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Assessment of Human Leukocyte Antigen Differences between Smokers with Reinke's Edema and Those with Laryngeal Cancer

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Abstract

Introduction:

The present study aimed to assess human leukocyte antigen (HLA) typing differences between smokers with Reinke's edema and those with laryngeal squamous cell carcinoma (SCC).

Materials and Methods:

The HLA class I, II alleles were examined in 76 unrelated Iranian patients using low-resolution polymerase chain reaction with the sequence-specific primer (PCR-SSP) method.

Results:

The frequency of the HLA-A*36 allele and HLA-B*35 was significantly higher in patients with SCC. The frequency of HLA-DRB1*01 alleles in Reinke's edema was significantly higher, as compared to that in others. In the volunteer group, HLA-DRB1*13 and HLA-DRB1*15 were significantly higher.

Conclusions:

As evidenced by the obtained results, HLA-A*36 was significantly higher in SCC, as compared to that in volunteers and Reinke's edema patients. It can be concluded that being positive for HLA-A*36 increases the chance of SCC by three times. This result should be further investigated in cohort studies conducted on larger samples. Furthermore, HLA-A*24 was significantly higher in the volunteer group, as compared to that in other groups. The HLADRB1*01 was remarkably higher in Reinke's edema, as compared to that in SCC.

Keywords:

Human leukocyte antigen, Hypertrophic edema, Iranian, Polymerase chain reaction sequence-specific primers, Reinke's edema.

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Introduction

The 14th most common cancer worldwide, laryngeal squamous cell carcinoma (SCC), which usually develops through the sixth to seventh decades of life, is responsible for over 3500 deaths per year (1). Furthermore, it is the second most common cause of upper aerodigestive tract cancer. Approximately 157,000 new cases are reported annually; therefore, the apparent and possible causes of this type of cancer have been under extensive research. Firstly, alcohol ingestion and tobacco consumption are two common risk factors with an interaction effect. Secondly, human papillomavirus and laryngopharyngeal reflux are yet to be investigated for their relationship with laryngeal cancer (2-6).

Hypertrophic laryngeal edema or Reinke's edema, the inflation of vocal fold in Reinke's space, is seriously related to smoking which is associated with a hoarse low-pitched voice and can result in stridor if edema is considerable. The correct diagnosis of disease and its progression in volunteer groups before and after treatment is only possible through observation of vocal-fold vibration (7,8). Human locus antigen (HLA) and their possible response to changes in the expression of class I and II antigens in human malignant cells have been debated by many scientists. Multiple studies of genetic elements, such as major histocompatibility complex (MHC), and reports of genetic predispositions for certain sorts of malignancy have been conducted on the development of laryngeal cancer in recent years (9,10).

The MHC can be found on the short arm of the chromosome, which is called the HLA region, including HLA class I (A, B, C) and HLA class II (DR, DQ) alleles. In general, the HLA class I phenotype is defined by serological assays, such as the micro cytotoxic test. Nonetheless, serological typing cannot distinguish many class I subtypes; for example, HLA-A*02 has been shown to comprise at least 18 subtypes by polymerase chain reaction (PCR)-based DNA typing (11).

Moreover, serological HLA-A class I typing makes typing errors, in comparison with DNA typing (12). Therefore, PCR-based DNA typing is required for the precise investigation of HLA-linked predisposition to the disease. An important feature which makes HLA an ideal marker for genetic studies is the fact that it is highly polymorphic(i.e., several alleles exist at each locus) (13,14). In light of the aforementioned issues, the present study aimed to assess HLA typing differences between smokers with Reinke's edema and those with laryngeal squamous cell carcinoma (SCC).

Materials and Methods

Study design and ethical issues

This case-control study was performed on patients admitted to the laryngology clinic at a tertiary academic referral center between March 2017 and October 2018. All the steps of the experiment were explained to the subjects, and written consent was obtained. The study was approved by the Medical Ethics Committee of Iran University of Medical Sciences, Tehran, Iran (Approval No: 1396.30602).

Inclusion and exclusion criteria

A total of 76 consecutive patients were recruited in the present study, and since it is a pilot study, the results are used to estimate the size of the studied sample. The first group consisted of 20 consecutive patients with laryngeal SCC confirmed by direct laryngoscopy and biopsy with a history of one pack per day smoking for at least 10 years. The second group comprises 20 consecutive patients with Reinke's edema confirmed by indirect laryngoscopy and the same history of smoking.

