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DISTRIBUTION OF HLA ALLELE FREQUENCIES IN PATIENTS WITH CYSTIC AND ALVEOLAR ECHINOCOCCOSIS IN LATVIA

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The aim of this study was to assess the relationship between HLA Class II alleles in two groups of patients in Latvia: patients with cystic and alveolar echinococcosis. The study included 37 patients from the Rīga East Clinical University Hospital with echinococcosis (29 patients with cystic echinococcosis and eight patients with alveolar echinococcosis) and 100 healthy control persons without echinococcosis. HLA Class II allele genotyping was performed using Real-time polymerase chain reaction–sequence specific primer (RT-PCR-SSP). The odds ratios (OR), with 95% confidence intervals (95% CI), were calculated using statistical analysis performed with IBM SPSS Statistics for Windows, Version 22.0, to evaluate the risk of developing the disease in an individual having a particular HLA genotype. In the case of cystic echinococcosis a more severe course of a disease can be anticipated in the presence of HLA-DRB1 *alleles* *17:01 *and* *07:01*,* -DQB1 *03:02*, *and* *03:01*,* -DQA1*04:01 and haplotypes HLA-DRB1*04:01/-DQB1*03:01/ -DQA1*03:01, HLADRB1*11:01/ -DQB1*03:01 /-DQA1*05:01*. However, in the group with alveolar echinococcosis it was associated with the* HLA-DRB1 *alleles* *17:01 *and* *07:01*,* -DQB1 *05:01 *and haplotypes* HLA- DRB1*17:01/-DQB1*02:01-2/-DQA1*01:01, HLA-DRB1*11:01/ -DQB1*03:01/-DQA1*01:03 *and* HLA-DRB1*11:01/-DQB1*03:01/-DQA1*03:01. HLA-DRB1*15:01/-DQĀ1*06:02-8/-DQA1*05:01 *and* HLA-DRB1*13:01/-DQB1*02:01-2/-DQA1*05:01 *haplotypes were protective in all patient groups. The limitations of this exploratory study indicate that a broader study needs to be conducted for revealing specific risk and protective HLA Class II haplotypes for patients with cystic and alveolar echinococcosis in Latvia.*

Key words: *echinococcosis, HLA Class II alleles, genotypes.*

INTRODUCTION

The incidence of echinococcosis in European countries varies from 0.1 to 10 cases per 100 000 residents (Eiermann *et al*., 1998; Lukmanova *et al*., 2011; Mosayebi *et al*., 2013). The number of cases of echinococcosis diagnosed for the first time is rather high in Latvia as well, despite a comparatively low population size. The location of Latvia next to endemic regions for this zoonotic disease, for example, Russia, Belarus, Poland is also an important factor (Eiermann *et al*., 1998, Lukmanova *et al*., 2011); however, no targeted studies on the incidence, risk factors, diagnostics and therapy have been conducted particularly in our country. Data on the quality of life of patients in cases where radical surgical extraction of the parasite was impossible, and on the number of years of medication therapy required, are also lacking.

The development of cystic, as well as alveolar echinococcosis is associated with individual factors of the host organism, as well as immunological reactions. It is known that the susceptibility in humans varies, as there are more-susceptible individuals and non-susceptible or resistant individuals. This is possibly connected with the major histocompatibility complex (MHC) system, which is directly involved in adaptive immune response formation. Since T

cells recognise only fragmented antigens displayed on cell surfaces, antigen processing must occur before the antigen fragment, bound to the MHC, is transported to the surface of the cell, a process known as presentation, where it can be recognised by a T cell receptor. Antigen presentation describes a vital immune process that is essential for T cell immune response triggering. MHC-II molecules are responsible for the presentation of peptides to T-lymphocytes, which initiate the response of different immune system cells. *HLA-DRB1/-DQA1/-DQB1* are the most polymorphic of the HLA class II genes and therefore can be used for individual identification. According to literature data, there are alleles of HLA that are associated with increased risk of developing a disease and alleles that are associated with decreased risk of the progress of the disease (Mosayebi *et al*., 2013).

