Study of HLA-class II Serotyping and cellular immunity CD4+T, CD8+T cells in Iraqi patients with Rheumatoid arthritis

Hanaa N. Abdullah*[1], Amina N. Al-Thuwani[2]& Mohammed Ibrahim Nadir[2]

College of Health and medical technology. Baghdad, Middle Technical University.

Genetics Engineering and biotechnology Institute for Postgraduate Studies, University of Baghdad.

(*) Corresponding address: E -mail: Dr.hanaa_gene2010@healthtech.mtu.edu.iq

Abstract

Rheumatoid arthritis is a complex polygenic disease whose environmental and genetic factors contribute to both of the predisposition and developing of disease. The current study was conducted to determine the frequency of human leukocyte antigen (HLA-DR, -DQ) and correlate to this variation with disease severity and detected on the role of cellular immune response in pathogenesis of rheumatoid arthritis. Fifty blood samples were collected from RA patients and 50 healthy control group with no history of inflammatory arthritis. The HLA class II (DR, DQ) estimated serologically by using micro lymphocytotoxicity test. The result indicated that a highly significant frequencies of HLA-DR4 and DR53 antigens were observed in RA as compared to healthy group ($P<0.001$). Our results revealed that there are significant higher frequencies of HLA-DQ3 antigen ($P<0.001$) in RA group as compared to control groups. Additionally, the percentage of CD8+ has been decreased in patients in comparison with healthy group, whereas the percentage of CD4+ T-cell has been slightly increased in patients in comparison with healthy control group with nonsignificant differences. The results of this study proved that non-significant correlation which was observed between cellular immune response (CD4 and CD8) among patients.

***Keywords*:** Rheumatoid arthritis, Human leukocyte antigen, Cluster of Differentiation (CD4, CD8).

دراسة التنميط المصلي لكريات الدم البيضاء و المناعة الخلوية للخلايا التائية المساعدة والسمية لدى المرضى العراقيين المصابين بالتهاب المفاصل الرثوي

د.هناء ناجي عبدالله[1], امنه نعمة الثويني[2], محمد ابراهيم نادر[2]

كلية التقنيات الصحية والطبية بغداد, الجامعة التقنية الوسطى[1]

معهدالهندسة الوراثية والتقنيات الاحيائية للدراسات العليا, جامعة بغداد

الملخص

يعد التهاب المفاصل الرثوي مرض اضطراب مناعي ذاتي وتساهم العوامل البيئية والوراثية مساهمة شديدة في الاستعداد للإصابة بالمرض. اجريت الدراسة الحالية للتحري عن مستضدات الكرية البيضاء البشري النمط الثاني-HLA) (DQ-, HLA-DR -II-Class, وعلاقتها في اختلاف شدة المرض والتحري عن دور الاستجابة المناعية الخلوية في التسبب في التهاب المفاصل الرثوي. جمعت 50 عينة دم من مرضى التهاب المفاصل الرثوي و50 عينة من الاشخاص الاصحاء ظاهريا. تم قياس تنميط مستضدات الكريات البيضاء مصليا(HLA-DR,-DQ) باستخدام فحص سمية الخلايا اللمفاوية (Lymphocytotoxicity). وأظهرت النتائج بأن هنالك زيادة معنوية في تكرار مستضدات الكريات البيضاء البشرية (HLA-DR53,HLA- DR4) لدى مرضى التهاب المفاصل الرثوي مقارنة بالأصحاء (P<0.001). فضلا عن وجود زيادة في تكرار مستضدات الكريات البيضاء البشرية (HLA-DQ3) وبفارق عالي المعنوية في مرضى التهاب المفاصل الرثوي مقارنة بالأصحاء (P<0.001). بالإضافة الى ذلك, انخفاض نسبة الخلايا التائية السمية (CD8+T cells) لدى مرضى التهاب المفاصل الرثوي مقارنة مع مجموعة الأصحاء ، بينما اظهرت النتائج ارتفاعا في نسبة الخلايا التائية المساعدة (CD4+Tcells) لدى المرضى مقارنة بمجموعة الأصحاء. تؤكد نتائج الدراسة الحالية عدم وجود علاقة معنوية بين الخلايا(CD4+T and CD8+T cells).