The third group (the volunteer group) consisted of 36 volunteers referred to Rasoul Akram Hospital, Tehran, Iran, with no history of laryngeal problems and no pathology in indirect laryngoscopy. All patients were evaluated by a laryngologist with indirect laryngotelescopy. Since SCC and Reinke's edema are affected in the age group of over 40 years, the participants in the age range of 40-85 age were included in the study. Patients' demographic characteristics are presented in Table 1. The inclusion criteria were the age range of 40-85 years and willingness to participate in the study.

On the other hand, the exclusion criteria entailed chronic cough, history of allergies, benign vocal cord lesion, odynophagia, and dysphagia, as well as a history of hypothyroidism and any contact with a chemical substance (such as asbestos and nickel).

DNA Extraction

Firstly, 5 cc blood was collected in tubes containing EDTA (PH=8) from each subject and the total DNA was then extracted according to the manufacturer's protocol, (QIAamp DNA Mini Kit Cat No 51304; Qiagen, Hilden, Germany). The DNA sample should have an A260/A280 ratio between 1.65 and 1.8 which can be used immediately after isolation or stored at ≤ -20°C for a long period of time (over 1 year) with no negative effects on the HLA typing results.

Polymerase chain reaction setup

The low-resolution HLA Morgan™ ABDR SSP kit (Texas Bio Gene, Inc, USA) was used in this study for the analysis of HLA class I loci A and B, as well as class II loci DR, and the steps were performed according to the manual instructions. The main steps for each sample were as follows: Taq DNA polymerase, DNA samples, and buffer were mixed and then dispensed into a 96-well plate (1-24 for A, 2-72 for B, 73-96 for DR) containing different primers for HLA genotyping. The PCR program was examined on initial DNA denaturation at 96°C for 2.5 min, followed by 10 cycles of denaturation at 96°C for 15 sec, annealing at 65°C for 60 sec, 22 cycles of denaturation at 95°C for 15 sec, annealing at 62°C for 50 sec, and extension at 72°C for 30 sec, and eventually final extension at 72°C for 10 min. After PCR amplification, all the PCR products were transferred from the 96-well plate to a 96-well agarose gel.

Following that, gel electrophoresis was performed and the photograph of the gel was saved. The electrophoresis results of 96 wells were imported into Morgan SSPal V.2.5 software (Texas BioGene, Inc.), and the HLA genotype of each sample was presented automatically (Figure1). All samples were repeated in the same way.

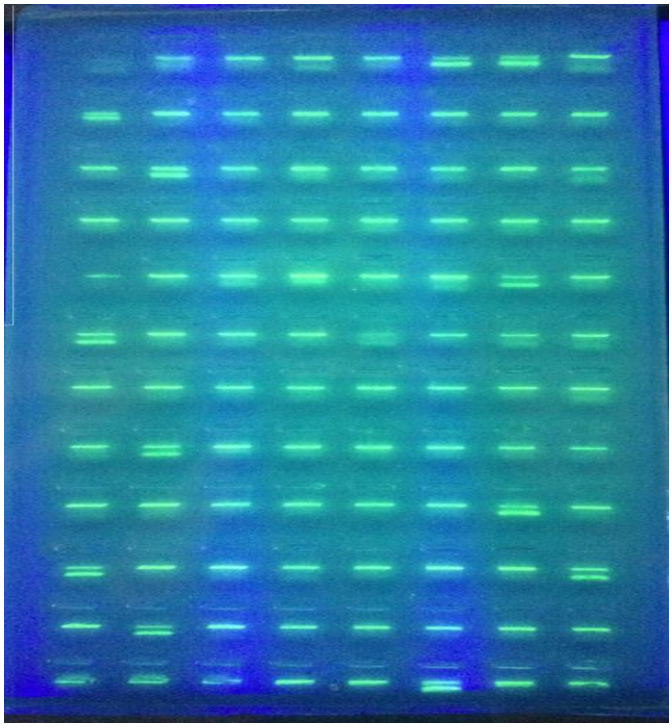

Fig 1: One sample of agarose gel electrophoresis of HLA-A, B, DRB1 alleles. The IC band (600 bp) is positive in all lanes excluding lane 1, lane 1 has not IC band because it is negative Control. Lanes: 7, 18, 24, 35,39,41,58,71,73,80,82,94 were positive (as explain in Morgan HLA SSP ABDR Typing Kit)(HLA, human leukocyte antigen; IC, internal control. (All samples were repeated in the same way).

