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Jamal Naser Farhood
Al Qadisiyah University,
Collage of Biotechnology, Iraq

Corresponding Author:
Jamal Naser Farhood
Al Qadisiyah University,
Collage of Biotechnology, Iraq

Molecular basis of vascular pathology in rheumatoid is it reasonable?

Jamal Naser Farhood

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Abstract
Objectives: Analyze HLA DRB1 genotype of rheumatoid arthritis patients using PCR-SSP analysis, concurrently measuring levels of P-ANCA vasculits markers using indirect ELIZA and trying to elucidate if there is any genetic basis.
Study protocol: This is a prospective cohort study comprised of 110 subjects (102 females and 8 males), with an age range of 24-71 years (average 45.6±12.245) fulfilling the American College of Rheumatology (ACR) criteria for RA. Blood samples were taken from all members of the study groups (110 RA patients and 50 healthy controls), and subsequently tested for HLA-DRB1 genetic analysis using PCR-SSP and P-ANCA using indirect ELIZA.
Results: DRB1 alleles (*10 and*15) showed higher frequency percentage in anti-cathepsin G positive patients, though non –significant. On the other hand, a particular allele (HLA-DRB*7) was found in higher proportion in rheumatoid patients with positive anti-lactoferrin autoantibodies. All HLA DRB1 allele groups (except *04 and *10) were found in a higher frequency in anti-elastase positive.
Conclusion: Possible role of non-shared epitope DRB alleles in severity of RA patients.

Keywords: Rheumatoid arthritis, HLA DRB1 genotype, PCR-SSP analysis, P-ANCA vasculitis markers, indirect ELISA, genetic basis

Introduction

Accumulated papers around the world pointed out to responsibility of heredity factors (genes) for increasing subjects' predisposion to get rheumatoid, especially HLA DRB1. The shared epitope (SE) in particular had been implicated greatly. It's simply a well-preserved particular sequence motifs of amino acids, which are positioned inside the third complementarity determining region (CDR) of the DR beta chains at amino acids series sequence 70–74. The amino acid sequence of shared epitope consists of glutamine-lysine-arginine-alanine-alanine (QKRAA) [1]. Higher predisposition to rheumatoid has been linked to up regulation of aforementioned genes, which predispose individual to aggressive course of the disease [2]. Scholars had found association between shared epitope genes and rheumatoid arthritis to be for ACCP antibody positive illness [3]. As well, Shared epitope increases susceptibility to ACCP antibody positive disease, and a greater disease's severity [1]. A higher susceptibility to rheumatoid arthritis was observed when there was double dose shared epitope genotype [4].

Anti –neutrophil cytoplasmic antibodies are against wide array of cytoplasmic antigens, they consisted of two major categories when examined by fluorescent microscope: c-ANCA, which targets cytoplasmic antigens, and p-ANCA, which gives peri-nuclear fluorescence on examined tissue sections [5]. Proteinase 3 (PR3) was identified as potential C-ANCA antigen, myeloperoxidase (MPO), lactoferrin (LF), leukocyte elastase (LE) for P-ANCA [6].

Some ANCA are specific diagnostic markers for certain vasculitis syndromes [7]. Also they had been found in other auto immune disorders as rheumatoid arthritis (RA) and inflammatory bowel diseases with various percentages [8]. In the former diseases, the p-ANCAs antigens were unknown, though possibly lactoferrin, cathpsin, elastase and lysozyme were suggested [9]. Proportions of ANCA among rheumatoid arthritis vary greatly around the globe, with a range between 0 to 72% recorded accordingly [10].

Anti-neutrophil cytoplasmic antibodies had been associated with aggressive RA course especially extra-articular manifestations [11, 12], radiographic joint destruction, and considered

as an initial indicator of growing destructive rheumatoid arthritis [10]. Anti-LF Abs was found in substantially higher amounts amongst subjects with rheumatoid arthritis who had vasculitis, and suggested as diagnostic marker of vasculitis in RA [12]. In addition, ANCA had found associated with pathogenesis by stimulating neutrophils and monocytes to release reactive oxygen species (ROS) subsequently causing tissue destruction and prolongation of chronic inflammation [13].

