A Preliminary Research on HLA-Association of Lumbar Spinal Stenosis

Lomber Spinal Stenozun HLA İlişkisi Üzerine Ön Araştırma

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ÖZ
Amaç: Biz bu çalışmada lumbar spinal stenosis (LSS) ile genetik HLA sistemi arasındaki ilişkiyi tespit etmeye amaçladık.

Materiyl ve Metod: Bu çalışmada LSS tanısi konmuş 41 olgudan (7 erkek, 8 kadın; yaş ortalaması: 60,27) 15inde HLA sınıf I ABC genlerini ve 41 olgunun tamamında (19 erkek, 22 kadın; yaş ortalaması: 60,46) HLA sınıf II DRDQB1 genlerini çalıştık. DNA ekstraksiyonundan sonra HLA A, B, C, DQ ve DR ye özgü primerlerle DNA amplifiye edildi ve SSP-PCR yöntemiyle çalışıldı. Allel tanımlamaları, resmi HLA Nomenclature Komitesine ait software ile yapıldı.

Bulgular: LSS grubunda HLA A2 allel (% 36,7) kontrol grubu (%17,2) göre istatistiksel anlamıyla yüksekti (p:0,039; p<0,05). B44 allelinin görülme sıklığı (%20), kontrol grubundan (%5,1) istatistiksel olarak anlamıyla tespit edildi. Ayrıca şekilde C6 allelinin görülme sıklığı (%26,7), kontrol grubundan (%8,6) istatistiksel anlamıyla yüksekti. (p:0,013; p<0,05).

Sonuç: Bu ön çalışma LSS ile HLA sınıf I genlerinin ilişkili olduğunu ortaya koymıştır. HLA A2, HLA B44 ve HLA C6 allelinin LSS için predispozan faktörlar olarak tespit edilmiştir.

Anahtar kelimeler: Human lókosit antijen, lomber spinal stenosis, sınıf I/II genler

ABSTRACT
Objective: We aim to identify the relationship lumbar spinal stenosis (LSS) and genetic HLA system.

Materials and Methods: The present study investigates 15 cases out of 41 (7 male, 8 female; average age: 60.27) who were studied for HLA class I ABC genes, as well as totally 41 cases (19 male; 22 female; average age: 60.46) who were studied for HLA class II DR.D QB1 genes among the cases with LSS. DNA extraction, the DNA was amplified with primers specific to HLA-A, B, C, DQ and DR, and studied using the SSP-PCR method. Allele identification was made using the official HLA Nomenclature Committee software.

Results: HLA A2 allele in LSS group (%36.7) is statistically significantly higher than control group (%17.2) (p:0.039; p<0.05). The frequency of the B44 allele was statistically significantly higher in the LSS group (20%) than in the control group (5.1%) (p:0.01; p<0.05). Likewise, the frequency of the C6 allele was statistically significantly higher in the LSS group (26.7%) than in the control group (8.6%) (p:0.013; p<0.05).

Conclusion: This preliminary study has suggested that LSS is associated with HLA class I genes. HLA A2, HLA B44, and HLA C6 alleles have been determined as predisposing factors for LSS.

Keywords: Class I/II genes, human leukocyte antigen, lumbar spinal stenosis

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INTRODUCTION

Lumbar Spinal Stenosis (LSS) is a common condition involving the spine that starts with the narrowing of the spinal canal and manifests in lower extremity pain caused by inflammation. There are ongoing studies seeking to identify the pathophysiology of this condition. It is known about the Major Histocompatibility Complex (MHC), a large gene complex, with an important role in the immune system. The MHC-encoded glycoprotein molecule in human is called as human leukocyte antigen (HLA) which is involved in antigen presentation of short peptides to T cells and play a key role in the body’s immune defence. HLA-disease association being an interesting subject over the recent several decades since long list of human diseases that are significantly more common among individuals that carry particular HLA alleles in Population studies. The main inspiration for the present study is Ankylosing Spondylitis (AS) – a disease that affects the spine. Previous studies have identified the presence of the HLA-B27 gene in 96% of AS patients. The role of HLA-B27, as the most well-known genetic predisposition factor for AS, in the pathogenesis is not exactly known, although it has been assumed that an arthritogenic peptide specific to the disease is presented to the immune system. On the other hand, several studies of Behçet’s disease (BD) to date have reported the HLA-B51 allele as the most potent genetic predisposition factor for BD. HLA-DQB1*0602 is associated with narcolepsy, HLA-DRB1 alleles (e.g.DRB1*04:01, DRB1*04:04, DRB1*04:05, DRB1*01:01) that code for a sequence motif in the DRB chain called ‘shared epitope’ (SE) are associated with seropositive rheumatoid arthritis (RA). Although many hypotheses have been postulated, the mechanism underlying HLA-disease association is still unclear unfortunately. In Type 1 Diabetes Mellitus (T1DM), as another HLA-related disease, the predisposition genes have been identified as HLA “DR3/DR4”. The protective alleles countering the disease have in turn been identified as A*11:01, A*32:01, A*66:01, B*07:02, B*44:03, B*35:02, C*16:01 and C*04:01. Studies of genetic factors have also established a significant association between several single-nucleotide polymorphisms (SNPs) and LSS in an Indian population. A previous study of a Han Chinese sample in turn identified an association between the COL11A1 (rs1337185) and ADAMTS5 (rs162509) gene polymorphisms with a predisposition to LSS.

