

## THE RELATIONSHIP IN BETWEEN HETEROTOPIC OSSIFICATION AND HLA TYPES IN PATIENTS WITH SPINAL CORD INJURY

### SPİNAL KORD YARALANMALI HASTALARDA HETEROTOPİK OSSİFİKASYON İLE HLA TİPLERİ ARASINDAKİ İLİŞKİ

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#### ABSTRACT

**Aim:** This study was planned in order to answer the question whether the development of heterotopic ossification (HO) following spinal cord injury (SCI) can be estimated by human leukocyte antigens (HLA).

**Methods:** Major Histocompatibility Complex (MHC) class I molecules of heterotopic ossification patients with SCI (20 patients) was compared with 20 healthy controls. The typing of the histocompatibility antigens was performed using the microcytotoxicity test.

**Results:** The HLA A2 locus was present in 11 patients (36.7%), while this ratio was 28.9% in the control group. While HLA A28 locus was present in 4 patients (13.3%), this ratio was 2.6% in the control group. The presence of HLA B18 locus in the patient group was less than the ratio seen in the control group. When these HLA types were analyzed in the study groups, there was no statistically significant difference in between these groups.

**Conclusion:** It was concluded that MHC class I molecules were not predictive of HO development in patients with SCI.

**Key words:** Heterotopic ossification, HLA antigens, MHC molecules, spinal cord injury.

#### ÖZET

**Amaç:** Bu çalışma, spinal kord yaralanması sonrasında gelişen heterotopik ossifikasyonun (HO), insan lökosit antijenleri (human leukocyte antigens (HLA)) ile tahmin edilip edilemeyeceği sorusuna yanıt bulmak için planlanmıştır.

**Metod:** Spinal kord yaralanması ve heterotopik ossifikasyonu olan 20 hastanın "Major Histocompatibility Complex (MHC)" sınıf I molekülleri, 20 sağlıklı kontrollerinki ile karşılaştırılmıştır. Histokompatibilite antijenlerinin tiplendirilmesi, mikrositotoksitesite testi kullanılarak yapılmıştır.

**Bulgular:** HLA A2 gen bölgesi kontrol grubunda %28.9 oranında gözlenirken, hasta grubunda 11 hastada (%36.7) saptanmıştır. HLA 28 gen bölgesi 4 hastada (%13.3) saptanırken, bu oran kontrol grubunda %2.6 olarak bulunmuştur. HLA B18 gen bölgesinin varlığı, kontrol grubunda saptanandan daha az olarak gözlenmiştir. Bu HLA tipleri çalışma gruplarında incelendiğinde, gruplar arasında istatistiksel önemli bir farklılık bulunamamıştır.

**Sonuç:** Spinal kord yaralanmalı hastalarda MHC sınıf I moleküllerinin HO gelişiminde prediktif değerinin olmadığı sonucuna varılmıştır.

**Anahtar kelimeler:** Heterotopik ossifikasyon, HLA antigens, MHC molecules, spinal cord injury.

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**INTRODUCTION**

Reidel first defined heterotopic ossification (HO) in 1883 and after him Dejerne and Cellier reported HO in soldiers with spinal cord injury during the 1st World War (1918) (1). The incidence of HO following SCI was 1653% (24). It is also named as paraarticular ossification or paraosteoarthropathy (POA) (5). HO can be defined as bone formation within the soft tissue (1). In clinical practice, HO is observed at the joints distal to the level of spinal injury, and in the early phase there is swelling and erythema at the joint and the range of motion is limited. It should be differentiated from cellulites, osteomyelitis, and thrombophlebitis (1,3). In the late phase, it could be debilitating depending on the limitation of the range of motion (2,6). Although the etiopathogenesis of HO is not yet completely understood, humoral, neural, and local factors have all been suggested to play a role in the pathophysiology. A reduction in tissue oxygenation and some unknown factors inducing the transformation into chondroblasts and osteoblasts in the multipotent connective tissue has been proposed (14,68). Genetic factors are rarely mentioned in the development of HO (3,9). Ossification of the posterior longitudinal ligament (OPLL) in the spine is characterized by heterotopic bone formation in spinal ligaments (10). In Japanese population, HO of the posterior longitudinal ligament (PLL) or in other words, the new bone formation in the spinal ligaments is shown to be related with the genetic locus around the HLA area on the short arm of the 6th chromosome (6p) (10,11). The prevalence of HLA B 18 (3,5,9,12), HLA B 27 (3,5,9,13), and HLA Dw7 (5,9) antigens is reported to increase in the patients with HO when compared with normal cases. The HLA B 27 allele is suggested to play a role in the new bone formation (3,13,14). Apart from the studies mentioned above, other studies performed don't confirm these findings (3,9,15). This study was planned to answer the question whether Major Histocompatibility Complex (MHC) class I molecules in patients following spinal cord injury can predict the development of HO.

