OTJHS
Online Turkish Journal of Health Sciences

e-ISSN: 2459-1467

OTSBD
Online Türk Sağlık Bilimleri Dergisi

Online Turkish Journal of Health Sciences 2020;5(4):683-692 *Online Türk Sağlık Bilimleri Dergisi 2020;5(4):683-692*

A Preliminary Research on HLA-Association of Lumbar Spinal Stenosis

Lomber Spinal Stenozun HLA İlişkisi Üzerine Ön Araştırma

[1]Mustafa EFENDİOĞLU, [1]Ezgi AKAR, [2]Emine Emel KOÇMAN, [3]Recep BAŞARAN, [2]Gulbu IŞITMANGİL

[1]Department of Neurosurgery, University of Health Sciences, Haydarpasa Numune Training and Research Hospital, Istanbul, Turkey.
[2]Department of Immunology and Tissue Typing, University of Health Sciences, Haydarpasa Numune Training and Research Hospital, Istanbul, Turkey.
[3]Department of Neurosurgery, University of Health Sciences, Ilhan Varank Training and Research Hospital, Istanbul, Turkey.

Mustafa Efendioglu: https://orcid.org/0000-0003-3663-047X
Ezgi Akar: //orcid.org/0000-0002-8582-2948
Emine Emel Kocman: https://orcid.org/0000-0003-3474-0878
Recep Basaran: https://orcid.org/0000-0001-5186-1116
Gulbu Isitmangil: https://orcid.org/0000-0003-4243-8003

ÖZ

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

Materyal ve Metod: Bu çalışmada LSS tanısı konmuş 41 olgudan (7 erkek, 8 kadın; yaş ortalaması: 60,27) 15 inde 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 gruba (%17,2) göre istatistiksel anlamlı yüksekti (p:0,039; p<0,05). B44 allelinin görülme sıklığı (%20), kontrol grubundan (%5,1) istatistiksel olarak anlamlı yüksek tespit edildi. (p:0,010; p<0,05) Aynı şekilde C6 allelin görülme sıklığı (%26,7), kontrol grubundan (%8,6) istatistiksel anlamlı yüksek bulundu. (p:0,013; p<0,05).

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

Anahtar kelimeler: Human lökosit 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.DQB1 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:0.010; 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

Sorumlu Yazar / Corresponding Author:
Ezgi Akar
Department of Neurosurgery, University of Health Sciences, Haydarpasa Numune Training and Research Hospital. Selimiye, Tıbbiye Cd No: 38, 34668 Üsküdar /Istanbul, Turkey.
Fax Number:+9002165323232
Tel: 05063444256
E-mail: ezgiaycicek@gmail.com

Yayın Bilgisi / Article Info:
Gönderi Tarihi/ Received: 26/06/2020
Kabul Tarihi/ Accepted: 24/11/2020
Online Yayın Tarihi/ Published: 30/12/2020

Atıf / Cited: Efendioglu M, et al. A Preliminary Research on HLA-Association of Lumbar Spinal Stenosis. *Online Türk Sağlık Bilimleri Dergisi* 2020;5(4):683-692. doi: 10.26453/otjhs.758530

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.[1] 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.[2] 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.[3] 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.[4] 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.[5] 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.[3] 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.[6] 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.[7]

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 Haydarpasa 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 Haydarpasa 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, intermittant neurogenic claudicasion and/or motor and sensory deficit. 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:0.039; 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:0.017; 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:0.013; 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.[8] 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.[9-11] Hyun SJ et al. declared that Korean population examined the thrombospondin 2 (THBS2) polymorphism, and revealed a significant link between haplotypes HAP4 and HAP5 and progression toward LSS, whereas HAP1, another haplotype, played a protective role against LSS.[8]

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.[9] 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, while HLA-B*13 displayed a significant association in Type 2 psoriasis.[12] 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.[13] 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 identify a protective allele for PV.[14] Zhang et al reported the expression of Platelet-Derived Growth Factor (PDGF-BB) in LSS.[15] Jirathanathornnukul 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 meriting 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 Haydarpasa 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.