Human leukocyte antigen analysis and statistics

A comparison was made between the number of patients (Reinke's edema and SCC) and volunteers. All results were statistically analyzed in SPSS software (version 17.0) (Chicago, IL, U.S.A.). The Chi-square test (or Fisher exact test as appropriate) was applied to compare the

observed frequency of alleles among different groups. The odds Ratio (OR) and 95% CI were also calculated. A p-value of less than 0.05 was considered statistically significant. Considering that the distribution of age was not normal, the nonparametric Kruskal-Wallis test was performed to compare three groups. To compare gender frequency among the three groups, Fisher's exact test was utilized.

Results

Patient's demographics

Considering that the present study is a pilot study, 40 patients were recruited in the first and second groups. Moreover, 36 cases were selected as the volunteer group. A total of 76 adult patients with a history of smoking were included in the study. Among them, 20 patients had SCC, 20 subjects had Reinke's edema, and 36 cases were selected as the volunteer group. While all the patients in both case groups were smokers, the volunteer group was selected from smoker individuals. The diagnosis of the disorders was performed by the same laryngology specialist. The mean age of participants was reported as 58±8.5 years (the age range of 43-85 years).

In addition, the mean age scores of patients with SCC and those with Reinke edema were obtained at 56.5±11.2 and 58.0±7.9 years, respectively. There was no significant difference in age among the three groups (P= 0.575); nonetheless, gender frequency was significantly different among the three groups (P=0.001; Table 1).

Table1: Frequency of age, sex in SCC, Reinke's edema, and volunteer's groups

Disorder	Age Mean ± SD	Sex Number (%)
SCC	56.50 ± 11.2	M: 20 (100%)
Reinke's edema	58 ± 7.9	M: 11(55%) F: 9 (45%)
volunteer's groups	58.5 ± 7.1	M; 29 (80.6%) F: 7 (19.4%)
Total	58 ± 8.5	M:60 F:16
P-value	0.575	0.001

SCC: squamous cell carcinoma, SD: standard deviation, M: male, F: female

Human leukocyte antigen typing in patients with laryngeal SCC

HLA-A in patients with squamous cell carcinoma and volunteer group

The most common alleles were HLA-A*02 in %45 and %38.9 of patients in SCC and volunteer groups, respectively.

The comparison of the frequency of HLA-A in SCC patients and volunteer group demonstrated that the frequency of HLA-A*36 alleles was significantly greater in patients with SCC (P= 0.041, Odds Ratio (OR)=3.18 ،CI 95% (2.107, 4.613), while in the volunteer group, the frequency of HLA-A*24 (12; %32.4) was significantly higher (P=0.011, Odds Ratio (O.R) =0.093 CI 95% (0.011, 0.778) (Table 2).

HLA-B in patients with squamous cell carcinoma and volunteer group

The most frequent alleles in patients with SCC were HLA-B*35 (18; %90) and HLA-B*51 (3; %15). Moreover, in volunteer group, HLA-B*35 (14; 38.9%) and HLA-B*51 (8; %22.2) were the most frequent ones.

The HLA-B*35 was significantly higher in patients with SCC, compared to that in the volunteer group (P< 0.001, Odds Ratio (O.R) 14.143, CI 95% (2.835-70.557). It was found that the frequency of HLA-B*08 (7; %19.4) was significantly higher in the volunteer group (P=0.042, Odds Ratio (O.R)= 1.690, CI 95% (1.339, 2.132)) (Table 3).