1. Introduction

A rheumatoid arthritis (RA) is a chronic disease, in which various joints in the body are inflamed, leading to swelling, pain, stiffness, and the possible loss of function [1]. RA is strongly associated with the human leukocyte antigen (HLA)-DRB1 locus that possesses the shared susceptibility epitope (SE) and the citrullination of self-antigens [2]. There are two popular theories regarding the pathogenesis of RA. The first holds that the T cell, through interaction with Antigen an as yet unidentified, is the primary cell responsible for initiating the disease as well as for driving the chronic inflammatory process. This theory is based upon the known association of RA with HLA class II antigens, the large number of CD4 cells and skewed T cell receptor gene usage in the RA synovium. The second theory states, while T cells may be important in an initiating the disease, chronic inflammation is self-perpetuated by macrophages and fibroblasts in a T-cell independent manner. This theory is based upon the relative absence of activated T cells phenotypes in chronic RA and the preponderance of activated macrophage and fibroblast phenotypes [3]. The HLA-II molecules are encoded by the highly polymorphic HLA-*DR*, *DQ*, and *DP* loci. The polymorphisms are found largely within the antigen-binding pocket of these molecules, but in HLA-DR they are confined to the DRB1 chain (DRB1,3,4,5 genes) with the DR chain being essentially monomorphic [4][5]. The HLA molecules have been suggested to play a role in the pathogenesis of autoimmune disease including RA , Insulin Dependent Diabetes Mellitus and Multiple Sclerosis [6]. The mechanisms of HLA association

with disease have not been adequately explained. Most studies that agree with the association are usually described in terms of susceptibility while negatively associated molecules are sometimes referred to as protective [7]. Selected CD8 T cells reportedly function as regulatory cells in immune responses [8], but it is not known whether any CD8 T cell subsets act as anti-inflammatory regulators in RA tissue lesions. The idea that CD8 T cells are capable of suppressing immune responses first emerged when it was found that adoptive transfer of T cells from mice tolerant to a known Ag into syngeneic hosts decreased subsequent immune responses to that same Antigen [9]. Subsequent studies have characterized human CD8 suppressor T cells as low responsive to TCR-mediated stimulation [10]. It is now clear that synovial CD8 T cells have several mechanisms through which they modulate the outcome of rheumatoid synovitis [11]. On the basis of this, HLA-II show preferential subsets, in most cases, CD8 T cell recognize antigen on Class-I molecule, while CD4 T cell see antigen, which are associated with HLA- II molecule. An important point should be observed that any defect in this antigen presentation predicts future disease expression with autoimmune diseases [12]. The current study was conducted to determine the frequency of human leukocyte antigen (HLA-DR, -DQ) and correlate to this variation with disease severity and detected on the role of cellular immune response in pathogenesis of rheumatoid arthritis.

2. Methods

2.1. Samples collection

Fifty ples were collected from RA patients and 50 healthy control group with no history of inflammatory arthritis. All patients had RA as defined by the American College of rheumatology criteria (ACR) for RA. Those patients were attending the consultant clinic for Rheumatology in Baghdad Teaching Hospital from March 2008 to March 2009. The mean age of RA patients was 46.75 ± 12.82 years, the mean duration of disease was 8.072 years. Forty-seven (47%) of RA were Rheumatoid factor positive.

H LA Serotyping

Laboratory indices measured included the Micro lymphocytotoxicity test for HLA class II (DR and DQ molecules). The test is complement dependent reaction, in which antibodies recognize antigens on the surface of lymphocytes and form antibody-Antigen complexes. The formed antigen-antibody complex thus are able to activate the added complement which results in death of reacted cells which permit adsorption of dye to score the reactions and to determine the HLA

phenotypes. Evaluation was done under phase contrast microscope. Living cells appeared light and shiny (negative reaction) while dead cells were stained with the dye and look dark and larger than living cells (positive reaction).

CD4 and CD8 T-cell assay

An immune enzymatic assay is based on the specific capture of the CD4+T cell and CD8+T lymphocytes, with paramagnetic micro particles coated with capture antibodies (8). The measurement of the absorbance values of the wells is performed with a spectrophotometer at 450 nm. The absorbance values are proportional to the number of CD4+Tand CD8+T cells in the blood samples.

2.2. Statistical Methods

The mean ± SD were given, difference between means of patients and healthy control group were assess by least significant differences (LSD). These statically analyses were done by using Pentium four computer through the SSPS program (version 10). The strength of an association between disease and genetic marker is generally expressed in terms of a relative risk value (RR). The level of significance (probability) is calculated by fisher's exact probability (p) through constructing 2X2 contingency tables from the previous four entries (a, b, c and d), to avoid a chance occurrence of an association (due to many comparisons). The P is multiplied by the number antigens tested at each HLA locus, therefore the corrected probability (Pc).