Peer-review: Externally peer-reviewed.

Acknowledgement: We thank to **'**Varyans Statistical Consulting Company'.

REFERENCES

1. Karaeminoğulları O, Aydınlı U. Dejeneratif Lomber Spinal Stenoz. TOTBİD. 2004;3(4):105-113.
2. Akçam FZ. HLA System: Review. Turkiye Klinikleri J Med Sci. 2005;25(6):829-34.
3. Mosaad YM. Clinical Role of Human Leukocyte Antigen in Health and Disease. Scandind J Immunol. 2015;82(4):283–306.
4. Bingöl Ü. Etiopathogenesis in Ankylosing Spondylitis. Türkiye Klinikleri, J Immunol Rheumatol. 2011;4(1):7-15.
5. Alpsoy E. Behçet Hastalığı: Etyopatogenezde Güncel Bilgiler. Turk J Dermatol. 2013;7:41-45.
6. Noble JA, Valdes AM. Genetics of the HLA Region in the Prediction of Type 1 Diabetes. Curr Diab Rep. 2011;11(6):533–542.
7. Jiang H, Yang Q, Jiang J, Zhan X, Xiao Z. Association between COL11A1 (rs1337185) and ADAMTS5 (rs162509) gene polymorphisms and lumbar spine pathologies in Chinese Han population: an observational study. BMJ Open. 2017;7 (5):1-8.
8. Hyun SJ, Park BG, Rhim SC, Jang JW, Jeon SR, Roh SW. Progression of lumbar spinal stenosis is influenced by polymorphism of thrombospondin 2 gene in the Korean population. Eur Spine J. 2014;23(1):57-63.
9. Hallioglu O, Mesci L, Ozer S. DRB1, DQA1, DQB1 genes in Turkish children with rheumatic fever. Clin Exp Rheumatol. 2005;23(1):117-120.
10. Zhang YG, Zhang F, Sun Z. A controlled case study of the relationship between environmental risk factors and apoptotic gene polymorphism and lumbar disc herniation. Am J Pathol. 2013;182:56–63.
11. Mayer JE, Iatridis JC, Chan D. Genetic polymorphisms associated with intervertebral disc degeneration. Spine J. 2013;13:299–317.
12. Atasoy M, Pirim I, Bayrak ÖF, et al. Association of HLA class I and class II alleles with psoriasis vulgaris in Turkish population. Influence of type I and II psoriasis. Saudi Med J. 2006;27(3): 373-376.
13. Doganay L, Katrinli S, Colak Y, et al. HLA DQB1 alleles are related with nonalcoholic fatty

liver disease. Mol Biol Rep. Article in Molecular Biology Reports. 2014;41(12):7937-7943.

14. Tunca M, Musabak U, Sagkan RI, Koc E, Akar A. Association of human leukocyte antigen class II alleles with pemphigus vulgaris in a Turkish population. J Dermatol. 2010;37(3):246-250.
15. Zhang Y, Chen J, Zhong ZM, Yang D, Zhu Q. Is platelet-derived growth factor-BB expression proportional to fibrosis in the hypertrophied lumber ligamentum flavum? Spine (Phila Pa 1976). 2010;35(25):1479-1486.
16. Jirathanathornnukul N, Limthongkul W, Yingsakmongkol W, Singhatanadgige W, Parkpian V, Honsawek S. Increased expression of vascular endothelial growth factor is associated with hypertrophic ligamentum flavum in lumbar spinal canal stenosis. J Investig Med. 2016;64(4):882-887.

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

	Control (n=116)		**LSS (n=30)**		**P**
HLA A	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.*

Table 3. HLA C comparison between LSS and control groups.

	Control (n=116)		**LSS (n=30)**		**P**
HLA C	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.

	Control (n=116)		**LSS (n=82)**		**P**
HLA DR	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; ++:Ki-square test, Continuity (yates) correction.

Table 5. HLA DQ comparison between LSS and control groups.

	Kontrol (n=116)		**LSS (n=82)**		**P**
HLA DQ	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

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