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Investigation of the HLA class I antigens in patients with primary spontaneous pneumothorax

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Aim: There is no report investigating the human leukocyte antigen system (HLA) class I alleles and haplotypes in the patients with primary spontaneous pneumothorax (PSP) without any familial history in the literature. We investigated the association of these alleles and haplotypes, and the occurrence of the PSP in Turkish patients.

Materials and methods: The study group consisted of 20 patients diagnosed as PSP (without any familial history), and 20 healthy volunteers as control group. All the participants were Turkish male nonsmokers. Their genomic DNAs were extracted from venous samples, and the HLA class I alleles and haplotypes were analysed.

Results: The HLA Bw4 allele was significantly increased in the study group (80% vs. 60%, P = 0.05). The frequencies of the HLA Cw7, and B18 alleles were higher in the study group (35% vs. 15%, and 20% vs. 0%, respectively, P > 0.05), and there was a high ratio of the Cw7 homozygotism (30% vs. 10%, P > 0.05).

Conclusion: HLA Bw4, B18, and Cw7 alleles may play a genetic role in the development of the nonfamilial PSP in the Turkish population, but further accumulation of the cases are necessary to clarify whether the HLA-typing can confirm the development of a nonfamilial PSP.

Key words: Antibody/antigen, Genes/polymorphism, Pneumothorax

Primer spontan pnömotoraks hastalarında HLA Sınıf I antijenlerinin incelenmesi

Amaç: Literatüde ailesel öyküsü bulunmayan primer spontan pnömotoraks (PSP) hastalarında HLA Sınıf I allel ve haplotiplerinin incelendiği bir çalışma mevcut değildir. Bu çalışmada, Türk halkında ilgili allel ve haplotiplerin PSP gelişimiyle ilişkisini inceledik.

Yöntem ve gereç: Ailesel PSP öyküsü olmayan 20 hasta çalışma grubunu, diğer 20 sağlıklı gönüllü kontrol grubunu oluşturdu. Tüm katılımcılar sigara kullanmayan erkeklerdi. Venöz kan örneklerinden genomik DNA çıkarıldı ve HLA Sınıf I allel ve haplotipleri analiz edildi.

Bulgular: Çalışma grubunda HLA Bw4 alleli anlamlı şekilde yüksekti (% 80 vs. % 60, P = 0,05). Aynı şekilde HLA Cw7 ve B18 allel frekansı yüksek saptandı (sırasıyla, % 35 vs. % 15 ve % 20 vs. % 0, P > 0,05) ve Cw7 homogizotluk oranında da artış mevcuttu (% 30 vs. % 10, P > 0,05).

Sonuç: HLA Bw4, B18 ve Cw7 allelleri Türk halkındaki ailesel olmayan PSP gelişiminde genetik role sahip olabilirler, fakat kesin bir ilişkilendirme yapabilmek için daha fazla sayıda vakanın incelenmesine gerek vardır.

Anahtar sözcükler: Antikor/antijen, genler/polimorfizm, pnömotoraks

Introduction

Primary spontaneous pneumothorax (PSP) usually occurs sporadically in healthy young adults, who mostly tend to be tall, to have an asthenic habitus, and to be smoker. The most common cause

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is the rupture of an apical subpleural bleb or bullae (1). Familial spontaneous pneumothorax (FSP) is a very rare disease, and the incidence is 2 to 10% of all cases of PSP (2).

The human leukocyte antigen system (HLA) is the name of the major histocompatibility complex, and the superlocus contains a large number of genes related to the immune system function in humans. These genes reside on chromosome 6, and encode cell-surface antigen presenting proteins and many other genes. Major HLA class I antigens (A, B, and C), which are the essential elements in immune function, present peptides from inside the cell, including viral peptides, if present. The diversity of the HLA antigens in human population is one aspect of the disease defense, and, as a result, the chance of two unrelated individuals having identical HLA molecules on all loci is very low (3, 4).

It has been proposed that there may be a relationship between the HLA, the alpha 1-antitrypsin phenotype deficiency, and the occurrence of FSP (5, 6). Although there exist several reports about the genetic background of the familial cases of the PSP, reviews of the medical literature reveal no report investigating the HLA class I antigens in the patients with PSP without any familial history. This study has been designed to investigate the association of the class I HLA antigens in Turkish people, and the occurrence of the PSP.