An Accumulated current research articles support hereditary root for vasculitis caused by anti-neutrophil cytoplasmic antibodies, with possible participation of human leuckocyte antigen, in particular HLA-DRB1*09:01 [14], scholars found former gene linked to different autoimmune disorders together with rheumatoid arthritis [14]. they also discovered ANCA associated vasculitis linkage to particular allelic variants [15], also, scientists had identified group of potential alleles, they subsequently pointed out to their responsibility for higher proportion of ANCA associated vasculitis around the globe [16, 17, 18, 19]. The suggested nominee were diverse alleles at major histocompatibility complex loci [17, 20, 21]. If the above mentioned alleles that increase susceptibility to various inflammatory disorders, they might apply to ANCA positivity in rheumatoid arthritis which this study tried to investigate is a matter of debate.

Methods

This is a prospective cohort study comprised of 110 subjects (Comprised of one hundred and two ladies against eight men), the age limits of participants vary between 24 to 71years, with mean age equals 45.6 years all participants matched the diagnostic standards of rheumatoid arthritis. All subjects were selected from attendee of Baghdad medical city, and their diagnosis was established by qualified physician (rheumatologist). The experiment lasted for 10 months, and carried out at 2012. Then I took a printed approval for participation from each person. Unrelated, 50 apparently fit, age and six matched subjects were selected as controls. Approximetly 5 ml Blood specimen were drawn from each participant. Every sample was divided into two portions; first is 2 ml serum for serological tests (ANCA autoantibodies using indirect ELIZA), the remaining second portion were whole blood exclusively used for DNA extraction for PCR-SSP.

As sample collection is time consuming, All specimens (including sera and whole blood) were preserved at -20 C till exmined, were refreezing was avoided completely once thawed all serum specimens subsequently tested for P-ANCA autoantibodies using indirect ELIZA.

ELISA reader (Biotech, ELX 800), and ELISA washer (Biotech, ELX 50), automated Eliza system from (Human, uno, Germany), Micro-centrifuge 18 tubes (Beekman, Germany), Thermo-cycler (Pxe 9700), Auto lipa (Belgium), centrifuge (5417, Germany) were used for blood processing. The following kits were used in this study; Inno-lipa HLA-DRB1 plus, QIAamp DNA mini kit (Innogenetics, Belgium), Anti elastase, anti-cathepsin, and anti-lactoferin screen from (Immuchem, Belgium).

serum levels of Anti elastase, anti-cathepsin, anti-lactoferin, and anti-elastase P-ANCA Abs titer were tested, measured and determined in both RA patients and control groups, and RA patients with different disease stages respectively. The results of this study were translated into a computerized database structure. SPSS10 was used solely for processing data, attaining results, and drawing conclusions.

Results

Table (1) illustrates connection between tested anti neutrophil cytoplasmic antibodies and suggested HLA DRB1 in RA patients.it is obvious that no correlation of aforementioned distribution of gene groups with anti-cathepsin were found, though certain HLA DRB1 alleles (*10 and*15) show higher frequency percentage in anti-cathepsin G positive (>10 U/ml) than anti-cathepsin negative (≤10 U/ml) RA patients.

One non-SE DRB1 allele group (*07) was found in a higher frequency percentage in rheumatoid arthritis patients whom tested positive for lactoferrin autoantibodies against those who didn't. All gene groups (except *04, *10) were found in a higher frequency in those patients tested positive for elastase autoantibodies against those who didnt.