Review of the conducted research on alveolar echinococcosis presents various results. The data of a study conducted in Germany suggest that *HLA-DRB1*11* is associated with decreased risk of developing alveolar echinococcosis, while *HLA-DQB1*09:01* with faster progression of the disease (Eiermann *et al*., 1998). However, in accordance with research conducted in China, *HLA-DRB1*04:0x* is associated with increased susceptibility, while *HLA-DRB1*07:01* with resistance (Mosayebi *et al*., 2013). A study of the Alaskan population showed that *HLA-DRB1*09:01* and *HLA-DRB1*16:01*, **16:02* are associated with elevated susceptibility to disease (Mosayebi *et al*., 2013). In a study conducted in 1998 in Europe, the *HLA-DRB1*11* allele group was associated with reduced risk of disease development, while *HLA-DQB1*02* was more frequently detected in patients with a progressive course of the disease (Aydinli *et al*., 2007). The data of a Turkish study showed that allele groups *HLA-DRB1*15*, *HLA-DQB1*02, *06, *07* were most commonly detected in patients, and that *HLA-DQB1*02, *06, *07* was more frequently detected in patients with a complicated course of the disease (Aydinli *et al*., 2007).

The review of research results dealing with cystic echinococcosis also presents varying results. Data from a study of cystic echinococcosis conducted in Iran showed that *HLA-DQB1*03* and *HLA-DRB1*11* were associated with decreased risk of the development of cystic echinococcosis (Mosayebi *et al*., 2013). A study on children in Russia demonstrated that *HLA-DRB1*07*, *-DQB1*09*, and *-DQB1*02* were associated with elevated risk of the progression of the disease and *HLA-DQB1*02* and *-DRB1*03* with a higher risk of developing a complicated condition (superinfection of the cyst content with bacterial pathogens) (Lukmanova *et al*., 2011). Data from Lebanon showed that *HLA-B*14* and *-DRB1*01* alleles were associated with lower risk of contracting the disease, while *HLA-B*35* — with high risk; the researchers noted that their results were similar to those from Russia (Chakhtoura *et al*., 2007). In the Saudi Arabia population, *HLA-DR16* and *HLA-DR7* alleles were associated with a higher risk of contracting the disease, while *HLA-DR1* and *HLA-DR10* alleles ensured resistance (Hussein *et al*., 2012). In Yemen, the *HLA-DR16* allele was associated with increased risk of getting infected, and *HLA-DR1*, *-DR8* and *-DR52* alleles with decreased risk (Al-Ghoury *et al*., 2010). An Egyptian study also linked *HLA-DR3* with increased risk of developing a complicated course of the disease (Azab *et al.,* 2004). A Turkish study in a child population showed that *HLA-DR15* and *HLA-B44* were associated with the development of the disease, *HLA-B18* and *HLA-DR1* with resistance, and *HLA-DR11* with a high probability of recovery (Yalcin *et al*., 2010). Taking into account that antigen presentation by HLA II molecules is an inductor of specific immune response (Janeway *et al*., 2001; Male *et al*., 2013), it is important, and the aim of this study was to assess the relationship between HLA Class II alleles in two groups of patients in Latvia: patients with cystic and alveolar echinococcosis. This has not been investigated in many previous studies in European countries and also in Latvia.

MATERIALS AND METHODS

Patient group. The study involved 144 patients with echinococcosis in the medical records available at the Rīga East Clinical University Hospital for the time period from 1 January 1999 to 1 February 2015. During selection, 28 patients were not included in the study, because the diagnosis could not be considered to be verified in the presence of positive serology analyses alone, because cross-reaction with other flatworm parasites was possible. Also, other patients were excluded from the study group, because the finding of a simple cyst (radiologically no signs typical of echinococcosis were found) in the liver could not be deemed to be echinococcosis, and furthermore, it is not confirmed by serological analyses. Thus, a total of 116 patients were selected for further analysis.

A total of 37 patients were included in the study of immunogenetic factors affecting the course of the disease (the study group was limited because 31 patients from the group died, and 48 patients did not wish to participate for various reasons). The patients were selected based on the following criteria: 1) confirmed case of parasitosis — positive serological finding and characteristic radiological findings (principally, ultrasound or computed tomography imaging data); 2) diagnosis occurred between the period of 1999 and 2015; 3) the patient had lived in Latvia for at least 15 years and was alive; 4) the patient gave their consent to participate in the study. We used the term "complicated" or severe course of the disease based on the following criteria: 1) echinococcosis lesion larger than 10 cm; 2) lesion count 5 or more; 3) inability to use radical treatment and 4) reoccurrence of disease after surgical removal of the parasite.