Materials and methods

Study Subjects

The study group included 20 Turkish male patients diagnosed as PSP (without any history of a FSP), and treated in the clinic of Thoracic Surgery from November 2002 to September 2003. None of the patients had marfanoid characteristics, and all of them were nonsmokers. Twenty Turkish nonsmoker male volunteers (without any known disease) were entered into the study as control group. The study was reviewed and approved by the instutitional review board, and informed consent was obtained from all the participants.

HLA typing

HLA analysis procedure was performed in the laboratory of the Department of Medical Biology and Genetics using polymerase chain reaction (PCR) analysis. A total of 40 samples of venous blood (3cc/person) were collected into tubes containing ethylenediaminetetraacetic acid. The genomic DNA was extracted by the use of the Promega Wizard Genomic DNA Extraction Kit (Promega, USA) according to manufacturer's instructions. The analysis of the HLA-A, -B, and -C alleles and haplotypes was performed by using In Vitro Diagnosticum Kit (Inno Train Diagnostic, Kronberg, Germany) according to manufacturer's instruction. A mixture of 1046.3 µl (624 µl distilled water, 312 µl PCR mixture, 8.3 µl amplitaq DNA polymerase, and 102 µl of 100 ng/µl DNA) was prepared. Then 10.8µl of the mixture was distributed into 96 tubes each containing specific low resolution-single strand primer. Amplification was performed in an Eppendorf Master Cycler Gradient PCR as follows: 10 cycles of 94°C for two minutes, 96°C for 15 seconds, 65°C for one minute, and then 20 cycles of 96°C for 15 seconds, 61°C for 50 seconds, and 72°C for 30 seconds. The products were resolved by electrophoresis in a 1.5% agarose gel at 120 V for 15 to 20 minutes. The gel products were investigated under the ultraviolet light, and the HLA antigens were detected according to the size of the products. The tested 70 HLA antigens were as follows: A1, A2, A3, A9, A11, A19, A23, A24, A25, A26, A28, A29, A30, A31, A32, A33, A34, A36, A43, A66, A68, A69, A74, A80, B7, B8, B13, B14, B18, B27, B35, B37, B38, B39, B41, B44, B49, B50, B51, B52, B53, B54, B55, B56, B57, B58, B60, B61, B62, B63, B64, B65, B70, B71, B72, B73, B76, B77, B78, Bw4, Bw6, Cw1, Cw2, Cw3, Cw4, Cw5, Cw6, Cw7, Cw8, Cw9, Cw10.

Statistical analysis

The distribution of HLA antigens in both groups was compared using chi-square test or Fisher's exact test, as appropriate. A P value of less than or equal to 0.05 was considered to be significant. There was no deviation from the Hardy-Weinberg law of equilibrium in the allele frequency distributions of the HLA-A, -B, and -C loci.

Results

The mean age of the patients in the study group was 28 ± 1 years, and that of the control group was 25 ± 4 years. The regional, environmental, and ethnic

characteristics of the patients in both groups were similar. The patients in the study group underwent a tube thoracostomy, but three (15%) patients required a thoracotomy due to prolonged air leak. None of the patients in the study group developed another morbidity, and there was no mortality.

The distribution of the HLA antigens in the groups was outlined in Table 1 and 2. The haplotype analysis indicated that the HLA Bw4 allele was significantly increased in the study group patients compared with controls (80% vs. 60%, P = 0.05). Although insignificant, the frequencies of the HLA Cw7, and

Table 1. The frequencies of the HLA-A, -B, and –C alleles in both groups

Alleles	Study	Control	Alleles	Study	Control	Alleles	Study	Control
A1	5 (25%)	4 (20%)	B8	2 (10%)	2 (10%)	CW1	4 (20%)	2 (10%)
A2	10 (50%)	12 (60%)	B7	1 (5%)	1 (5%)	CW2	2 (10%)	4 (20%)
A3	4 (20%)	6 (30%)	B13	1 (5%)	1 (5%)	CW3	2 (10%)	1 (5%)
A9	ND	ND	B14	ND	ND	CW4	8 (40%)	10 (50%)
A11	3 (15%)	4 (20%)	B15	ND	ND	CW5	ND	ND
A23	1 (5%)	ND	B17	ND	ND	CW6	5 (40%)	6 (35%)
A24	7 (35%)	8 (40%)	B18	4 (20%)	ND	CW7	7 (35%)	3 (15%)
A26	ND	ND	B27	1 (5%)	ND	CW8	ND	1 (5%)
A29	2 (10%)	ND	B35	7 (35%)	10 (50%)			
A28	ND	ND	B38	2 (10%)	1 (5%)			
A30	ND	ND	B37	ND	ND			
A31	ND	ND	B39	ND	ND			
A32	2 (10%)	ND	B40	ND	1 (5%)			
A33	1 (5%)	ND	B41	ND	ND			
A34	ND	ND	B44	1 (5%)	1 (5%)			
A35	ND	ND	B48	ND	ND			
A36	ND	1 (5%)	B49	ND	ND			
A66	ND	ND	B50	ND	ND			
A68	2 (10%)	1 (5%)	B51	9 (45%)	10 (50%)			
A69	ND	ND	B52	ND	ND			
A74	ND	ND	B53	ND	ND			
			B54	ND	ND			
			B55	ND	ND			
			B56	ND	ND			
			B57	ND	ND			
			B58	ND	ND			
			B61	2 (10%)	1 (5%)			
			B63	ND	ND			
			B64	ND	ND			
			B65	ND	ND			
			B70	ND	1 (5%)			
			B72	ND	ND			
			B73	ND	ND			
			B75	ND	ND			
			B78	ND	ND			
			Bw4[a]	16 (80%)	10 (60%)			
			Bw6	16 (80%)	19 (95%)			