**METHODS**

Twenty patients, who developed HO following spinal cord injury, were screened for MHC class I molecules. The presence of HO was confirmed in all patients with radiological methods. Five patients were women. The mean age of all patients was 31 years (range 18-53 years). The histocompatibility antigens typing of the patients were performed at the Tissue Typing Laboratory of the Yüksek İhtisas Hospital, Ankara, Turkey, with the microdroplet lymphocyte toxicity test (16). Every sample was investigated for the 68 separate locus within the MHC class I A, B, C

antigens system. This system of the patients with HO and 20 healthy controls were compared. The relative risk (RR), etiologic fraction method (EF), chi square test and Fisher's exact test (FET) were used in the statistical analyses (17,18). The homozygoteness of all antigens was not determined, as there was no family history and the missing values were extracted from the study. The  $p < 0.05$  was accepted as statistically significant.

**RESULTS**

The HLA A 9, HLA A10, HLA B 5, HLA B 14, HLA B27, HLA B 38, HLA B 52, and HLA C2 loci were present in 6 patients (20.0%), 3 patients (10.0%), 3 patients (8.8%), 3 patients, 2 patients (5.9%), 2 patients (5.9%), 2 patients (5.9%) and 3 patients (15%), respectively. These antigens were not observed in the control group (Table 1). HLA A2 locus was present in 11

**Table-1**  
The frequencies of MHC class I antigens in 20 healthy controls and in 20 patients with heterotopic ossification following spinal injury.

CONTROLS			PATIENTS		
HLA	n	%	HLA	n	%
A1	4	10.5	A1	4	13.3
A2	11	28.9	A2	11	36.7
A3	5	13.2	A9	6	20.0
A11	5	13.2	A10	3	10.0
A24	7	18.4	A28	4	13.3
A26	1	2.6	A29	1	3.3
A28	1	2.6			
A29	1	2.6			
A30	2	5.3			
A32	1	2.6			
B7	1	2.6	B5	3	8.8
B8	1	2.6	B7	3	8.8
B13	2	5.3	B8	1	2.9
B18	3	7.9	B13	1	2.9
B35	8	21.1	B14	3	8.8
B39	2	5.3	B18	2	5.9
B41	1	2.6	B27	2	5.9
B44	4	10.5	B35	2	5.9
B49	6	15.8	B38	2	5.9
B50	1	2.6	B39	1	2.9
B51	3	7.9	B42	1	2.9
B55	1	2.6	B44	4	11.8
B57	2	5.3	B51	4	11.8
B60	1	2.6	B52	2	5.9
B63	2	5.3	B57	2	5.9
C1	1	4.5	C2	3	15
C3	1	4.5	C3	2	10
C4	8	36.4	C4	4	20
C5	1	4.5	C5	2	10
C6	5	22.7	C6	1	5
C7	6	27.3	C7	5	25
			C8	1	5

**Tablo-II**

The comparisons of MHC class I antigens in between the group of patients with heterotopic ossification and the control group.

ANTIGEN	hp	RR	EF	p
A1	0.13	1.31	0.03	0.724*
A2	0.37	1.42	0.11	0.499**
A28	0.13	5.69	0.11	0.162*
A29	0.03	1.28	0.01	1.000*
B7	0.09	3.58	0.06	0.338*
B8	0.03	1.12	0.00	1.000*
B13	0.03	0.55	-0.02	1.000*
B18	0.06	0.73	-0.02	1.000*
B35	0.06	0.23	-0.19	0.090*
B39	0.03	0.55	-0.02	1.000*
B44	0.12	1.13	0.01	1.000*
B51	0.12	1.56	0.04	0.700*
B57	0.06	1.13	0.01	1.000*
C3	0.10	2.33	0.06	0.598*
C4	0.20	0.44	-0.26	0.241**
C5	0.10	2.33	0.06	0.598*
C6	0.05	0.18	-0.23	0.187*
C7	0.25	0.89	-0.03	0.867**

hp: the frequency of antigens in patients, RR: relative risk, EF: etiologic fraction