All patients participating in the study were divided into two groups based on subtype of the parasite — infection of *Echinococcus granulosus* and *Echinococcus multilocularis*, respectively 29 and 8 patients. Most of the patients (75%) were females. The mean age of females was 51.1 years and

55.4 years in males and was not significantly different between genders.

This study started in October 2015. Approval of the Rīga Stradiņš University (RSU) Ethics Committee was obtained to perform this study. Each participant signed an informed consent to participation and approval of the Central Medical Ethics Committee, Rīga, Latvia for the genetic analysis was also obtained.

Methods. *HLA-DRB1;-DQA1;-DQB1* typing detection was performed in the Joint Laboratory of Clinical Immunology and Immunogenetics of the Rīga Stradiņš University. For HLA Class II alleles detection, 4 ml of peripheral blood with EDTA were collected and stored at –20 ^{O}C before detection. For human DNA extraction QIAamp® DNA Blood Kit (Germany) was used in accordance with the methodology approved by the manufacturer**.**

Material from the data base of the Joint Laboratory of Clinical Immunology and Immunogenetics of RSU was used for the comparison of patient HLA test results with HLA of healthy blood donors (n = 100) determined in the laboratory. This control group included blood donors aged from 18 to 65 years, without HIV infection, viral hepatitis C and cytomegalovirus infection. No relatives were involved in this group. In both subsamples, clinical and control, the same protocol for DNA isolation and HLA typing was applied. The analysis was conducted in the Joint Laboratory of Clinical Immunology and Immunogenetics of RSU.

The investigation of HLA class II genotype of each patient was performed in the Joint Laboratory of Clinical Immunology and Immunogenetics of RSU. Peripheral blood samples (5 ml) were collected in EDTA containing tubes and stored at –20 °C. The QIAamp® DNA Blood Kit (QIAGEN, Germany) was used for human DNA extraction according to manufacturer's instruction (Anonymous, 2016b). DNA amplification was performed by Real-Time polymerase chain reaction (RT-PCR) with low resolution sequence specific primers (DNA-Technology, Russia) according to manufacturer's instructions (Anonymous, 2018). Each kit contained an internal control for evaluation of RT- PCR quality and positive control. HLA typing included identification of *HLA-DRB1** alleles (01 to 18), *HLA-DQA1**alleles (01:01, 01:02; 01:03; 01:04, 02:01; 03:01;04:01, 05:01, 06:01), and *HLA-DQB1** alleles (02:01-02:02; 03:01-04; 04:01-04:02; 05:01-04; 05:02-03; 06:01;06:02-08). Amplification was performed using a Real-Time PCR Thermal Cycler "DT-Lite" with four channels and 48 wells (DNA-Technology, Russia). The reaction mixture was subjected to 35 amplification cycles, each consisting of denaturation at 94 °C (60 s), followed by one cycle, annealing at 94 °C (20 s), 67 °C (2 s) followed by seven cycles and extension at 93 °C (5 s), 65 °C (4 s), with a final extension in step with 35 cycles.

Statistical analysis. IBM SPSS Statistics for Windows, Version 22.0, was used for the statistical analysis. Frequencies of alleles were compared with the Pearson's chi-square test. Data were considered statistically significant when a p value was less than or equal to 0.05. The odds ratios (OR), with 95% confidence intervals (95% CI), was calculated using the Simple Interactive Statistical Analysis (SISA) statistics online (http://home.clara.net/sisa/) website, to evaluate the risk of an individual developing the disease while having a particular HLA type (Anonymous, 2016a).

RESULTS

A quantitative analysis of *HLA-DRB1*, *-DQA1* and *-DQB1* alleles of HLA Class II was conducted during immunogenetic testing in all patients with echinococcosis, as well as separately in cystic and alveolar echinococcosis groups.

The predisposition to develop cystic echinococcosis in the patients group was associated with the *HLA-DRB1*17:01* (OR = 4.63; $p < 0.000$) and *HLA-DRB1*07:01* (OR 5.65; $p < 0.004$) alleles (Table 1). Moreover, the allele *-DRB1*15:01* (OR = 0.19; $p = 0.001$) was rarer in the cystic echinococcosis patient group and significantly more frequent in the control group. The detected *HLA-DRB1* differences were not significant after Fisher's correction for *-DRB1*01:01*, *-DRB1*08:01* and *-DRB1*13:01* alleles (Table 1). Probably, only *-DRB1*15:01* has a protective effect in the pathogenesis of cystic echinococcosis.