[a]: P = 0.05, ND: Non Detected

Table 2. The distribution of the HLA class I antigens in the study group

Patient number	HLA class I antigens
1	A2, A23, B51, Bw4, Cw1
2	A2, A24, B27, B61, Bw4, Bw6, Cw2, Cw3
3	A1, A2, B51, Bw4, Cw4, Cw7
4	A3, A11, B35, B51, Bw4, Bw6, Cw4, Cw7
5	A3, A29, B8, B18, Bw4, Bw6, Cw7
6	A3, B51, Bw4, Bw6, Cw7
7	A2, A3, B38, Bw4, Bw6, Cw7
8	A1, A24, B51, Bw4, Cw7
9	A24, A32, B35, B38, Bw4, BW6, Cw4, Cw6
10	A2, A11, B8, B61, Bw6, Cw4
11	A1, A2, B18, B35, Bw6, Cw4, Cw6
12	A2, A11, B35, Bw6, Cw4
13	A24, B35, B55, Bw6, Cw1, Cw4
14	A32, A33, B13, Bw4, Bw6, Cw1, Cw2
15	A2, A29, B7, B35, Bw4, Bw6, Cw3
16	A1, A24, B35, B51, Bw4, Bw6, Cw4
17	A2, A68, B18, B51, BW4, Bw6, Cw7
18	A68, B13, B51, Bw4, Cw1, Cw6
19	A1, A24, B51, B18, Bw4, Bw6, Cw7
20	A3, A24, B8, B44, Bw4, Bw6, Cw4, Cw6

B18 alleles were also higher in the study group (35% vs. 15%, and 20% vs. 0%, respectively). Finally, in the study group, there was a Cw7 homozygotism in six patients (30%), compared to two patients (10%) in the control group, but the difference was insignificant.

Discussion

To date, several associations have been reported between certain HLA class I alleles and some diseases. Keleş et al. (7) reported that the frequencies of HLA A24, and HLA Cw12 alleles were significantly elevated in the patients with nasal polyposis. It has been known that HLA B27 increases the risk of developing ankylosing spondylitis (8). HLA A3 is a secondary risk factor for myasthenia gravis (9).

The HLA allele and haplotype frequencies in the Turkish population were investigated in a large study between 1980 and 1998 concerning 1095 living organ donors. According to that study, it was detected that HLA A1, A2, A3, A9, B5, B12, B35, and Cw4 alleles, and HLA A2-B5, A1-B35, and B35-Cw4 haplotypes were more common in the Turkish population (10). The frequencies of HLA A2, A3, A24, A26, B35, B44, and B51 alleles were higher in another study investigating 228 Turkish people (11). Here in the present study, the common alleles were HLA A1, A2, A3, A24, B35, B51, and Cw4.

FSP was first described by Faber in 1921 (12). Many studies suggested a relationship between the HLA haplotype A2B40 and the development of PSP (5, 13, 14), while a few did not find such a relationship (15-17). Sugiyama et al. (18) reported two brothers with PSP having both an HLA A2B15Cw3/A11-Bw55 haplotype. The frequency of an HLA A2B70 haplotype was 75% in the study of Yamada et al. (13).

To our knowledge, the current study is the first study investigating HLA Class I antigens in the cases with the PSP without any familial history. In this study it was found that the HLA Bw4 allele frequency was significantly higher in the patients with PSP ($P = 0.05$). Also, there was an increased ratio of HLA B18 and Cw7 alleles, and Cw7 homozygotism in those patients ($P > 0.05$).

