estimate the TNF- α, and IL-10 Levels by using the three ml of blood to extract the serum. Another two ml was used for DNA extraction, and then HLA-Class Il genotyping was performed by polymerase chain reaction-sequence specific oligonucleotide probes (PCR-SSO).

Results: A highly statistically significant variation both in TNF – α levels, and IL-10 between RA patients group and healthy control group was observed, the P value was <0.001

No statically significant differences between males and females in frequency of the RA with
0.119 P value.

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

Key words: RA, TNF- α, IL-10, Genotyping, alleles

1-INTRODUCTION

RA is an autoimmune-diseases worldwide and is characterized by the inflammation of synovial tissues, which is capable of severe damge of adjacent cartilage and bone that results in subsequent joint destruction (Goldring, 2000). RA results from interaction between environmental and genetic factors (Gregersen, 1999).

progressive joint damage and disability in patients with Rheumatoid arthritis (RA) is a systemic disease is and histologically showed 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, and antigen-presenting cells are massive producer of proinflammatory mediators, such as TNF and IL-1, are implicated. Histopathologically RA synovial tissue appearse infiltration by macrophages and T cells, hyperplasia, neoangiogenesis and pannus formation (Arend, 1995, Goronzy, 2005, Maqsood and Jamal, 2011).

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 (Thomas, 999, Santiago-Schwarz, 2001) Also, RA is associated with the production of autoantibodies such as the rheumatoid factor and antibodies against cyclic citrullinated peptide (Vossenaar 2004, Sutton, 2000) 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 (Urayama, 2013). 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 (Kindt 2007). Numerous studies in Iraq reported associations of HLA and RA diseases (Al-Karkhi 2017).

2-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).

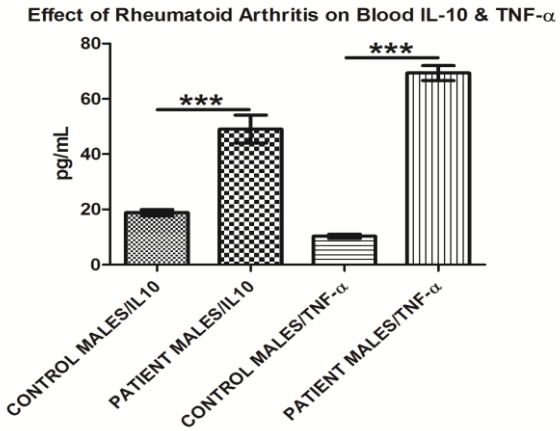
Serum levels of TNF-α, IL-10: Commercial kits were utilized for estimation of serum levels of IL-10, TNF-α, (Demeditec Diagnostic/Germany) and using automated ELISA apparatus (Diagnostic Automation Inc, USA) and following the manual protocol supplied with each kit.

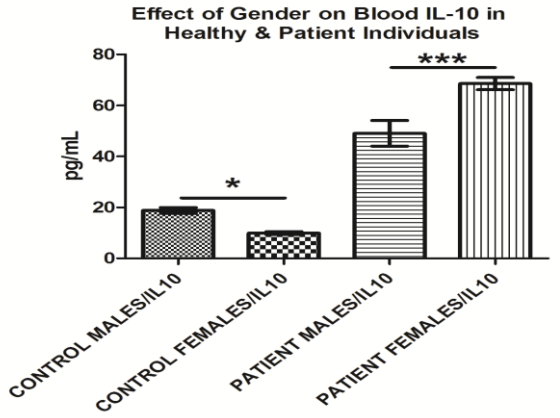
Statistical Analysis: The Statistical Package for the Social sciences (SPSS, version 14) was used for statistical analysis. The association between different alleles and the development of RA was calculated through adjusted odd ratio and 95% confidence intervals using Chi-square test. Serum levels of 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.

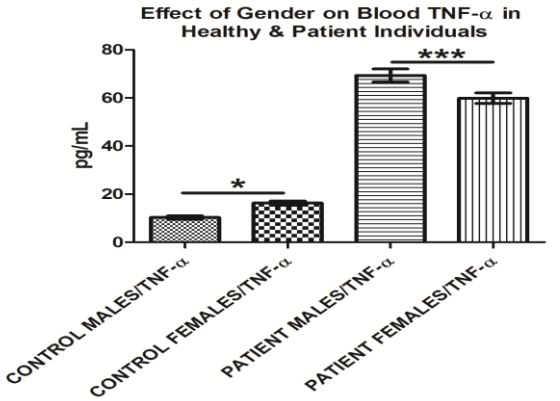
3- RESULTS

Regarding statistical analysis of serum cytokines levels: a significant elevation was noticed in the median serum level of Th2-cells related cytokines (TNF-α, 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 figures.







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 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 3-1: 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
*0403	2	6.67%	7	35.00%	0.158	6.333	-10.67	0.91	0.020
*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

OR: odds ratio, NS: non-significant, IOR inverse odd ratio, EF= Etiological Factor PF= Preventive Factor

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 (Stern 2009).

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 predisposetion 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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