The alveolar echinococcosis in the patient group showed significant association after Fisher correction with the *HLA-DRB1*17:01* (OR = 3.07; $p = 0.033$) and *-DRB1*07:01* (OR=7.0; $p = 0.014$) alleles (Table 1), but not for *-DRB1* 11:01* allele (Table 1). Probably, only *HLA-DRB1*17:01* and *-DRB1*07:01* alleles have a positive effect in the pathogenesis of alveolar echinococcosis in the patient group. The alleles *-DRB1*13:01* (OR = 0.39; $p = 0.591$), *-DRB1*15:01* (OR = 0.23; $p = 0.106$) and *-DRB1*01:01* (OR = 0.78; $p = 0.545$) were rarer in alveolar echinococcosis in the patient group and significantly more frequent in the control group, but the differences were not significant after Fisher correction (Table 1).

In the patient group, the allele combinations **04:01/*11:01* (OR = 8.75, $p = 0.002$), **17:01/*13:01*(OR = 8.11, p = 0.037) and **11:01/*13:01* (OR = 4.90, $p = 0.044$) was associated with more severe disease presentation. In the cystic echinococcosis patient group, the allele combination **04:01/*11:01* (OR = 7.78, $p = 0.010$) was associated with a more complicated disease presentation. Also, in the alveolar echinococcosis group, the severity of disease was linked to three allele combinations **17:01/*13:01* (OR = 16.33, $p = 0.001$) (Table 2). We did not identify any protective alleles in this group.

Cystic echinococcosis in the patient group was associated with *HLA-DQB1*03:02* (OR = 3.67; $p = 0.02$) and *HLA-DQB1*03:01* (OR = 2.01; $p = 0.033$). Also, the allele *-DQB1*05:01* (OR = 0.25; $p = 0.047$) and *-DQB1*06:02-8* (OR = 0.31; $p = 0.013$) was more rare in the cystic echinococcosis in patients group (Table 3). This suggests that

Table 1

PROTECTIVE AND RISK *DRB1* ALLELES OF HLA CLASS II IN IN-PATIENTS WITH CYSTIC ECHINOCOCCOSIS (n = 29), ALVEOLAR ECHINOCOCCOSIS (n = 8) AND A CONTROL GROUP (n = 100) GROUPS

Alleles *DRB1**	Patient group with the cystic echinococcosis (alleles n = 58)	Gf	OR	95% CI	*p*	Patient group with the alveolar echinococcosis (alleles n = 16)	Gf	OR	95% CI	*p*	Control group (alleles n = 200)	Gf
**01:01*	7	0.12	0.75	0.26-1.87	< 0.516	2	0.13	0.78	0.08-3.67	< 0.545**	31	0.15
****15:01***	3	0.05	**0.19**	0.04-0.63	< 0.001	1	0.06	0.23	0.01-1.58	< 0.106**	45	0.23
**** 17:01***	15	0.26	**4.63**	1.91-11.16	< 0.000	3	0.19	**3.07**	0.91-17.15	< 0.033**	14	0.07
**04:01*	7	0.12	1.06	0.36-2.73	< 0.905	2	0.13	1.10	0.55-9.38	< 0.122**	23	0.12
**11:01*	14	0.24	1.61	0.73-3.41	< 0.184	5	0.31	2.30	0.58-7.75	< 0.141	33	0.17
**13:01*	5	0.09	0.56	0.16-1.56	< 0.243	1	0.06	0.39	0.01-2.76	< 0.591**	29	0.15
****07:01***	6	0.10	**5.65**	1.28-28.03	< 0.004	2	0.13	**7.0**	0.57-52.99	< 0.014**	4	0.02
**08:01*	1	0.02	0.23	0.01-1.60	< 0.110**	-	-	ND	-	-	14	0.07
**09:01*	-	-	ND		-	-	-	ND	-	-	2	0.01
**10:01*	-	-	ND		-	-	-	ND	-	-	5	0.02

The alleles in bold are statistically significant ($p < 0.05$). Gf, gene frequency; ND, not defined; OR, odds ratio; CI, confidence interval; *p*-value (probability); **An extract cell value is less than 5, used Fisher extract results.