HLA Class I antigen system is the most polymorphic genetic system in human (19). The linkage disequilibrium between the alleles and haplotypes can vary in every ethnical population, so every ethnic group has different gene frequencies and characteristic haplotype arrangements (20). Acorrding to the results of the current study we can conclude that HLA Bw4, B18, and Cw7 alleles may play a genetic role in the development of the PSP.

HLA typing may be helpful in determining a possible genetic link in nonfamilial cases of PSP. Our limited data on the HLA make up permit not exact but limited conclusions. Further accumulation of the cases and close observation will be necessary to clarify whether the HLA-typing can confirm the development of a nonfamilial PSP.

References

1. Fry WA, Paape K. Pneumothorax. In: Shields TW, LoCicero III J, Ponn RB, Rusch VW, editors. General Thoracic Surgery. 6th ed. Philadelphia: Lippincott Williams&Wilkins; 2005. p.794-805.
2. Mitsuo A, Hiroyuki M. Clinic of spontaneous pneumothorax. Jpn J Chest Dis 1980; 39: 102-110.
3. Klein J, Sato A. The HLA system. First of two parts. N Eng J Med 2000; 343: 702-709.
4. Klein J, Sato A. The HLA system. Second of two parts. N Eng J Med 2000; 343: 782-786.
5. Sharpe IK, Ahmad M, Braun W. Familial spontaneous penumothorax and HLA antigens. Chest 1980; 78: 264-268.
6. Abolnik IZ, Lossos IS, Zlotogora J, Brauer R. On the inheritence of primary spontaneous pneumothorax. Am J Med Genet 1991; 40: 155-158.
7. Keles B, Cora T, Acar H, Arbag H, Inan Z, Ozturk K et al. Evaluation of HLA-A, -B, -Cw, and –DRB1 alleles frequency in Turkish patients with nasal polyposis. Otolaryngol Head Neck Surg 2008; 139: 580-585.
8. Reveille JD. The genetic basis of ankylosing spondylitis. Curr Opin Rheumatol 2006; 18: 332-341.
9. Machens A, Löliger C, Pichlmeier U, Emskötter T, Busch C, Izbicki J. Correlation of thymic pathology with HLA in myasthenia gravis. Clin Immunol 1999; 91: 296-301.
10. Tokgöz G, Düzgün N, Turgay M, Kınıklı G, Duman M, Ölmez Ü et al. The distribution of HLA-A, B and C antigens in Turkish population. Turk J Med Res 1993; 11: 32-36.
11. Arnaiz-Villena A, Karin M, Bendikuze N, Gomez-Casado E, Moscoso J, Silvera C et al. HLA alleles and haplotypes in the Turkish population: relatedness to Kurds, Armenians and other Mediterraneans. Tissue Antigen 2001; 57: 308-317.
12. Faber EE. Spontaneous pneumothorax in two siblings. Hospitalstid 1921; 64: 573-574.
13. Yamada A, Takeda Y, Hayashi S, Shimizu K. Familial spontaneous pneumothorax in three generations and its HLA. Jpn J Thorac Cardiovasc Surg 2003; 51: 456-458.
14. Satoshi N, Yasumasa O, Naoki T, Tetsuya Y, Katsuhiro A. Case of spontaneous pneumotohorax in twins. Jpn J Chest Dis 1985; 44: 1040-1043.
15. Cheng YJ, Chou SH, Kao EL. Familial spontaneous pneumothorax- report of seven cases in two families. Gaoxiong Yi Xue Ke Xue Za Zhi 1992; 8: 390-394.
16. Lenler-Petersen P, Grunnet N, Jespersen TW, Jaeger P. Familial spontaneous pneumotohrax. Eur Respir J 1990; 3: 342-345.
17. Morrison PJ, Lowry RC, Nevin NC. Familial primary spontaneous pneumothorax consistent with true autosomal dominant inheritence. Thorax 1998; 53: 151-152.
18. Sugiyama Y, Maeda H, Yotsumoto H, Takaku F. Familial spontaneous pneumothorax. Thorax 1986; 41: 969-970.
19. Joint Reports. In: Albert ED, Baur M, Mayr W, editors. Histocompatibility testing. Cophenhagen: Munksgaard, 1984. p.118-209.
20. Bodmer W, Thomson G. Population genetics and evaluation of the HLA system. In: Dausset J, Svejgaard A, editors. HLA and disease. Cophenhagen: Munksgaard, 1977. p.280-292.