3. Results

The HLA typing is conducted in this study for two groups (RA patients, healthy control) by using the serological method for HLA-typing. The results are read by the inverted microscope. The bright cells are considered as negative results, while the non-bright cells have meant a positive result. The frequency distribution of various HLA class II-DR antigens for RA group and healthy controls. Highly significant frequencies of HLA-DR4 and DR53 antigens are observed, with P value (0.001) for both groups. Hence these frequencies have elevated relative risk (RR) (7.21and 6.24) and etiological fraction (EF) (0.41 and 0.38) respectively with positive association, as well as the following antigen DR1 was presented with higher frequencies in RA patients than healthy control groups with RR (1.28) and EF (0.048). In addition, there were some antigens that showed higher frequency in healthy controls as compared with patients, but statistically that were not significant like HLA- R3, DR5, DR7, DR8, DR9, DR10, DR11and DR52, as mentioned in Table (1).

Table 1: Frequencies of HLA class II-DR antigen among RA patients and control group

HLA-DR	RA patients (50)		healthy group (50)		RR	EF	PF	P-value	PC
	N	%	N	%					
1	11	22	9	18	1.28	0.04	-	0.619	NS
2	2	4	2	4	1.00	-	-	0.1	NS
3	6	12	9	18	0.621	-	0.06	0.403	NS
4	27	54	7	14	7.21	0.41	-	2.1×10^{-5}	2.1×10^{-4}
5	7	14	11	22	0.57	-	0.09	0.300	NS
6	2	4	3	6	0.65	-	0.02	0.648	NS
7	2	4	5	10	0.37	-	0.06	0.242	NS
8	1	2	3	6	0.31	-	0.04	0.310	NS
9	10	20	12	24	0.79	-	0.05	0.631	NS
10	4	8	7	14	0.53	-	0.06	0.34	NS
11	0	0	2	4	-	-	-	-	-
14	1	2	1	2	1.00	-	-	-	-
52	11	22	16	32	0.59	-	0.13	0.262	NS
53	23	46	6	12	6.24	0.38	-	1.6×10^{-4}	0.002

More to the point, the results revealed that there were high frequencies of HLA-DQ3 antigen ($P<0.001$) in RA group as compared to control groups with RR of (5.687) , EF of (0.428), while HLA-DQ1 and DQ2 showed higher frequencies in healthy control than patients ,but all did not attained statistical significance, as listed in Table (2).

Table 2: Frequencies of HLA class II-DQ antigen among RA patients and control group.

HLA-DQ	RA (50)		Control (50)		RR	EF	PF	P-value	PC
	N	%	N	%					
1	11	22	16	32	0.59	-	0.13	0.26	NS
2	22	44	29	58	0.56	-	0.25	0.356	NS
3	26	52	8	16	5.68	0.42	-	1.3×10^{-4}	0.002

The mean of CD8+ and CD4+ in RA patients and healthy control groups have been presented in table (3). The result of this study appeared high significant decrease (P<0.001) in the percentage means the CD8+ cells in RA patients, in comparison with the healthy group, despite of increase of CD4+ T cells in blood of patients but statistically non-significant. The result of this study proved that no significant correlation which was observed between cellular immune response (CD4 and CD8) among patients, as mentioned in Table (4).

Table 3: Mean of CD4 and CD8 in blood of studied groups

Cellular parameters		Number	Mean	Sd. Deviation	P-value	Sig.
CD8 TCD8/ul	Healthy group	50	1402.67	285.96	0.00	HS
	RA patients	50	685.67	313.38		
CD4 TCD4/ul	Healthy group	50	1445.67	272.8	.629	NS
	RA patients	50	1394.77	555.23		

Table 4: The correlation between cellular immunity (CD4 and CD8) among patients

Correlation		CD4 TCD4/ul
CD8 TCD8/ul	Pearson Correlation	
	P-value	.189
	Significant	.060
		NS

4. Discussion

The HLA is a vital genetic factor that initiates or regulates immune response by presenting foreign or self-antigens to T-lymphocyte, therefore lower or higher representation of some HLA alleles may contribute to developing of rheumatoid arthritis. The frequency distribution of HLA-DR for studied groups showed that a highly significant frequency of DR4 antigen in patients as compared to control group. Different findings regarding this association were reported, highly significant association between DR4 and RA but in less percentage [13,14]. Almeida *et.al.* found the frequencies HLA-DR1(55.7%) HLA-DR4 represent (43.3%), in comparison HLA-DR3 (22.7%) and HLA-DR1 and HLA-BR13 (both in 20.6% of patients) [15]. However, the association with certain HLA-DR antigen is subject to ethnic variation due to the different baseline frequencies of the antigens in the populations affected. This antigen may regulate immune responsiveness, but not all, the environmental triggers that may initiate the disease [16,17], also high frequency of DR53 observe significantly in RA patients and this result agrees with some studies [13]. Furthermore, HLA-DR1 appeared as risk factor of (1.28), in particular, tended to be more frequent in patients than control. Our results generally agree with the findings