In this study, we aimed to analyse the association of HLA alleles with LSS patients and continue with larger series if results are encouraging. Also, we aimed to analyze the association of HLA alleles with LSS patients as a preliminary report.

MATERIALS AND METHODS

Study Design and Data Collection: The project was approved by the Clinical Research Ethics Committee of the Haydarpaşa Numune Training and Research Hospital (TRH) (Date: 14/02/2019, decision no: HNEAH KAEK 2019/16) and informed consent forms were obtained from all patients. In order to make allele identification in the HLA region of the Lumbar Spinal Stenosis cases, the study included patients diagnosed clinically and radiologically in the Neurosurgery Department of the Istanbul Haydarpaşa Numune TRH. The patients did not have any inflammatory comorbidities with spine disorder and did not have any accompanying autoimmune disease. Romatoid factor (RF) was negative in all except one patient and HLA B27 and 51 was negative in all of the patients. In detailed history, there was not any familial history and patients were not doing routine exercise. The symptoms of patients were long-term back pain, leg pain, intermittent neurogenic claudicasion and/or motor and sensory deficits. Exclusion criteria were acute lumbosyatalgia, discal hernia without spinal stenosis in radiologic imaging. The patients, who were selected from the same geographical region, included 15 cases out of 41 studied for HLA class I ABC genes (7 male, 8 female; average age: 60.27 years), and totally 41 cases studied for HLA class II DR/DQB1 genes (19 male, 22 female; average age: 60.46 years). The number of these two groups will be equal in larger series if results are encouraging in the present study. The control group included 58 healthy individuals. Exclusion criteria for control group were any kind of spinal disorder and inflammatory disease. Inclusion criteria for control group was among donors of renal transplantation over 50 years old which were routinely examined for HLA genes. The median age of patient and control group was comparable.

DNA Isolation: DNA was isolated from peripheral blood samples with EDTA using a manual kit, as per the manufacturer’s instructions (Quick-DNA tm miniprep plus kit (made in USA), Zymo Research). For amplification, 30 polymerase chain reaction (PCR) cycles were followed by 20 further cycles in a thermal cycler (Techne Flexigene version 32.02, Oxford,Cambridge, UK). Denaturation, annealing
and extension stages were included when the PCR products were separated on a 2.5% agarose.

**PCR-SSP (Sequence Specific Primer):** The presence of specific-locus amplification was analyzed using allele-specific primers using the PCR-SSP method. Low-resolution tissue typing was carried out using Micro SSP kits HLA ABC, HLA DRQ and DRB1, as per the manufacturer’s instructions. The kits used were Generic HLA class I DNA Typing Tray, Lot 011; Generic HLA class II DNA Typing Tray, DRB only, Lot 004;and Generic HLA class II DNA Typing Tray, DQB1 only, Lot 001 (One Lambda, Canoga Park, CA, USA). A PCR analysis was carried out to study A (22 alleles), B (36 alleles), C (14 alleles), DR (12 alleles) and DQ (5 alleles). Allele identification was made using the official HLA Nomenclature Committee software (HLA Fusion version 4.1.0.13925, One Lambda).

**Statistical Analysis:** IBM SPSS Statistics 22 (IBM SPSS, Turkey) software was used for the statistical analyses of the study data. The study data was assessed using a Chi-Square test, a Fisher’s Exact Chi-Square test and a Continuity (Yates) Correction. A p value of <0.05 was considered statistically significant.

**RESULTS**

Tables 1, 2, 3, 4 and 5 present the findings of our study for the HLA Class I (A, B, C) and Class II (DR, DQ) alleles comparing among the LSS patients and control groups to analyse the association of HLA and LSS. Table 1 shows reveals no statistically significant difference in the frequency of the A1, A3, A11, A24, A25, A26, A29, A30, A32 and A68 alleles between the control and LSS groups (p>0.05). HLA A2 allele in LSS group (%36.7) is statistically significantly higher than control group (%17.2) (p<0.05).

Table 2 shows that the frequency of B44 allele is statistically significantly higher in the LSS group (20%) than in the control group (5.2%) (p<0.05). No statistically significant difference was noted in the frequency of the B7, B8, B14, B18, B27, B35, B38, B50, B51 and B58 alleles between control and LSS groups (p>0.05).