Table 2

IMPORTANT *HLA-DRB1* ALLELE COMBINATIONS ASSOCIATED WITH SEVERITY OF DISEASE

Alleles group	****17:01/*11:01***	****17:01/*13:01***	****04:01/*11:01***	****11:01/*13:01***
All patients (n = 37) gf&OR(p)	**0.13**	**0.05/8.11(0.037)**	**0.15/8.75 (0.002)**	**0.09/4.90 (0.044)**
Cystic echinococcosis patients (n = 29) gf&OR(p)	**0.17/3.26(0.071)**	**0.07/7.33(0.640)**	**0.14/7.78(0.010)**	**0.054**
Alveolar echinococcosis patients (n = 8) gf&OR(p)	0.03	-	-	0.25/16.33(0.001)
Control subjects (n = 100) gf	0.06	0.01	0.02	0.02

The alleles in bold are statistically significant ($p < 0.05$). Gf, gene frequency; ND, not defined; OR, odds ratio; *p*-value (probability).

*HLA-DQB1*05:01* and *-DRB1*06:02-8* have a protective effect in the pathogenesis of cystic echinococcosis in the patients group, compared to the control group (gf = 0.12 and 0.23).

Alveolar echinococcosis in the patient group showed association with the *HLA-DQB1*05:01* (OR = 3.18; $p = 0.037$) alleles (Table 3). Probably, only these alleles have risk for pathogenesis of alveolar echinococcosis. The alleles *-DQB1*03:02* (OR = 8.03; $p = 0.643$), *-DQB1*04:01-2* (OR = 11.48; $p = 0.545$) and *-DQB1*06:02-8* (OR = 1.49; $p = 0.091$) were more rare in the alveolar echinococcosis patients group and more frequent in controls, but the differences were not significant after Fisher correction (Table 3).

Regarding the *HLA-DQB1* allele group, we found that **03:01/*03:02* (OR = 17.68, $p = 0.0004$), **02:01-2/*03:01* (OR = 3.84, $p = 0.047$) and **03:02/*06:02-8* (OR = 4.02, $p = 0.08$) allele combinations were linked with severe clinical symptoms in all groups.

In the cystic echinococcosis group, the following genotypes were related to more severe disease: **02:01-2/*03:01* (OR = 5.17, p = 0.024), **03:01/*03:02* (OR = 33.00, $p = 0.014$) and **03:02/*06:02-8* (OR = 5.94, p = 0.042), but in the alveolar echinococcosis group with **03:01/*03:02* (OR = 25.83, $p = 0.0004$) (Table 4).

For *HLA-DQA1* group alleles, in the cystic echinococcosis group the most frequent and possibly with higher risk for more severe course of the disease was allele **04:01* (OR = 3.84, $p = 0.006$), while allele **01:02* (OR = 0.28, $p = 0.013$) was rare and possibly protective. Alleles **01:03* (OR = 1.81, $p = 0.181$) and **02:01* (OR = 1.69, $p = 0.204$) was less frequent than in the control group, but without statistical significance (Table 5).

In the *HLA-DQA1* allele group, for alveolar echinococcosis patients we found that alleles **01:01* (OR = 1.89, $p = 0.291$) and **03:01* (OR = 1.48, $p = 0.388$) were more rare that in the control group and possibly protective but without statistical significance (Table 5).

Table 3

PROTECTIVE AND RISK *DQB1* ALLELES OF HLA CLASS II IN IN-PATIENTS WITH CYSTIC ECHINOCOCCOSIS (n = 29), ALVEOLAR ECHINOCOCCOSIS (n = 8) AND A CONTROL GROUP (n = 100) GROUPS

Alleles *DQB1**	Patient group with the Cystic echinococcosis (alleles n = 58)	Gf	OR	95% CI	*p*	Patient group with the alveolar echinococcosis (alleles n = 16)	Gf	OR	95% CI	*p*	Control group (alleles n = 200)	Gf
**02:01-2*	13	0.22	1.77	0.78-3.88	< 0.123	2	0.12	0.88	0.09-4.16	< 0.612**	28	0.14
****03:01***	19	0.33	**2.01**	0.98-4.02	< 0.033	6	0.37	2.48	0.69-8.03	< 0.088	39	0.19
****03:02***	11	0.19	**3.67**	1.36-9.66	< 0.002	1	0.06	1.04	0.02-8.03	< 0.643**	12	0.06
**03:03*	5	0.09	1.25	0.34-3.9	< 0.077	-	-	ND		-	14	0.07
**04:01-2*	1	0.02	0.37	0.01-2.80	< 0.300**	1	0.06	1.41	0.03-11.48	< 0.545**	9	0.04
****05:01***	2	0.03	**0.25**	0.03-1.06	< 0.047	5	0.31	**3.18**	0.79-10.92	< 0.037	25	0.12
**05:02-4*	-	-	ND		-	-	-	ND	-	-	13	0.06
**06:01*	2	0.03	0.61	0.06-2.94	< 0.529**	-	-	ND	-	-	11	0.05
****06:02-8***	5	0.09	**0.31**	0.09-0.83	< 0.013	1	0.06	0.22	0.01-1.49	< 0.091**	47	0.23