in Korean RA patients (13) and similar to report (14) of which showed the prevalence of DR1 in whole population as well as in DR4 negative patients that was also significantly increased in Asian, Greek origins with rheumatoid patients. Generally, it was found that presence of HLA-DR3, DR5, DR7, DR8, DR9, DR10, DR11, DR52 antigens in high frequencies in healthy control more than patients. Concerning the DR3 was in agreement with some studies, in Canada, HLA-DR3 was observed positively associated with RA patients, especially with severe disease which developed gold induced toxic reactions [18]. It is likely that the difference of DR in different groups of patients with RA is related to the severity of disease or may be due to difference in the alleles primarily associated with RA patients, reflecting the prevalence of this allele in the control populations.

Furthermore, DQ1 and DQ2 appeared as high frequencies in healthy individuals than patients. Thus, the development of RA would depended upon the expression of the susceptibility DQ allele and the non-protection DRB1 allele, along with environmental factors that stimulate the autoimmune process [18], further study was suggest that polymorphism in DQB1 genes might determine predisposition to RA while the DRB1 polymorphism might dictate severity /protection of the disease. In our study this fact is true since DQ3 is associated with RA while DQ1 and DQ2 correlated with protection in the presence or absence of DR4 antigen. The present study also emphasized on the role of cellular immune response in pathogenesis of rheumatoid arthritis. The classical paradigm for RA pathogenesis holds that CD4 cells mediate joint damage both directly and by driving non - T effectors cells to release inflammatory cytokines. Additionally, T cells and macrophage are considered to play an important role in the initiation and perpetuation of inflammatory responses in RA [14]. A role for CD8+ T cells in the pathogenesis of rheumatoid arthritis has been suggested, the precise nature of their involvement is not fully understood. CD8+ T cells were associated with the presence of germinal centers in RA synovium, suggesting a role for CD8+ T cells in the formation or maintenance of those lymphoid structures in the synovium [16,19]. Our results do not agree with Mahir who found no significant difference in mean percentage of T–helper (CD4+) and T– cytotoxic (CD8+) in RA patients than healthy group (P>0.005.(20), while Lee reported that the percentage number of circulating T–lymphocyte (helper CD4+) increase mildly in circulation of patients with RA, with a slight decrease in (cytotoxic CD8+) [12]. Whereas other investigators showed the percentages of CD4+ and CD8+ T cell that were similar in patients with RA and healthy [13]. Another study found that peripheral blood central memory CD8+T cells were more frequent in RA patients when compared to healthy controls whereas the opposite profile was seen with effector memory CD8+T cells (22). Additionally, CD4+ T cells contribute to autoantibody production, inflammation, synovial

angiogenesis, hyperplasia, and cartilage and bone destruction in RA, and T regulatory cells are implicated in the suppression of cellular and humoral responses in RA. Furthermore, abnormalities in T cell homeostasis and *invitro* responses have been described in RA and may be genetically determined [13]. Our results suggest that CD8+T cells play a bigger role in RA than recognized in disease pathogenesis and maintenance, according to which pathogenic T cells are HLA class II-restricted, i.e. CD4+.

5. Conclusions

1-There are high frequencies of HLA- DR4 and HLA-DR53 among RA patients. This emphasizes the role of HLA–DR antigen in rheumatoid arthritis susceptibility.

2-CD8+T were highly significantly decreased in RA patients and no significant correlation between cellular immune response (CD4 and CD8) among Ra patients.

References

[1] Goronzy, J. and Weyand, E.Developments in the scientific understanding of Rheumatoid arthritis. Arthritis Res & Ther.2009; 11:249.

[2] Scally, J.; Soi Cheng. L. and Nadine, L. Dudek. A molecular basis for the association of the HLA-DRB1 locus, citrullination, and rheumatoid arthritis J. Nel, J. Exp. Med.;2002; 210 (12):2569-2582.

[3] Arend, W. The pathophsiology and treatment of Rheumatoid arthritis. Arthritis & Rheum.1997;40:595-59.

[4] Viatte, S., D. Plant, and S. Raychaudhuri.Genetics and epigenetics of rheumatoid arthritis. Nat Rev Rheum.2013;9:141–153.