Table 3 shows that the frequency of the C6 allele is statistically significantly higher in the LSS group (26.7%) than in the control group (8.6%) (p<0.05). There is no statistically significant difference in the frequency of the C1, C2, C4, C7, C8, C14, C15 and C16 alleles between the control and LSS groups (p>0.05). Table 4 shows that there is no statistically significant difference in the frequency of the DR1, DR3, DR4, DR7, DR11, DR12, DR13, DR14, DR15 ve DR16 alleles between the control and LSS groups (p>0.05).

Table 5 shows that there is no statistically significant difference in the frequency of the DQ2, DQ3, DQ5 and DQ6 alleles between the control and LSS groups (p>0.05).

**DISCUSSION AND CONCLUSION**

Degenerative LSS was formerly believed to be a mechanical condition related to old age however several genetic analyses such as tryptophan alleles in COL9A2 and COL9A3 and polymorphisms in the vitamin D receptor gene (VDR) (FokI and TaqI) have been performed to understand the LSS pathogenesis in the Finnish and in the Japanese population in recent years.

In the present study we investigate the association between the HLA Class I (A,B,C) and Class II ( DR, DQ) genes and predisposition to LSS in Turkish patients. Since congenital spinal stenosis is associated with chondrodysplasia, the degenerative type of LSS which occurs in the elderly is also believed to be caused by genetic factors. Hallioğlu et al. conducted a study of Turkish children with rheumatic fever, and reported the HLA DQA1*03 allele to be a protective factor that may be even more potent when presenting with the DRB1*04 and DQA1*03 alleles. Hallioğlu et al. and Atasoy et al. noted an increase in the HLA-A*30 and A*68, B*7, B*13, B*57, Cw6 and DRB1*07 antigens of psoriatic patients when compared to controls. Moreover, the B*57, Cw6 and DRB1*07 alleles were found to be more significant in Type I psoriasis, whereas HLA-B*13 displayed a significant association in Type 2 psoriasis.

In a study by Doganay et al. on “Non-alcoholic fatty liver disease”, the authors identified HLA DQB1*06:04 allele as the predisposition gene and DQB1*03:02 as the protective gene. Tunca et al. established the HLA DRB1*04 and DRB1*14 alleles and the HLA DRB1*04/DQB1*03 and HLA DRB1*14/DQB1*05 haplotypes as the genetic predisposition markers for pemphigus vulgaris in a Turkish population, but the authors could not identi-
fy a protective allele for PV.14 Zhang et al reported the expression of Platelet-Derived Growth Factor (PDGF-BB) in LSS.15 Jirathathanhornkul et al. suggested that Vascular Endothelial Growth Factor (VEGF) may play a role in the pathogenesis of LSS.16 These studies suggest that the association of HLA with diseases is an area warranting further research in terms of both facilitating the understanding of the disease and identifying a means of prevention. It is known that the HLA tissue groups may cause predispositions to certain clinical conditions affecting the spine, such as ankylosing spondylitis, and may render individuals more likely to contract the disease depending on which allele is present. We previously ascertained from the familial history of our patients that considerable numbers of family members had similar complaints. This led us to analyze the HLA tissue groups in the patients who presented at our Hospital who were diagnosed with LSS. Based on the findings of our study, we established a statistically significant association between the LSS cases and the HLA A2, HLA B44 and HLA C6 alleles. Apparently HLA class I gene is responsible for LSS pathogenesis leading to cytotoxic T cell response. One case with romatoid factor positive is unrelated to our results since this case were not analysed for class I HLA.

**Conclusion:** HLA A2, HLA B44 and HLA C6 alleles is a genetic correlation for LSS not the cause of LSS. We recommend a re-study of the alleles identified in the present study as predisposition genes to LSS involving a larger case series and using more advanced technologies. This study has some limitations. This was a “cross-sectional case-control” study, and was not intended to establish a direct cause of disease. The disease-HLA association should be confirmed through high-resolution sequencing studies with further techniques applied. Furthermore, the number of cases was low and unequal between the groups in the present study.

**Ethics Committee Approval:** Our study was approved by the Clinical Research Ethics Committee of the Haydarpaşa Numune Training and Research Hospital (Date:14/02/2019, decision no: HNEAH KAEK 2019/16).

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Author Contributions:** Concept – GI, ME, RB; Supervision – GI, EA, RB; Materials – EA, EEK; Data Collection and/or Processing – EA, EEK, ME; Analysis and/ or Interpretation – GI, ME, RB, EEK; Writing -GI.