Alleles in bold are statistically significant ($p < 0.05$). Gf, gene frequency; ND, not defined; OR, odds ratio; CI, confidence interval; *p*-value (probability). **An extract cell value is less than 5, used Fisher extract results.

Table 4

IMPORTANT *HLA-DQB1* ALLELE COMBINATIONS ASSOCIATED WITH SEVERITY OF DISEASE

Alleles group	***02:01-2/*03:01**	***03:01/*03:02**	***03:01/*06:02-8**	***03:02/*06:02-8**
All patients (n = 37) gf&OR(p)	0.13/3.84 ($p = 0.047$)	0.08/17.68 ($p = 0.000$)	0.13	0.08/4.02 ($p = 0.008$)
Cystic echinococcosis, patients (n = 29) gf&OR(p)	**0.14/5.17 (0.024)**	**0.07/33. 0 (0.014)**	0.17	0.03
Alveolar echinococcosis patients (n = 8) gf&OR(p)	0.07	0.12	**-**	**0.25/5.22(0.048)**
Control subjects (n = 100) gf	0.03	0.01	0.07	0.03

The alleles in bold are statistically significant ($p < 0.05$). Gf, gene frequency; OR, odds ratio; *p*-value (probability).

Table 5

PROTECTIVE AND RISK *DQA1* ALLELES OF HLA CLASS II IN IN-PATIENTS WITH CYSTIC ECHINOCOCCOSIS (n = 29), ALVEOLAR ECHINOCOCCOSIS (n = 8) AND A CONTROL GROUP (n = 100) GROUPS

Alleles *DQA1**	Patient Group with the Cystic echinococcosis (alleles n = 58)	Gf	OR	95% CI	*p*	Patient Group with the Alveolar echinococcosis (alleles n = 16)	Gf	OR	95% CI	*p*	Control group (alleles n = 200)	Gf
**01:01*	5	0.09	0.53	0.15-1.49	< 0.211	4	0.25	1.89	0.41-6.77	< 0.291**	30	0.15
****01:02***	4	0.07	**0.28**	0.07-0.82	< 0.013	2	0.12	0.54	0.06-2.49	< 0.417**	42	0.21
**01:03*	8	0.14	1.81	0.63-4.79	< 0.181	1	0.06	0.77	0.02-5.57	< 0.635**	16	0.08
**02:01*	10	0.17	1.69	0.66-4.01	< 0.204	2	0.12	1.16	0.12-5.58	< 0.552**	22	0.11
**03:01*	7	0.12	0.88	0.31-2.23	< 0.777	3	0.19	1.48	0.25-5.88	< 0.388**	27	0.13
****04:01***	8	0.14	**3.84**	1.18-12.31	< 0.006	1	0.06	1.6	0.03-13.35	< 0.506**	8	0.04
**05:01*	16	0.26	1.21	0.58-2.43	< 0.578	3	0.19	0.73	0.13-2.82	< 0.451**	48	0.24

The alleles in bold are statistically significant ($p < 0.05$). Gf, gene frequency; ND, not defined; OR, odds ratio; CI, confidence interval; *p*-value (probability). **An extract cell value is less than 5, used Fisher extract results.

We found association of **01:03/05:01* (OR = 5.65, p = 0.040) and **03:01/05:01* (OR = 3.47, p = 0.031) with severe disease course in all groups and also in the alveolar echinoccosis group **03:01/*05:01* (OR = 4.23, p = 0.016), but only for *01:01/02:01* (OR = 10.78, p = 0,044) in the alveolar echinoccosis group (Table 6).

Regarding *HLA-DRB1*/-DQB1*DQA1** genotypes, we found that Echinococcus infection in all groups was associated with the following genotypes:

1) *HLA-DRB1*17:01/-DQB1*02:01-2/-DQA1*01:01*, gene frequency 0,0.16, OR = 6.26, p = 0.006; compared with control group 0.03,

2) *HLA-DRB1*04:01/-DQB1*03:01/-DQA1*03:01* gene frequency 0.22, OR = 6.62, p = 0.001 compared with control group 0.04 and

3) *HLA-DRB1*11:01/-DQB1*03:01/-DQA1*05:01* gene frequency 0.22, OR = 2.79, p = 0.047 compared with control group 0.09.