[5] Raychaudhuri, S., C.; Sandor, E.A.:Stahl, J.; Freudenberg, H.S.; Lee, X.; Jia, L. ;Alfredsson, L.; Padyukov, L.; Klareskog, J. ;Worthington, et al. Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis. Nat. Genet.2012; 44:291–296.

[6] Forbes, S. & Trowsdale, J. (1999). The MHC quarterly report. Immunogenetics; 50: 152-159.

[7] Clark, A. and Vyes, T. Genetics of rheumatic disease. Arth. Res. Ther.2009;11:248.

[8] Chang, C. C., R. Ciubotariu, J. S. Manavalan, J. Yuan, A. I. Colovai, F. Piazza,S. Lederman, M. Colonna, R. Tolerization of dendritic cells by TS cells: the crucial role of inhibitory receptors ILT3 and ILT4. Nat. Immunol.2002; 3: 237–243.

[9] Dorf, M. E.; V. K. Kuchroo and M. Collins.Suppressor T cells: some answers but more questions. Immunol. Today; .1992. 13: 241–243.

[10] Arosa, F. A. (2002). CD8CD28 T cells: certainties and uncertainties of a prevalent human T-cell subset. Immunol. Cell Biol.;80: 1–13.

[11] Koetz, K.; E. Bryl; K. Spickschen; W. M., O'Fallon, J. J. Goronzy, and C. M. Weyand. T cell homeostasis in patients with rheumatoid arthritis. Proc. Natl. Acad. Sci. USA2002; 97: 9203–9208.

[12] Buch, M. and Emery, P.The Etiology and Pathogenesis of rheumatoid arthritis. J. Hospital Pharmacist2002; 9: 5-10,

[13] Skapenko, A.; Leipe , J.; Lipsky , P. & Schulze-Koops , H. The role of the T cell in autoimmune inflammation. Arthritis Res. & Ther.2005; 7(Suppl 2): S4-S14doi.

[14] Berner, B.; Akca, D.; Jung, T.; Muller,G. & Reuss-Borst, MA. Analysis of the Th1 and Th2 cytokines expressing CD4 and CD8 T cells in rheumatoid arthritis by flow cytometry. J. Rheum.2000 ;27 (5): 1128-35.

[15] Almeida, M.; Almeida, J.; Bertolo,M.;s Pires, C. and Campelo,V. HLA-DR Frequency in Individuals with Rheumatoid by TS cells: the crucial role of inhibitory receptors ILT3 and ILT4. Nat. Immunol.2011;3 :237–243.

[16] FU, Y.;Yan, G.; Shi, L. & Faustman, D.Antigen processing & autoimmunity , salivary gland biogenetics. Ann. New York. Acad. Sci.1989;842: 138-154,

[17] Molkentin , J.; Gregersen, P.; Zhu, N.; Wang, Y.; Chen, S.; Chen, S.; Baxter-Low ,L.& Sliver, J. Molecular analysis of HLA-DRB and DQB polymorphism in Chinese with RA. Genes & Immunity2003; 5:240-245.

[18] Kapitany, A.; Zilahi , E.; Szanto,S.; Szucs,G.; Szabo, Z.; Vegvari, A.; Rass, P.; Sipka, S.; Szegedi, G. & Szekanecz, Z. Association of rheumatoid arthritis with HLA-DR1 and DR4 in Hungary .Ann. North Y. Acad. Sci.2005;1051: 263-270.

[19] Carvalheiro,H.; Duarte,C.;Silva-Cardoso,S.; da Silva, J. et al. CD8+T CELL PROFILES IN PATIENTS WITH RHEUMATOID ARTHRITIS AND THEIR RELATIONSHIP TO DISEASE ACTIVITY. Arthritis & Rheumatism2014; DOI 10.1002/art.38941.

[20] van Roon, J.; Glaudemans, C., Bijsma, J. & Lafeber, F. Differentiation of naïve CD4 T Cells Towards T helper 2 cells is not impaired in Rheumatoid arthritis patients. Arthritis research & Therapy2003;5(5): R269-276.

[21] Maldonado, A.; Mueller, YM.; Thomas, P.; Bojczuk, P.; O'Connors, C. and Katsikis, PD. Decreased effector memory CD45RA+ CD62L- CD8+ T cells and increased central memory CD45RA- CD62L+ CD8+ T cells in peripheral blood of rheumatoid arthritis patients. Arthritis Res Ther. 2003;5(2): R91-6.

[22] Cho BA, Sim JH, Park JA, Kim HW, Yoo WH, Lee SH, et al. Characterization of Effector Memory CD8(+) T Cells in the Synovial Fluid of Rheumatoid Arthritis. J Clin Immunol. 2012; 188 (1 Supplement)69.1.