Table 2: Frequency of HLA-A in patients with SCC, Reinke's edema, and volunteer's groups

Patients HLA-A type	SCC (Number of cases) (n=20)		Reinke's edema (Number of cases) (n=20)		Volunteer 's group (Number of cases) (n=36)		Comparison of HLA-A between SCC and Volunteer 's group			Comparison of HLA-A between Reinke's edema and Volunteer 's group		
	Number	Percent	Number	Percent	Number	Percent	P-	Odd Ratio (O.R)	Confidence interval (CI) 95%	P-Value	Odd Ratio (O.R)	CI 95%
A01	8	38.1	3	14.3	8	11.1	0.219	2.333	0.709 7.675	0.728	0.618	0.144-2.652
A02	9	49.2	8	38.1	14	19.4	1.000	1.048	0.343 -3.203	0.935	1.048	0.343 -3.203
A03	2	9.5	7	33.3	6	8.3	0.697	0.556	0.101 -3.052	0.186	2.692	0.756 -9.586
A11	7	33.3	5	23.8	8	11.1	0.536	1.500	0.435 -5.172	0.814	1.167	0.324 -4.202
A24**	1	4.8	7	33.3	12	16.7	0.020	0.105	0.13 - 0.883	0.900	1.077	0.341 -3.404
A26	3	14.3	2	9.5	1	1.4	0.125	6.176	0.597-63.874	0.288	3.889	0.330-45.832
A30	2	9.5	3	14.3	2	2.8	0.611	1.889	0.245 -14.549	0.336	3.000	0.457 -19.691
A31	1	4.8	1	4.8	3	4.2	1.000	0.579	0.056 - 5.965	1.000	0.579	0.056 - 5.965
A32	0	0	2	9.5	4	5.6	0.285	1.625	1.311 - 2.015	1.000	0.889	0.148 - 5.340
A33	1	4.8	0	0	5	6.9	0.405	0.326	0.35 - 3.010	0.148	1.645	1.320 - 2.051
A36**	3	14.3	0	0	0	0	0.041	3.118	2.107 - 4.613	-	-	-
A5 *	0	0	0	0	1	1.4						
A23*	1	4.8	0	0	1	1.4	P-Value: 1,000 OR: 1.038 (CI): (0.462-2.329)			P-Value :0.300 OR: 1.571 (CI): (1.422-1.737)		
A29*	1	4.8	0	0	1	1.4						
A68*	1	4.8	1	4.8	0	0						
A69*	0	0	0	0	1	1.4						

*These alleles had low frequency and analyzed together ** Significant alleles

Table 3: Frequency of HLA-B in patients with SCC, Reinke's edema, and volunteer groups

Patients HLA-B type	SCC (Number of cases) (n=20)		Reinke's edema (Number of cases) (n=20)		Volunteer 's group (Number of cases) (n=36)		Comparison of HLA-B between SCC and Volunteer 's group			Comparison of HLA-B between Reinke's edema and Volunteer 's group		
	Number	Percent	Number	Percent	Number	Percent	P-Value	Odd Ratio (OR)	Confidence interval 95%	P-Value	Odd Ratio (OR)	Confidence interval 95%
B08**	0	0	1	4.8	7	9.7	0.042	1.690	1.339 -2.132	0.236	0.218	0.025 -1.917
B15	2	9.5	2	9.5	1	1.4	0.288	3.889	0.330-45.832	0.288	3.889	0330 -45.832
B18	1	4.8	2	9.5	3	4.2	1.000	0.579	0.056 - 5.965	1.000	1.222	0.187 - 8.003
B35**	16	76.2	10	47.6	14	19.4	0.010	4.714	1.400 -15.870	0.421	1.571	0.521 - 4.736
B38	1	4.8	2	9.5	2	2.8	1.000	0.895	0.76 - 10.528	0.611	1.889	0.245 -14.549
B40	1	4.8	1	4.8	2	2.8	1.000	0.895	0.076 -10.528	1.000	0.895	0.076 -10.528
B44	0	0	2	9.5	3	4.2	0.545	1.606	1.302 - 1.981	1.000	1.222	0.187 - 8.003
B49	1	4.8	1	4.8	4	5.6	0.645	0.421	0.044 - 4.050	0.645	0.421	0.044 - 4.050
B50	0	0	2	9.5	3	4.2	0.545	1.606	1.302 - 1.981	1.000	1.222	0.187 - 8.003
B51	3	14.3	7	33.3	8	11.1	0.728	0.618	0.144 - 2.652	0.301	1.885	0.563 - 6.314
B52	2	9.5	0	0	6	8.3	0.697	0.556	0.101 - 3.052	0.078	1.667	1.329 - 2.090
B53	2	9.5	2	9.5	0	0	0.123	3.000	2.057 - 4.375	0.123	3.000	2.057 - 4.375
B55	4	19.0	1	4.8	3	4.2	0.234	2.750	0.549 -13.780	0.545	1.606	1.302 - 1.981
B57	0	0	2	9.5	3	4.2	0.545	1.606	1.302 - 1.981	1.000	1.222	0.187 - 8.003
B7 *	1	4.8	0	0	1	1.4						
B13*	1	4.8	0	0	0	0						
B28*	0	0	0	0	1	1.4						
B37*	1	4.8	0	0	0	0	P-Value: 0.717 OR: 0.696 (CI): (0.208-2.327)			P-Value: 0.054 OR:1.571 (CI): (1.457-1.695)		
B39*	0	0	0	0	1	1.4						
B41*	0	0	0	0	1	1.4						
B42*	0	0	0	0	1	1.4						
B56*	0	0	0	0	1	1.4						
B58*	1	4.8	0	0	1	1.4						

