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Association of HLA Type With Pseudoexfoliation of the Lens Capsule

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Abstract: Exfoliation of the lens capsule is asymptomatic but it is associated with the development of glaucoma and blindness if undetected and untreated. The occurrence of exfoliation varies widely in different countries where the population is ethnically homogeneous. It is suggested that both genetic and environmental influences are important in the development of exfoliative syndrome (ES).

This study was carried out to determine if there was any relation between ES and HLA.

HLA antigen types were determined in 45 patients with exfoliative syndrome. We found a strong association between HLA-A2, HLA-DR5, HLA B21 and HLA-DR7 and the existence of ES (Chi - squares were 4. 849, 11.072, 7.200, 5.404 respectively and $p < 0.05$).

Data were analysed for linkage disequilibrium (LD). LD was found positive for HLA-A2DR5, HLA-A2DR7, HLA-B21DR7 and HLA-A2B21DR7 were the most frequent haplotypes. However, the frequencies of HLA-A3 and HLA-A10 were higher in the control subjects than in the patients (P exacts < 0.05).

These findings showed that there were close association between HLA-A2, B21, DR5 and DR7 antigens and HLA-A2DR5 , HLA-A2DR7, HLA-B21DR7 and HLA-A2B21DR7 haplotypes and exfoliative syndrome.

Key Words: Exfoliative syndrome , histocompatibility antigens, HLA.

Introduction

Exfoliative syndrome (ES) was first reported in 1917 by Lindberg (1), who noted greyish flakes at the pupillary border in most of his patients with chronic glaucoma. Although this entity has been recognised for more than 78 years, the essential nature of ES is still obscure. Many investigations have been made to determine the nature of ES. Extensive electron microscopic evaluation of the exfoliative material has indicated that it consists of fibrils and filaments embedded in an amorphous substance. These fibrils possess a characteristic cross banding, which usually exhibits a periodicity of 17-56 nm (2, 3). Ultrastructurally these fibrils do not resemble interstitial collagen, and this material was not soluble in a variety of enzymes such as collagenase, trypsin, pepsin and papain. Amyloid deposits resembling exfoliative material have been found on the equatorial lens capsule and the anterior and posterior surfaces of the iris in patients with primary systemic non familial amyloidosis (4).

The aqueous humour IgG of patients has been found to increase significantly with senile exfoliation, indicating that there could be an immunological participitant in the pathogenesis of the exfoliative material (5). Histopathological and histochemical examinations conducted on the conjunctiva, iris and lens tissue of 15 senile pseudo-exfoliation cases and on the trabecular tissue of 3 patients with capsular glaucoma. Demonstrated these tissues to be composed of glycoproteins and proteoaminoglycans (6). The detection of positive staining of exfoliative material (EM) and zonule with aldehyde fuchsine and chrome hemotoxylin after prior oxidation has led to the proposal that fibrils of EM are related to the microfibrillary component of elastic tissue (7). All of the studies mentioned above still do not adequate explain the true causes and the pathogenesis of EM.

According to most authors, the occurrence of ES varies widely in different countries (8, 9). Pseudo

Table 1. Characteristics of the study group and the control group

	Study Group	Control Group
Age range (years)	45-65	40-60
Mean age (years)		
Males	54 ± 4.32	53 ± 4.88
Females	52 ± 5.6	52 ± 2.9
Sex		
Males	29	25
Females	16	20

exfoliation is seen more frequently in some countries where the population is ethnically homogeneous. It is suspected that both genetic and environmental influences are important in the development of exfoliation syndrome (10).

Pseudoexfoliation of the lens capsule is asymptomatic but is associated with the development of glaucoma and blindness if undetected and untreated. Early identification could reduce the incidence of visual loss and blindness.

Major histocompatibility complex antigens (MHC) make up a genetic system which codes Class I and Class II antigens, the latter of which is found on the surface of almost all cells of an organism. The MHC region is in the H-2 region in mice and in the HLA region in humans (11, 12). Some association with MHC types and many presumed genetic and/or immune system diseases have been reported. The association between some HLA types and the some diseases are shown in Table 7 (12).

ES is seen in a high frequency in people above 50 years of age in Turkey (8, 13, 14). This study was carried out to determine if there was any relation between ES and HLA.