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### Table 1. HLA A comparison between LSS and control groups.

| HLA A | Control (n=116) | LSS (n=30) | P     |
|-------|----------------|------------|-------|
|       | n   | %     | n   | %   |       |
| A1    | 19  | 16.4 | 2   | 6.7 | 0.247 |
| A2    | 20  | 17.2 | 11  | 36.7| +0.039* |
| A3    | 16  | 13.8 | 4   | 13.3| 1.000 |
| A11   | 9   | 7.8  | 4   | 13.3| 0.469 |
| A24   | 20  | 17.2 | 2   | 6.7 | 0.250 |
| A25   | 2   | 1.7  | 1   | 3.3 | 0.501 |
| A26   | 8   | 6.9  | 1   | 3.3 | 0.686 |
| A29   | 6   | 5.2  | 1   | 3.3 | 1.000 |
| A30   | 4   | 3.4  | 1   | 3.3 | 1.000 |
| A32   | 4   | 3.4  | 1   | 3.3 | 1.000 |
| A68   | 1   | 0.9  | 2   | 6.7 | 0.107 |

*p<0.05, Fisher’s Exact Test; + Continuity (Yates) correction.
Table 2. HLA B comparison between LSS and control groups.

|       | Control (n=116) | LSS (n=30) | P     |
|-------|----------------|------------|-------|
| HLA B | n   | %       | n   | %   |     |
| B7    | 8   | 6.9     | 3   | 10  | 0.697|
| B8    | 5   | 4.3     | 1   | 3.3 | 1.000|
| B14   | 4   | 3.4     | 1   | 3.3 | 1.000|
| B18   | 5   | 4.3     | 2   | 6.7 | 0.633|
| B27   | 2   | 1.7     | 2   | 6.7 | 0.187|
| B35   | 24  | 20.7    | 4   | 13.3| +0.514|
| B38   | 2   | 1.7     | 3   | 10  | 0.059|
| B44   | 6   | 5.2     | 6   | 20  | 0.017*|
| B50   | 3   | 2.6     | 1   | 3.3 | 1.000|
| B51   | 17  | 14.7    | 4   | 13.3| 1.000|
| B58   | 1   | 0.9     | 2   | 6.7 | 0.107|

*p<0.05, Fisher’s Exact Test; +: Continuity (Yates) correction.
| HLA C | Control (n=116) | LSS (n=30) | P     |
|-------|----------------|------------|-------|
|       | n  | %   | n  | %   |       |
| C1    | 5  | 4.3 | 1  | 3.3 | 1.000 |
| C2    | 5  | 4.3 | 3  | 10  | 0.362 |
| C4    | 20 | 17.2| 6  | 20  | +0.933|
| C6    | 10 | 8.6 | 8  | 26.7| **0.013*** |
| C7    | 17 | 14.7| 6  | 20  | 0.574 |
| C8    | 3  | 2.6 | 1  | 3.3 | 1.000 |
| C14   | 5  | 4.3 | 1  | 3.3 | 1.000 |
| C15   | 15 | 12.9| 2  | 6.7 | 0.525 |
| C16   | 7  | 6   | 2  | 6.7 | 1.000 |

*p<0.05, Fisher’s Exact Test; +: Continuity (Yates) correction.
Table 4. HLA DR comparison between LSS and control groups.

| HLA DR | Control (n=116) | LSS (n=82) | P     |
|--------|----------------|------------|-------|
|        | n   | %  | N   | %   |       |
| DR1    | 7   | 6.0| 6   | 7.3 | 0.946 |
| DR3    | 15  | 12.9| 4  | 4.9 | 0.099 |
| DR4    | 13  | 11.2| 10  | 12.2 | 1.000 |
| DR7    | 10  | 8.6 | 8  | 9.8 | 0.982 |
| DR11   | 25  | 21.6| 21  | 25.6 | ++0.505 |
| DR12   | 2   | 1.7 | 1  | 1.2 | +1.000 |
| DR13   | 8   | 6.9 | 9  | 11.0 | 0.452 |
| DR14   | 9   | 7.8 | 8  | 9.8 | 0.813 |
| DR15   | 8   | 6.9 | 7  | 8.5 | 0.875 |
| DR16   | 4   | 3.4 | 7  | 8.5 | +0.206 |

+: Fisher’s Exact Test; ++: Chi-square test, Continuity (Yates) correction.
Table 5. HLA DQ comparison between LSS and control groups.

| HLA DQ | Kontrol (n=116) | LSS (n=82) | P   |
|--------|----------------|------------|-----|
|        | n   | %   | n   | %   |     |
| DQ2    | 14  | 12.1| 12  | 14.6| 0.754|
| DQ3    | 54  | 46.6| 36  | 43.9| +0.712|
| DQ5    | 26  | 22.4| 19  | 23.2| 1.000|
| DQ6    | 22  | 19  | 14  | 17.1| 0.878|

+: Chi-square Test, Continuity (yates) correction.