*These alleles had low frequency and analyzed together, ** Significant alleles

HLA-DRB1 in patients with squamous cell carcinoma and volunteer group

The most frequent alleles in patients with SCC were HLA-DR B1*03 (11; %55). In the volunteer group, HLA-DRB1*03 (12; %33.3) was the most frequent one, similar to that in patients with SCC. Thereafter, the frequency of HLA-DRB1 in patients with SCC and volunteer subjects was compared. The HLA-DRB1*01 (P=0.002, Odds Ratio (O.R)= 1.333, CI 95% (2.105, 61.020)) allele was significantly greater in patients with SCC. In the volunteer group, HLA-DRB1*13 (P=0.039, Odds Ratio (O. R=0.120 CI 95% (0.014, 1.009)) and HLA-DRB1*15 (P=0.010, Odds Ratio (O.R) =1.769, CI 95% (1.373, 2.280) were significantly greater (Table 4).

Human leukocyte antigen typing in patients with Reinke's edema

HLA-A in patients with Reinke's edema and volunteer's group

The most frequent alleles in patients with Reinke's edema were HLA-A *02 (8; %40). The most frequent alleles in the volunteer group were HLA-A*02 (14; %38.9) and HLA-A*24 (12; %32.4).

The patients with Reinke's edema and volunteers were compared for the frequency of HLA-A. Their frequency was not significantly different between Reinke's edema and volunteer groups (Table 2).

HLA-B in patients with Reinke's edema and volunteer group

The most frequent alleles in patients with Reinke's edema were HLA-B*35 (9; %45), which was also observed in the volunteer group (14; %38.9). In addition, patients with Reinke's edema and volunteers were compared for the frequency of HLA-B and no significant difference was observed (Table 3).

HLA-DRB1 in patients with Reinke's edema and volunteer group

The most frequent alleles were HLA-DRB1*01 and HLA-DRB1*03 in patients with Reinke's edema (%60) and volunteers (%33.3). Following that, the frequency of HLA-DRB1 was compared in patients with Reinke's edema and volunteers.

The frequency of HLA-DRB1*01 alleles was significantly higher in Reinke's edema (P≤ 0.001; Odds Ratio (O.R) =25.500; CI 95% (4.736, 137.295) (Table 4).

Table 4: Frequency of HLA-DRB1 in patients with SCC, Reinke's edema, and volunteer groups

Patients HLA-DRB1 type	SCC (Number of cases) (n=20)		Reinke's edema (Number of cases)(n=20)		Volunteer 's group (Number of cases) (n=36)		Comparison of HLA-DRB1 between SCC and Volunteer 's group			Comparison of HLA-DRB1 between Reinke's edema and Volunteer 's group		
	Number	Percent	Number	Percent	Number	Percent	P-Value	Odd Ratio (O.R)	CI (95%)	P-Value	Odd Ratio (O.R)	CI(95%)
*01**	8	38.1	12	57.1	2	2.8	0.002	11.333	2.105 -61.020	< 0.001	25.500	4.736 - 137.295
*03	11	52.4	6	28.6	11	15,3	0.114	2.444	0.797 - 7.498	0.798	0.857	0.263 - 2.792
*04	2	9.5	0	0	4	5,6	1.000	0.889	0.148 - 5.340	0.285	1.625	1.311 - 2.015
*07	2	9.5	1	4.8	7	9,7	0.466	0.460	0.086 - 2.465	0.236	0.218	0.025 - 1.917
*08	0	0	2	9.5	4	5.6	0.285	1.625	1.311 - 2.015	1.000	0.889	0.148 - 5.340
*11	7	33.3	10	47.6	11	15.3	0.822	0.867	0.249 - 3.017	0.096	2.600	0.831 - 8.132
*13**	1	4.8	2	9.5	11	15.3	0.039	0.120	0.014 - 1.009	0.106	0.253	0.050 - 1.281
*14	0	0	1	4.8	2	2.8	0.532	1.588	1.294 - 1.949	1.000	0.895	0.076 - 10.528
*15**	0	0	2	9.5	11	15.3	0.010	1.769	1.373 - 2.280	0.188	0.289	0.056 - 1.479
*16	1	4.8	1	4.8	3	4.2	1.000	0.576	0.056 - 5.965	1.000	0.579	0.056 - 5.965
*10 *	0	0	0	0	1	1.4	P-Value: 1.000 OR:0.775 (CI) : (0.192- 3.119)			P-Value: 1.000 OR:0.775 (CI) : (0.192-3.119)		
*12 *	1	4.8	1	4.8	0	0						