Materials and Methods

This study was carried out on 45 patients with ES (29 males, 16 females, aged between 45 and 65 yrs) and in a control group of 45 healthy volunteers (25 males, 20 females, aged between 40 and 60 yrs). We were unable to not examine the patients ' relatives to determine whether ES was familial or not, since most were from rural areas and their economic means were not sufficient for them to bring their relatives for an ophthalmic examination.

Control subjects were randomly selected and known to have no immunological disease.

The anetior segments of the eyes were examined by slit-lamp after the pupils were dilated with 10%

Table 2. The frequencies of HLA-A locus antigens in the patients with exfoliative syndrome (n=45) and in the control group (n=45)

Types of the HLA-A antigens	Study Group			Control Group			X^2	P Exact
	n	%	% GF	n	%	% GF		
A 1	10	22. 2	12	6	13. 3	7	0. 317	NS
A 2	34	75. 5	51	24	53. 2	32	4. 849	<0.05
A 3	-	-	-	7	15. 5	8	7. 590	<0.05
A 9	12	26. 6	14	12	26. 6	14	0. 080	NS
A10	2	4. 44	2	8	17. 76	9	4.050	<0.05
A 11	7		8	2	4. 44	2		NS
A 19	10	22. 2	12	6	13. 3	7	1. 216	NS
A 28	4	8. 88	5	4	8. 88	5		NS

NS: Not significant, GF: Gene frequency

Table 3. The frequencies of HLA-B locus antigens in the patients with exfoliative syndrome (n=45) and in the control group (n=45)

Types of the HLA-B antigens	Study Group			Control Group			X^2	P corrected	p exact
	n	%	% GF	n	%	% GF			
B 5	17	37.74	21	10	22.2	12	2. 593		NS
B 7	2	4.44	2	1	2.22	1			NS
B 8	3	6.66	3	2	4.44	2			NS
B 12	8	17.76	9	6	13.3	7	0. 338		NS
B 13	2	4.44	2	-	-	0			NS
B 14	1	2.22	1	2	4.44	2			NS
B 15	7	15.5	8	3	6.66	3	1. 800		NS
B 16	3	6.66	3	5	11.10	6			NS
B 17	1	2.22	1	1	2.22	1			NS
B 18	4	8.88	5	9	19.98	11	2.248		NS
B 21	9	19.98	11	1	2.22	1	7. 200		<0.05
B 27	5	11.10	6	3	6.66	3			NS
B 35	12	26.6	14	8	17.76	9			NS
Bw 41	1	2.22	1	-	-	0			NS
Bw 47	1	2.22	1	-	-	0			NS
Bw 53	1	2.22	1	-	-	0			NS
Bw 4	28	62.2	39	29	64.4	40		0. 096	NS
Bw 6	31	68.8	44	36	80	55		1. 518	NS

NS: Not significant, GF: Gene frequency

Table 4. The frequencies of HLA-C locus antigens in the patients with exfoliative syndrome (n=45) and in the control group (n=45)

Types of the HLA-A antigens	Study Group			Control Group			P corrected	P Exact
	n	%	% GF	n	%	% GF		
Cw 1	1	2.22	1	1	2.22	1		NS
Cw 2	5	11.10	6	2	4.44	2		NS
Cw 3	4	8.88	5	3	6.66	3		NS
Cw 4	10	22.2	12	6	13.32	7	1.292	NS
Cw 5	-	-	0	2	4.44	2		NS
Cw 6	4	8.88	5	4	8.88	5		NS
Cw 7	4	8.88	5	5	11.10	6		NS

NS: Not significant, GF: Gene frequenc

Table 5. The frequencies of HLA-DR locus antigens in the patients with exfoliative syndrome (n=45) and in the control group (n=45)

Types of the HLA-A antigens	Study Group			Control Group			X^2	P corrected	P Exact
	n	%	% GF	n	%	% GF			
DR 1	2	4.44	2	3	6.66	3			NS
DR 2	10	22.2	12	4	8.88	5		3.045	NS
DR 3	7	15.54	8	11	24.44	13		1.181	NS
DR 4	16	35.55	20	14	31.11	17		0.250	NS
DR 5	23	51.5	30	8	17.76	9		11.072	< 0.05
DR 6	7	15.54	8	6	13.32	7		0.090	NS
DR 7	14	31.11	17	5	11.10	6		5.404	< 0.05
DRw 8	1	2.22	1	-	-	0			NS
DRw9	1	2.22	1	-	-	0			NS
DRw10	2	4.44	2	1	2.22	1			NS
DR w 51	2	4.44	2	3	6.66	3			NS
DR w 52	29	64.4	40	32	71.1	46	0.509		NS
DRw 53	23	51.5	30	21	46.6	27	0.222		NS

