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THE MEANING OF TWO LOCI HLA CLASS II HAPLOTYPES DISTRIBUTION IN CHRONIC GLOMERULONEPHRITIS AND CHRONIC KIDNEY DISEASE IN UZBEK POPULATION

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ABSTRACT

End-Stage Renal Disease (ESRD) is a worldwide public health problem. There are many studies have found the association between human leukocyte antigen (HLA) and glomerulonephritis. As it is well known, big role in the disease progression plays single alleles, but some times more important role are playing there allelic combinations from different HLA loci. The aim of this study was to find out if there are any two-loci haplotypes are playing role in the development of glomerulonephritis and ESRD in Uzbek patients.

KEYWORDS: Chronic kidney disease, End Stage Renal Disease, HLA.

Chronic kidney disease (CKD) is a global worldwide health problem particularly in elderly people.[1,2] According to global WHO data at the moment kidneys diseases are diagnosed for 300–600 million people — about 5-10% of the population of the planet [http://who.int/mediacentre]. As of 2016, 11,854 people suffer from chronic renal failure in the republic (37.5 patients per 100,000 population) and at the moment there are 1888 patients requiring hemodialysis program.[3]

There are many studies, showing the association between the human leukocyte antigen (HLA) and glomerulonephritis over the world populations[4–7], and most of them show the association between HLA and ESRD. However, this problem is still needs further research as soon as it is still unknown how important is the role of immune system in renal diseases.[4] As we can see in many studies there are some significant associations with HLA II alleles. HLA genotypes are correlating with a risk of alloantibody sensitization in ESRD candidates for the kidney transplantation.[4] Also, more than 40 diseases have the links with different HLA genotypes.[5,6] But, as we can often see, not only markers of protection or susceptibility are playing the main role, but sometimes haplotypes combinations are more meaningful. So the aim of the current study is to analyze the differences in the distribution of HLAII class two loci haplotypes in patients with chronic glomerulonephritis and ESRD in the Uzbek population.

MATERIALS AND METHODS

The study was performed at the Institute of Immunology of the Academy of Sciences of Uzbekistan in the laboratory of human genomics named after Professor R.M. Ruzybakiev in the period from 2010 to 2016y.y. The selection of patients in outpatient and inpatient treatment was carried out on the basis of RSCS named acad. V.V. Vahidov and SDC "Immunogen Test" at the Institute of Immunology of the AS of Uzbekistan. We have examined 542 people of Uzbek nationality. During the process of selecting individuals for this study we took into account their national identity in three generations, according to the recommendations VII Workshop on HLA (1977). The 225 people of these have been diagnosed with chronic glomerulonephritis, chronic renal failure complicated with end-stage, to whom the chronodialize was recommended. The control group consisted of 317 healthy individuals who are relatives of the first line of kinship. The patients were 51 women and 174 men, mean age was 34,35 ± 11,41. Among the donors were 163 men and 154 women, mean age was 40,84 ± 11,12.

The DNA Isolation was carried out using the method of alcohol-salt treatment by S. Miller et al (1988). HLA-typing of alleles of genes DRB1, DQA1 and DQB1 was performed using kits "of HLA-DNA-TECH" (« DNA Technology SPA», Moscow) using PCR mSSP method (polymerase chain reaction with sequence-specific primers) in modification of Institute of Immunology MOH RF (D.Yu. Trofimov, 1996) and with Q-PCR. As a result

of the behavior of the reactions were determined following DRB1 specificity: DRB1 *01, *04, *05, *07, *08, *09, *10, * 11, *12, *13, *14, *15, *16, *17 (Splits option *03) *18 (*Splits version 03). During the process of typing of the DQA1 locus the following specificities were found: *0101, *0102, *0103, *0201, * 0301, *0401, *0501, *0601. On locus DQB1: *0201, *0301, *0302, *0303, *0304, *0305, *0401/02 *0501 * 0502/04 *0503, * 0601, *0602-08.

For evaluating of the results obtained, we held the statistical processing of the data, with the help of Arlequin 3.5.2.2 software package, Excel 2007, SISA and a number of formulas to calculate the OR (Odds Ratio) is the index of relative risk, EF (Etiologic fraction) - etiologic fraction, PF (Preventive fraction) - preventive fraction, χ2-confidence index Pearson, 95% CI-confidence interval.

RESULTS AND DISCUSSION

In this study have been identified differences in the distribution of two loci haplotypes frequencies of HLA class II genes. As can be seen from Table 1, there are three haplotypes combinations, DRB1*/DQA1*, DRB1*/DQB1* and DQA1*/DQB1*.