*These alleles had low frequency and analyzed together ** Significant alleles

Human leukocyte antigen typing in patients with Reinke's edema and squamous cell carcinoma

HLA-A in patients with Reinke's edema and patients with squamous cell carcinoma

The most frequent alleles in patients with SCC and Reinke's edema were HLA-A*02 (9; %45) and HLA-A*02 (8; %40). The frequency of HLA-A in patients with Reinke's edema was compared with that in patients with SCC. The HLA-A*24 was significantly lower in the SCC patients (P=0.044, Odds Ratio (O.R) =0.098, CI 95% (0.011, 0.892)) (Table 5).

HLA-B in patients with Reinke's edema and patients with squamous cell carcinoma

The most frequent alleles in patients with SCC and Reinke's edema were HLA-B*35 (18; %90) and HLA-B*35 (9; %45).

The frequency of HLA-B was compared in patients with Reinke's edema and those with SCC, and no significant difference was found in the frequency of alleles (Table 6).

HLA-DRB1 in patients with Reinke's edema and patients with squamous cell carcinoma

The most frequent alleles in patients with SCC and Reinke's edema were HLDR- B1* 03 (11; %55) and HLA-DR-B1*01 (12; %60). Subsequently, the frequency of HLA-DR B1 was compared in patients with Reinke's edema and SCC patients. No statistically significant difference was found between these disorders (Table 7).

Table 5: Frequency of HLA-A in patients with SCC and those with Reinke's edema

Patients HLA-A type	SCC (Number of cases) (n=20)		Reinke's edema (Number of cases)(n=20)		Comparison of HLA-A between SCC and Reinke's edema		
	Number	Percent	Number	Percent	P-Value	Odd Ratio (O.R)	Confidence interval 95%
A1	8	38.1	3	14.3	0.155	3.778	0.827 - 17.252
A2	9	49.2	8	38.1	1.000	1.227	0.350 - 4.307
A3	2	9.5	7	33.3	0.127	0.206	0.037 - 1.159
A11	7	33.3	5	23.8	0.737	1.615	0.412 - 6.338
A24 **	1	4.8	7	33.3	0.044	0.098	0.011 - 0.892
A26	3	14.3	2	9.5	1.000	1.588	0.236 - 10.704
A30	2	9.5	3	14.3	1.000	0.630	0.093 - 4.244
A32	0	0	2	9.5	0.487	1.111	0.960 - 1.286
A36	3	14.3	0	0	0.231	2.176	1.535 – 3.087
A68	1	4.8	1	9.5	1.000	0.474	0.039 - 5.688
A23*	1	4.8	0	0			
A29*	1	4.8	0	0			
A31*	1	4.8	1	4.8		P-Value:0.367 (OR): 0.392 (CI): (0.068-2.280)	
A33*	1	4.8	0	0			

*These alleles had low frequency and analyzed together ** Significant alleles

Table 6: The frequency of HLA-B in patients with SCC and those with Reinke's edema