NS: Not significant, GF: Gene frequency

Table 6. The frequencies of HLA-DQ locus antigens in the patients with exfoliative syndrome (n=45) and in the control group (n=45)

Types of the HLA-A antigens	Study Group			Control Group			X^2	P corrected	P Exact
	n	%	% GF	n	%	% GF			
DQw 1	25	33.30	33	24	53. 28	31	0.045		NS
DQw 2	8	17.76	7	15	33.30	18		2.862	NS
DQw 3	43	95.46	78	38	84.4	60	8.459		NS
DQw 4	1	2.22	1	-	-	0			NS

NS: Not significant, GF: Gene frequency

epinephrine HCl. Each patient enrolled in the study presented typical exfoliative material on the pupillary margin and/or on the lens surface.

HLA typing was performed on patients and control subjects by lymphocytotoxicity assay (15). Test plates were prepared by Behringwerke AG, Marburg, Ch.-B / lot No. 020505 V.

To determine the statistical significance of the observed difference in HLA status between the study and control populations, the X2, corrected Chi-square and

Table 7. Selected HLA-disease associations.

Disease	Antigen
Ancylosing spondylitis	B27
Anterior Uveitis	B27
Behçet's disease	B-51 (01)
Birdshot retinopathy	B 29
Sympathic ophthalmia	A11

control subjects are given in Table 2. We found a strong association between HLA-A2 and ES, and the Chi-square was 4.849 and $p<0.05$ However, the frequencies of HLA-A3 and HLA-A10 were higher in the control subjects than in the patients (P exacts <0.05).

Types of HLA-B antigens in the patients and in the control subjects are shown in Table 3. We found a strong association between HLA-B21 and ES, and the Chi-square was 7.200 and $p < 0.05$

The HLA-C antigens in the patients and in the control subjects are given in Table 4 . No statistically significant difference was found between the two groups.

Table 8. Linkage Disequilibriums (LD) of some two loci allele combinations of HLA-A2, HLA-B21, HLA-DR5 and HLA-DR7 antigens.

	Control Group			Study Group		
Antigens	%	%GF	LD x1000	%	%GF	L x1000
A2B21	1	1	6.8	11	6	3.9
A2DR5	3	3	27.2	40	23	77
A2DR7	2	2	18.0	20	11	23.3
B21DR5	1	1	9.1	2.2	1	-23
B21DR7	0	0	-0.6	13.3	7	51.3
DR5DR7	1	1	4.6	6.7	3	-21

GF: Gene frequency, LD: Linkage disequilibrium

Fisher's exact Chi-square tests were used.

Since the frequency of HLA-A2, HLA-DR5, HLA-DR7 and HLA-B21 antigens were high in patients with EM, we searched for a possible genetic coding between these antigens and the ES, calculating the linkage disequilibriums (LD) among antigens of high frequency (11, 12).

Results

The study group consisted of 29 males, 16 females and the mean ages (in years) of which were 54 ± 4.32 and 52 ± 5. 6, respectively. The control group consisted of 25 males and 20 females, the mean ages (in years) of which were 53 ± 4.88 and 51.9 ± 2.90, respectively (Table 1). All of the patients had senile cataract. Bilateral EM was present in 39 (85 %) patients, but none of them had capsular glaucoma.There were no statistically significant differences between the age and sex characteristics of the patients and the control subjects.

Types of the HLA-A antigens in the patients and in the

HLA-DR antigens in the patients and in the control subjects are given in Table 5 . HLA-DR 5 and HLA-DR 7 were statistically higher in the patients than in the control subjects (Chi-square= 11.072, 5.404, respectively and P cor < 0. 05).

Types of HLA-DQw antigens in the patients and in the control subjects are given in Table 6. No statistically significant difference was found between the two groups.