Thus, when we have analyzed combinations of DRB1* and DQA1* genes, we have found four significant combinations. As it is shown in the Table 1 there are three significant haplotypes showing positive association and one significant protective haplotype. From the sum of 79 possible haplotypes, there were observed 29 most often combinations in patients group and 24 haplotypes of 79 possible were observed in the group of healthy donors. Haplotype DRB1*08/DQA1*0103 was observed in patients group significantly more often than in the group of healthy donors (OR=12,918; EF=0,018; χ^2=9,773; 95% CI: 1.631≥12.918≥102.331). Another one haplotype was also observed in patients group significantly more often than in the group of healthy donors, that was DRB1*13/DQA1*0103 (OR=1.594; EF=0.044; χ^2=5.062; 95% CI:1.059≥1.594≥2.399). One more significant predisposing haplotypes is DRB1*14/DQA1*0101 (OR=2.088; EF=0.019; χ^2=3.592; 95% CI:0.96≥2.088≥4.543). The only significant protective combination was DRB1*04/DQA1*0301 (OR=0.316; PF=1.943; χ^2=25.721; 95% CI: 0.198≥0.316≥0.503).

Table 1. Two loci haplotypes in patients with glomerulonephritis and ESRD and in control group.

HLA	Frequencies				RR	EF	PF	χ^2	*P*
	Patients, n=225		Control (donors), n=317						
	HF	PhF	HF	PhF					
DRB1*/DQA1*									
01 / 0101	0.095556	43	0,07886	50	1,234	0,018		0,935	0,333
04 / 0301	0.053300	24	0,15142	96	0,316		1,943	25,721	≤0,001
07/0201	0.122222	55	0,15773	100	0,744		0,331	2,661	0,1
08 / 0103	0.019449	9	0,00157	1	12,918	0,018		9,773	≤0,001
08 / 0601	0.004444	2	0,00473	3	0,939	0,000	0,065	0,005	1,0
09 / 0301	0.035556	16	0,03943	25	0,898		0,113	0,109	0,74
10 / 0101	0.031111	14	0,02366	15	1,325	0,008		0,561	0,74
11 /0501	0.093320	42	0,09937	63	0,933		0,071	0,11	0,74
12 / 0501	0.042222	19	0,05047	32	0,829		0,204	0,4	0,52
12 / 0601	0.006667	3	0,01735	11	0,380		1,613	2,356	0,124
13 / 0102	0.034516	15	0,02709	17	1,252	0,007		0,39	0,53
13 / 0103	0.116498	53	0,07701	49	1,594	0,044		5,062	0,024
13 / 0501	0.024540	11	0,02997	19	0,811		0,232	0,298	0,584
14 / 0101	0.035542	16	0,01735	11	2,088	0,019		3,592	0,05
15 / 0102	0.080521	37	0,0707	45	1,173	0,012		0,476	0,49
15 / 0103	0.041701	18	0,04286	27	0,937		0,067	0,044	0,83
16 / 0102	0.013333	6	0,00789	5	1,700	0,005		0,77	0,37
17 / 0501	0.086572	39	0,08202	52	1,062	0,005		0,074	0,78
DRB1*/DQB1*									
01 / 0501	0,095556	43	0,07886	50	1,234	0,018		0,935	0,33
04 / 0302	0,04	18	0,08043	51	0,476		1,053	7,222	0,007
07 / 0201	0,1	45	0,13722	87	0,699		0,414	3,41	0,06
07 / 0303	0,02	9	0,02050	13	0,975		0,026	0,003	1
08 / 0301	0,006667	3	0,00473	3	1,412	0,002		0,179	0,672
08 / 0601	0,019443	9	0,00157	1	12,918	0,018		9,773	0,001
09 / 0303	0,026667	12	0,03785	24	0,696		0,431	1,026	0,311
10 / 0501	0,031111	14	0,02365	15	1,325	0,008		0,561	0,45

11 / 0301	0,084444	38	0,10249	65	0,807		0,234	1	0,31
11 / 0502		0	0,00948	6	0,233		3,266	7,357	0,006
12 / 0301	0,066667	30	0,06782	43	0,982		0,019	0,006	1
13 / 0301	0,026667	12	0,06782	43	0,377		1,586	9,256	0,002
13 / 0602	0,151111	68	0,10410	66	1,532	0,052		5,369	0,002
14 / 0502	0,002222	1	0,0094	6	0,233		3,266	2,151	0,14
14 / 0503	0,033333	15	0,00630	4	5,431	0,027		11,162	≤0,001
15 / 0502	0,008889	4	0,00950	6	2,816	0,006		0,01	1
15 / 0601	0,040557	18	0,03943	25	4,229	0,031		0,311	0,577
15 / 0602	0,072776	33	0,06463	41	1,877	0,034		0,311	0,577
16 / 0502	0,013333	6	0,00788	5	0,207		3,638	0,777	0,377
17 / 0201	0,077778	35	0,08200	52	9,817	0,070		0,064	0,8
DQA1*/DQB1*									
0101 / 0501	0,128889	58	0,10568	67	1,252	0,026		1,39	0,238
0101 / 0502	0,002222	1	0,00938	6	0,233		3,266	2,151	0,142
0101 / 0503	0,033333	15	0,00631	4	5,431	0,027		11,162	0,0008
0102 / 0502	0,028889	13	0,02689	17	1,080	0,002		0,042	0,83
0102 / 0602	0,111111	50	0,08825	56	1,290	0,025		1,548	0,21
0103 / 0601	0,068889	31	0,04101	26	1,730	0,029		4,106	0,04
0103 / 0602	0,117778	53	0,08044	51	1,526	0,041		4,23	0,03
0201 / 0201	0,1	45	0,14024	89	0,680		0,449	3,961	0,04
0201 / 0303	0,02	9	0,01434	9	1,417	0,006		0,543	0,46
0301 / 0201	0	0	0,02035	13	0,106		8,245	14,736	0,0001
0301 / 0301	0,004526	2	0,04767	30	0,090		9,690	16,887	0,0001
0301 / 0302	0,053333	24	0,08038	51	0,644		0,537	3,003	0,08
0301 / 0303	0,026585	12	0,04402	28	0,593		0,674	2,267	0,132
0501 / 0201	0,077778	35	0,08546	54	0,906		0,103	0,191	0,662
0501 / 0301	0,153251	69	0,17631	112	0,844		0,180	0,464	0,49
0601 / 0301	0,011111	5	0,02208	14	0,498		0,998	1,84	0,175