Patients / HLA-B type	SCC (Number of cases) (n=20)		Reinke's edema (Number of cases)(n=20)		Comparison of HLA-B between SCC and Reinke's edema		
	Number	Percent	Number	Percent	P-Value	Odd Ratio (o.R)	Confidence interval 95%
B14	0	0	3	14.3	0.231	1.176	0.979 - 1.414
B15	2	9.5	2	9.5	1.000	1.000	0.127 - 7.893
B18	1	4.8	2	9.5	1.000	0.474	0.039 - 5.688
B27	0	0	3	14.3	0.231	1.176	0.979 - 1.414
B35	16	76.2	10	47.6	0.096	4.000	0.983 - 16.271
B38	1	4.8	2	9.5	1.000	0.474	0.039 - 5.688
B44	0	0	2	9.5	0.487	1.111	0.960 - 1.286
B50	0	0	2	9.5	0.487	1.111	0.960 - 1.286
B51	3	14.3	7	33.3	0.273	0.328	0.071 - 1.518
B52	2	9.5	0	0	0.487	0.900	0.0778 - 1.142
B53	2	9.5	2	9.5	1.000	1.000	0.127 - 7.893
B55	4	19.0	0	0	0.106	0.800	0.643 - 0.996
B57	0	0	2	9.5	0.487	1.111	0.960 - 1.286
B7 *	1	4.8	0	0			
B8 *	0	0	1	4.8			
B13 *	1	4.8	0	0	P-Value: 0.369 OR: 0.394 (CI): (0.068-2.286)		
B37 *	1	4.8	0	0			
B58*	1	4.8	0	0			

*These alleles had low frequency and analyzed together

Table 7: The frequency of HLA-DRB1 in patients with SCC and those with Reinke's edema

Patients HLA-DRB1 type	SCC (Number of cases) (n=20)		Reinke's edema (number of cases)(n=20)		Comparison of HLA-DRB1 between SCC and Reinke's edema		
	Number	percent	Number	Percent	P-value	Odd Ratio (o.R)	CI 95%
*1	8	38.1	12	57.1	0.343	0.444	0.125 - 1.575
*2	2	9.5	1	4.8	1.000	2.111	0.176 - 25.349
*3	11	52.4	6	28.6	0.200	2.852	0.777 - 10.467
*4	2	9.5	0	0	0.487	0.900	0.0778 - 1.142
*6	2	9.5	0	0	0.487	0.900	0.0778 - 1.142
*7	2	9.5	1	4.8	1.000	2.111	0.176 - 25.349
*8	0	0	2	9.5	0.487	1.111	0.960 - 1.286
*11	7	33.3	10	47.6	0.523	0.538	0.151 - 1.917
*13	1	4.8	2	9.5	1.000	0.474	0.039 - 5.688
*15	0	0	2	9.5	0.487	1.111	0.960 - 1.286
*16	1	1	1	1	1.000	1.000	0.058 - 17.181
14	0	0	1	4.8	1.000	2.053	1.488-2.832

*These alleles had low frequency and analyzed together

Discussion

Some studies reinforce the premise that Reinke's edema, the most common voice problem in smokers, occurs by alterations in the epithelial barrier function, as well as inflammatory responses in the Reinke space**,**

where edema may be protective against malignant transformation. Cigarette smoking also leads to SCC cancer; moreover, tobacco acts as a polycyclic aromatic hydrocarbon and can bind directly to DNA (15). Researchers have investigated the pathogenesis of tumors and detected an association between tumor pathogenesis and abnormal HLA class I and II molecules(16). The HLA I gene variations cause the loss of HLA I antigen expression, resulting in tumor formation and development. We decided to assess HLA typing differences between smokers with Reinke's edema and those with laryngeal SCC. We compared HLA class I and II allele distribution among three groups (SCC patients, Reinke's edema patients, and volunteer group).

Based on the results of this study, the distribution of the HLA-A*01 allele appeared to be common in the SCC and volunteer groups. The HLA-A*36 was only observed in the SCC group. It may be concluded that being positive for HLA-A*36 increases the chance of SCC by three times. This result can be further investigated in cohort studies conducted on larger samples. In another research, HLA typing was performed in Adult T-cell leukemia/lymphoma disease, pointing to the high frequency of HLA-A*36 in this disease (17). In a cohort study performed on a normal Chinese population, HLA-A*36 was not found in the normal population (18).