Linkage disequilibriums of HLA-A2, HLA-DR5, HLA-DR7 and HLA-B21 in the patients and in the control subjects are were given in Tables 8 and 9. Positive linkage disequilibriums were observed between A2-DR5, B21-DR7, A2-B21-DR7, and A2-DR7 (77, 51.3 , 40.5 and 23.3, respectively).

Discussion

The frequency of EM varies from one population to another (16-18). ES is seen more frequently in some

Table 9. Linkage Disequilibriums (LD) of some three and four loci allele combinations of HLA-A2, HLA-B21, HLA-DR5 and HLA-DR7 antigens.

	Control Group			Study Group		
Antigens	%	%GF	LD x1000	%	%GF	LD x1000
A2B21DR5	0	0	-0.03	1	1	-6.83
A2B21DR7	0	0	-0.02	4	5	40.5
A2DR5DR7	2	2	9.82	3	3	3.99
B21DR5DR7	0	0	-0.05	1	1	4.4
A2B21DR5 DR7	0	0	-0.001	0	0	-2.86

GF: Gene frequency, LD: Linkage disequilibrium

countries where the population is ethnically homogenous (17, 18). In Turkey, ES is seen in 7.2 %-12.8 % of the population, especially in people older than 50 years (8). The incidence was found to be 12.8 % in a clinical population of 654 patients over 40 years of age (19). In our clinic, the incidence of ES was found to be 25% in a clinical population of 630 patients who had cataract (14). Similar to the studies cited above Many more reports similar to those cited above have not presented on the frequency of EM in almost all countries throughout the world. We wondered why EM is seen in some people but not in others.

We have pointed out above that some associations have been reported between HLA antigen types and many presumed genetic and/or immune system disease, some of which associations are listed in Table 7 (12). For this reason , we want to research the effects of the HLA antigen types on the development of the EM.

Olivius and Polland (19) have investigated the relation between HLA types and capsular glaucoma cases in Sweden. In this study, HLA-Bw 35 was found more frequently in patients with capsular glaucoma than in control subjects. However, the researchers suggested that connection between this antigen and the disease was weak.

In another study, this one from Norway, HLA-B12 was found in a higher frequency in patients with capsular glaucoma from a homogenous population (20).

In a study from Ireland, HLA A1, A33, B8, B47, B51, B53, B57, B62, DR3, DR12 and DR 13 were found to be significantly more common in the pseudoexfoliation group. Of these antigens the strongest and the most statistically significant associations appear to be between HLA A 33, B 47, B 53 and DR 12 (21).

HLA-A2, HLA-DR5, HLA-DR7 and HLA-B21 were found in a higher frequency in patients with ES than in control subjects. Statistical analyses showed that this finding was significant. We thought that genetic coding might exist between these HLA types and ES. For this reason, linkage disequilibrium was calculated and given in Table 8 and 9. Positive linkage disequilibriums were found between A2DR5, B21DR7, A2 B21DR5 DR7 and A2DR7 (77, 51.3, 40.5 and 23.3, respectively). HLA phenotype and haplotype frequencies were determined in Antalya-Turkey, and HLA-A2B5, HLA-A1B8 and HLA-B8DR3 showed the highest positive LD (22). Our LD and HLA antigen types are not in concordance with other studies mentioned above.

Several hypotheses, including the four discussed below, have been proposed to explain HLA-disease associations:

1-HLA molocules are receptors of etiologic agents. Particular HLA molecules may act as receptors for etiologic agents such as viruses, toxins, or other foreign substances.

2-The antigen-binding groovs only of particular HLA molecules can accept the processed antigenic peptide fragment that is ultimately responsible for causing the disease.

3-T cell receptor determines disease predisposition. The T cell antigen receptor is actually responsible for disease predisposition, but since T cell recognition is restricted by an HLA molecule, an apparent association is seen between the disease and HLA.

4-Causative agents mimic HLA molecules. Disease-associated HLA antigen is immunologically similar to the causative agent for the disease (12).

In this study we sought evidence for a genetic influence in the pathogenesis of this disorder. A positive association with the HLA system lends support to the genetic inheritance theory, as there is either a direct susceptibility to the disease in the presence of a certain HLA antigen or a genetically determined factor which is linked to HLA antigen conferring individual disease susceptibility (19, 20, 23).

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