*PhF – Phenotype frequency; HF – haplotypes frequency; OR – Odds Ratio; EF – Ethiologyc fraction; PF – Preventive fraction; χ^2 –Chi square; *P* – confidence index by Fisher.

In the combinations of DRB1* and DQB1* genes, we have found four significant combinations. As it is also shown in the Table 1 there are three significant haplotypes showing positive association and three significant protective haplotypes. From the sum of 103 possible haplotypes, there were 32 most often observed haplotypes in the group of patients. Haplotype DRB1*08/DQB1*0601 was observed in patients group significantly more often than in the group of healthy donors (OR=12,918; EF=0,018; χ^2=9,773; 95% CI: 1.631≥12.918≥102.331). Also, haplotype DRB1*13/DQB1*0602 was also observed in patients group significantly more often than in the group of healthy donors (OR=1.532; EF=0.052; χ^2=5.369; 95% CI: 1.066≥1.532≥2.202). Another one significant predisposing haplotypes is DRB1*14/DQB1*0503 (OR=5.431; EF=0.027; χ^2=5.369; 95% CI: 1.79≥5.431≥16.474). Significant protective combinations were DRB1*04/DQB1*0302 (OR=0.476; PF=1.053; χ^2=7.222; 95% CI:1. 79≥5.431≥16.474), DRB1*11/DQB1*0502 (OR=0.233; PF=3.266; χ^2=7.357), DRB1*13/DQB1*0301 (OR=0.377; PF=1.586; χ^2=9.256; 95% CI:0.196≥0.377≥0.723). Also we have analyzed DQA1*/DQB1* combinations, where we have observed 24 haplotypes from 63 possible combinations in patients and 22 haplotypes from 63 possible combinations in healthy donors. Six significant haplotypes were found in DQA1*/DQB1*, from that three haplotypes are having positive association and three haplotypes are showing negative association. Haplotype DQA1*0101/DQB1*0503 was observed in patients group significantly more often than in the group of healthy donors (OR=5.431; EF=0,027; χ^2=11.162; 95% CI: 1.79≥5.431≥16.474). Also haplotype DQA1*0103/DQB1*0601 was observed in patients group significantly more often than in the group of healthy donors (OR=1.730; EF=0.029; χ^2=4.106; 95% CI: 1.012≥1.73≥2.957). One more significant predisposing haplotype is DQA1*0103/DQB1*0602 (OR=1.526; EF=0.041; χ^2=4.23; 95% CI: 1.018≥1.526≥2.288). Significant protective combinations were DQA1*0201/DQB1*0201 (OR=0.680; PF=0.449; χ^2=3.961; 95% CI: 0.465≥0.68≥0.996), DQA1*0301/DQB1*0201 (OR=0.106; PF=8.245; χ^2=14.736), DQA1*0301/DQB1*0301 (OR=0.090; PF=9.690; χ^2=16.887).

So, as we can see in the result of the study, two loci haplotypes are playing a huge role in the development of glomerulonephritis and ESRD. It is shown, that sometimes it is even more important, than a single allele distribution and sometimes we can observe some interesting differences. As we can see in the Table 1 we have DRB1*13/DQB1*0301 and DRB1*13/DQB1*0602-8

haplotypes, but the first one has negative association and the second one is predisposing haplotype. As we have published in our previous publication both DRB1*13 and DQB1*0301 are neutral alleles and in combination they are giving a significant protective effect. At the same time in the DRB1*13/DQB1*0602-8 haplotype DQB1*0602-8 allele has strong association with the development of glomerulonephritis and ESRD. This data shows the important role of analyzing not only a single markers, but also an important role are playing there combinations.

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