This study may be able to support the result obtained for HLA-A*36 in SCC patients; nonetheless, the sample size must be larger to find a logical connection between HLA-A*36 and SCC patients. In agreement with the results of other studies, in the present research, the HLA-A*02 (37.8%), A*24 (32.4%), HLA-A*11(21.6%) were the most frequent alleles in the volunteer group. In their study, Shaiegan et al. demonstrated that HLA-A*02 was the most frequent allele in the normal Iranian population. In the same context, Farjadian referred to HLA-A*02(19.8%), HLA-A*03(13%), HLA-A*11, and HLA-A *24 (12.5%) as frequent alleles in the normal Iranian. Ghashghaie et al. also pointed to HLA-A*02 (18.16) and HLA-A *24 (16.41) as the most frequent alleles in the Iranian population (18-20). In line with the results of the study by Farjadian, in the current research, the frequency of HLA-A*24 was significantly higher in the volunteer group.

Furthermore, HLA-B typing was also performed, revealing that HLA-B*15 was found in patients with SCC and Reinke's edema with the same frequency. It has also been shown that HLA-B*15 is associated with human papillomaviruses in humans. This information can be of great help in the development of therapeutic vaccines(21). We found HLA-B*8 and HLA-B*49 in the volunteer group. Moreover, HLA-B*14 and HLA-B*27 was observed with the same frequency in Reinke's edema. The frequency of HLA-B*35 was significantly higher in patients with SCC in the present study. In another research on human leukocyte antigen and genetic susceptibility in human diseases, HLA-B*35 was found in hepatocellular carcinoma.(22). Ghashghaie et al. also referred to B*35(21.66) and B*51(13.35) as the most frequent alleles in the normal Iranian population.

Contrary to the present study which reported that HLA-B*35 was the most frequent allele in SCC patients, Ghashghaie et al. indicated that this allele was more frequent in the normal population(20). Further studies with a high sample size on HLA-typing and SCC patients will help us understand if there is a significant relationship between HLA-B*35 and SCC in Iranian patients. The HLA-B*51 was frequent in the volunteer group. Consistent with the results of a study by Baloch and Brahui in Pakistan, Farjadian et al. referred to HLA-B*04 as the most frequent allele in the Baloch people in Iran (23). Khazei et al. also detected HLA-B05(63.38%) and HLA-B16 (21.13%) in southeast Iran; nonetheless, these results were not obtained in the present study(24). The role of HLA class I molecules in malignant tumors has been investigated (25).

Furthermore, the study of HLA Class II molecules is under review (26). The conducted studies have addressed the polymorphism of genes of HLA class II in multiple diseases (27, 28). In the current study, The frequency of HLA-DRB1*01 was significantly higher in patients with Reinke's edema, as compared to that in the volunteer group. To the best of our knowledge, the current study was the first to investigate HLA-DRB1*01 alleles and Reinke's edema in Iranian patients. The association between HLA-DRB1*01 and other genetic factors can be valuable in the early diagnosis and treatment of patients with

Reinke's edema. The HLA-DRB1*03 had a higher frequency in patients with SCC, as compared to that in those with Reinke's edema; nonetheless, this difference was not statistically significant. The HLA-DRB1*13, HLA-DRB1*07, and HLA-DRB1*15 were most frequent in the volunteer group.

Along the same lines, in the study by Amirzargar et al., the most common DRB1 alleles were DRB1*11, DRB1*15, and DRB1*04 with a frequency of 25.0%, 14.5%, and 10.5%, respectively. In the meantime, in agreement with the findings of the current research, in a study by Yari et al., HLA-DRB1*11 was increased in normal patients. Shaiegan et al. also pointed to HLA-DRB1*11(20.8%) as the most common allele in their study. Farjadian et al. reported DRB1*11 as the most frequent allele in people residing in Fars. The DRB1*11 was not found in this study (27, 29).

The identification of HLA alleles with the next-generation sequence method may help us understand genetic differences between SCC and Reinke's edema in different ethnic groups. The literature review did not provide an article on the relationship between Rinke's edema and HLA typing; therefore, further studies are required to be carried out in this field.

The most notable strength of the present study was the assessment of HLA typing in Reinke's edema and SCC in the Iranian population. On the other hand, among the major limitations of the study, we can refer to the small sample size and uneven proportion of genders in different groups. Consequently, it is suggested that further larger cohort studies be conducted in this field.

Conclusion

The present study aimed to assess HLA typing differences between smokers with Reinke's edema and those with laryngeal SCC. Since no study has been carried out on HLA typing in Reinke's edema patients, it is worth further investigation. Furthermore, we look forward to sharing our information with research groups interested in this project.

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