\* results of Fisher's exact test

\*\* results of chi square test

patients (36.7%). The anticipated phenotypic ratio of this locus in our control group was 28.9%. The frequency of HLA A2 locus in our patients was not statistically significant when compared with our control group when analyzed with the chi square test and  $p < 0.05$  was accepted as statistically significant. Although the anticipated incidence was 2.6% in the control group, HLA A 28 locus was present in 4 patients (13.3%). The HLA B 7 locus was present in 3 patients (8.8%) while its anticipated incidence was 2.6%. HLA B 51 locus was present in 4 patients (11.8%) and B 18 locus was present in 2 patients (5.9%) while the anticipated incidences for both antigens were 7.9%. When the presence of this antigen was analyzed with

**Tablo-III**

Eğitim düzeyi, sigara kullanımı, egzersiz alışkanlığı, periferik eklem, kalça ve eklem dışı tutulumuna göre ayrılan grupların BASDAI ve BASFI değerlerinin beş yıllık değişimlerinin karşılaştırılması

	BASDAI değişimi p	BASFI değişimi p
Eğitim düzeyi	0.677	0.988
Sigara kullanımı	1.0	0.243
Egzersiz alışkanlığı	0.668	0.769
Periferik eklem tutulumu	0.922	0.932
Kalça tutulumu	0.797	0.645
Eklem dışı tutulum	0.614	0.166

Fisher's exact test within the groups, there was no statistically significant difference ( $p > 0.05$ ) (Table 2).

## DISCUSSION

The gene region encoding the tissue histocompatibility antigens (the Major Histocompatibility ComplexMHC) is on the short arm of the 6th chromosome and is responsible from an important part of the cell proliferation. This special region consists of important clinical information, which can affect certain diseases. The equivalence of the MHC complex in men is identified as human leukocyte antigens (HLA) and the gene region is defined as the HLA locus (3,19). The human leukocyte antigen system (HLA system) consists of a series of glycoprotein molecules present on cell surfaces. The estimation of patients who are under increased risk for the development of HO by the HLA system focused interest by means of the preventive treatment (7).

Minaire et al (12), investigated 11 antigens of the HLA A locus and 15 antigens on the HLA B locus and reported that the levels of the HLA B 18 antigen were increased in 43 patients with spinal cord injury and in 23 patients with head trauma and these 66 patients had paraosteoarthropathy. The rate of HLA B 18 was 25.7% in the patient group while it was 7.6% in the control group. In this study, it was stated that the presence of HLA B 18 could have a relationship with the increase in the risk of paraosteoarthropathy development. Larson and colleagues (13) investigated only the HLA B 27 antigen in 21 SCI patients with HO developed following spinal cord injury and found that its incidence was 0% (22 cases) in the control group, while this number was 24% in the patient group. Thus, they postulated that the HLA B 27 allele had a relationship with heterotopic bone formation in patients with posttraumatic spinal cord injury. Calvert et al (15) screened 23 patients with a genetic disorder named as fibroplasia ossificans progressiva for the HLA B 27 allele. In this study, the presence of the HLA B 27 allele showed no difference in the fibroplasia ossificans progressiva group when compared with the general population. Hunter et al (20), demonstrated that there was no statistically significant increase in the frequency of 30 different HLA A and B antigens in 21 patients with HO following spinal cord injury and HO following head trauma when compared with the normal population. Weiss et al (21) investigated 20 different HLA A and B antigens in their group consisting of 12 patients with HO fol-

lowing head trauma and 8 patients with HO following spinal injury. They didn't find any statistically significant increases. In the study of Garland et al (7), the frequency of a total of 66 antigens including the HLA B 18 and HLA B 27 was investigated in a total of 30 patients, 20 with spinal cord injury and 10 with head trauma and no increase was reported. In the same study, although the frequency of the HLA A2 antigen was high in the patient group when compared with the normal population, this rise was not statistically significant.

The results of our study support the findings of Weiss, Garland, and Hunter. There was no statistically significant increase in the frequency of the MHC class I antigens investigated. Although the frequency of the HLA A2, HLA A28, HLA B7, and HLA B51 was significant in the patient group when compared with controls, this rise was not statistically significant. When compared with controls, unlike the findings of Minaire et al, the frequency of HLA B 18 locus was less; this was present only in a few patients. While A9, A10, B5, B14, B27, B38, B52, and C2 antigens were present in our patients with varying levels, these antigens were not observed at the control group.

## CONCLUSION

Depending on this study, we conclude that the MHC class I antigens system is not predictive of HO in the patients with spinal cord injury. As a result, starting specific treatment on the basis of HLA system is not convenient. In order to explain the effect of the MHC class I molecules on the risk of HO development in patients with spinal cord injury, controlled studies performed on large patient groups are needed.

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