Table 1: Proportion of high and low *p*-ANCA vasculitis risk markers amongst rheumatoid arthritis subjects and their correlation with HLA DRB1 gene groups

Allele (FR)	Anti-cathepsin G FR (%)		Anti-lactoferrin FR (%)		Anti-elastase FR (%)	
	>10 U/ml	≤10 U/ml	>10 U/ml	≤10 U/ml	>10 U/ml	≤10 U/ml
01(6)	2(33.3)	4(66.7)	0 (0)	6 (100)	6(100)	0(0)
03(50)	18(36)	32(64)	14(28)	36(72)	32(64)	18(36)
04(28)	8(28.6)	20(71.4)	12(42.9)	16(57.1)	12(42.9)	16(57.1)
07(14)	2(14.3)	12(85.7)	8(57.1)	6(42.9)	10(71)	4(28.6)
08(12)	4(33.3)	8(66.7)	2(16.7)	10(83.3)	10(83.3)	2(16.7)
10(2)	2(100)	0(0)	0 (0)	2(100)	0 (0)	2(100)
11(34)	12(35.3)	22(64.7)	12(35.3)	22(64.7)	24(70.6)	10(29.4)
13(36)	8(22.2)	28(77.8)	6(16.7)	30(83.3)	28(77.8)	8(22.2)
14(14)	6(42.9)	8(57.1)	2(14.3)	12(85.7)	14(100)	0(0)
15(6)	4(66.7)	2(33.3)	0(0)	6(100)	6(100)	0(0)
16(2)	0 (0)	2(100)	0(0)	2(100)	2(100)	0(0)
Total	66(32.4)	138(67.6)	56(27.5)	148(72.5)	144(70.6)	60(29.4)
p-value	>0.05 for all		>0.05 for all except alleles 04 and 07		>0.05 for all except alleles 04 and 14	

Discussion

The results of the current study, especially those associated with HLA-DRB1*04 allele were in partial mismatching with some other studies.one study referred to possible link between genes of shared epitope (in particular HLA DRB1*04) and severity of vascular damage in RA [22], with a central role for p-ANCA since it is associated with systemic complications of RA such as pulmonary and blood vessels damage [23]. This was true for other studies, as in one of them a genetic basis of anti-neutrophil cytoplasmic

autoantibodies susceptibility in Wegener's Granulomatosis (WG) was found and that Human leukocyte antigen - DRB1*04 gene was highly up-regulated in German subjects with Wegener's Granulomatosis [24], moreover other study found this risk association to be located in the allele HLA-DPB1*0401 [25].

For anti-elastase antibodies, all HLA DRB1 allele groups (except *04 and *10) were found in a higher frequency among rheumatoid arthritis subjects who tested positive for elastase autoantibodies against those who didn't. This exceptional result related to *04 allele might refer to its dual function in the pathogenesis of *p*-ANCA vasculitis by having enhancement effect on anti-LF autoantibodies and inhibitory effect on anti-elastase autoantibodies in RA patients.

It was reported that SE genes are responsible for the production of ACPA positive RA only [26] and subsequent erosive disease [27], however, this study couldn't establish a stable connection between aforementioned shared epitope genes and the p-ANCA except for DRB1*014 allele group,in which significant association with anti-elastase had been found.

A result which might reshape the possible role of shared epitope genes in the etiology of rheumatoid arthritis and its contribution to pathogenesis. Apart from the weak p-ANCA positivity result with the *14 allele which is another SE-allele. Such result might point out a potential link of *14 allele with RA severity by means of the increased p-ANCAs. Some authors had referred to possible role of p-ANCA particularly those directed against lactoferrin, cathpsin G and certain activity markers mainly RF with clinical, lab findings and progressive X-rays erosive changes [28].

One non-SE DRB1 allele group (*07) was found in a higher frequency percentage with anti-lactoferrin positive than anti-lactoferrin negative RA patients, and might be responsible for elevated anti-lactoferrin autoantibodies in RA. This is in accordance with DRB115, the non-shared epitope allele which was found associated with elevated titers of ACCP autoantibodies amongst RA subjects, this observation with our study supports the hypothesis for a possible role of non-shared epitope DRB alleles in pathogenesis of RA patients [29].

Our result further supports the conclusion that there is individuality for each ethnic group, and topographical area. And while certain shared epitope alleles might increase risk and severity of RA in specified region, they might have opposite effect on the others [30], a result that questions the validity of shred epitope theory for RA pathogenesis. Especially if we take into account that correlation concerning SE genes, rheumatoid factor and other severity markers was previously mentioned by scholars from Europe, North America amongst rheumatoid arthritis subjects [31-34].

Conflict of Interest

Not available

Financial Support

Not available

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