In further analysis, we found that in the cystic echinococcosis group, more severe presentation was associated with presence of the following genotypes:

1) *HLA-DRB1*04:01/-DQB1*03:01/-DQA1*03:01*, gene frequency 0.28, OR = 9.14, p = 0.001 in control group 0.04,

2) *HLA-DRB1*11:01/-DQB1*06:02-8/-DQA1*01:03*, gene frequency 0,08, OR = 8.74, 0.03, in control group 0.01 and

3) *HLA-DRB1*11:01/-DQB1*03:01/-DQA1*05:01* gene frequency 0.28, OR = 3.85, p = 0.015 compared with control group 0.09.

In the alveolar echinococcosis group, the following genotypes were significant:

1) *HLA-DRB1*17:01/-DQB1*02:01-2/-DQA1*01:01*, gene frequency 0.75, OR = 97.0, p = 0.000 in control group 0.03,

2) *HLA-DRB1*11:01/-DQB1*03:01/-DQA1*01:03*, gene frequency 0.17, OR = 20.63, p = 0.000, control group 0.01 and

3) *HLA-DRB1*11:01/-DQB1*03:01/-DQA1*03:01*, gene frequency 0.63, OR = 165.0, p = 0.000, control group 0.01.

Protective haplotypes in all groups were: *HLA-DRB1*15:01/-DQĀ1*06:02-8/-DQA1*03:01* gene frequency 0.03, OR = 0.15, p = 0.027, in control group 0.16 (Table 7).

DISCUSSION

One of the objectives of the study was to determine HLA Class II allele occurrence among echinococcosis patients and their association with the severity of the disease. The literature data on the development of echinococcosis and immunogenetic factors of patients are limited. In the PubMed database only ten publications could be used, as some studies were not available in English or Russian, and some of the original publications could not be obtained.

Table 6

IMPORTANT *HLA-DQA1* ALLELE COMBINATIONS ASSOCIATED WITH SEVERITY OF DISEASE

Alleles group	****01:03/*-05:01***	****03:01/-*05:01***	****01:01/*02:01***
All patients (n = 37) gf&OR(p)	0.08	0.16	0.05
Cystic echinococcosis patients (n = 29) gf&OR(p)	**0.10/5.65(0.040)**	**0.21/3.47(0.031)**	-
Alveolar echinococcosis patients (n = 8) gf&OR(p)	**-**	**0.241/4.23 (0.016)**	**0.25/10.78(0.044)**
Control subjects (n = 100) gf	0.020	0.002	0.03

The alleles in bold are statistically significant ($p < 0.05$). Gf, gene frequency; OR, odds ratio; p-value (probability).

Table 7

THREE LOCUS, *DRB1/-DQB1/-DQA1*, ALLELE DISTRIBUTION IN ECHINOCOCCOSIS AND CONTROL GROUPS

Group Haplotypes	All patients (n = 37) gf/OR(p)	Cystic echinococcosis patients (n = 29) gf/OR(p)	Alveolar echinococcosis patients (n = 8) gf/OR (p)	Control subjects (n = 100) gf
****11:01/*03:01/*05:01***	**0.22/2.79(0.047)**	**0.28/3.85(0.015)**	**-**	0.09
****15:01/*06:02-8/*05:01***	**0.03/0.15(0.027)**	**0.03/0.19(0.064***)**	-	0.16
****04:01/*03:01/*03:01***	0.22/6.62(0.001)	**0.28/9.14(0.001)**	-	0.04
****13:01/*02:01-2/*05:01***	**0.03/0.19(0.065***)**	**0.03/0.24(0.128***)**	-	0.13
****17:01/*02:01-2/*01:01***	0.16/6.26(0.006)	-	**0.75/97.0(0.000)**	0.03
****11:01/*03:01/*01:03***	0.11	0.05	**0.17/20.63 (0.0001)**	0.01
****11:01/03:01/*03:01***	0.19	0.03	**0.63/165(0.000)**	0.01

The alleles in bold are statistically significant ($p < 0.05$). Gf, gene frequency; OR, odds ratio; p-value (probability).

The results showed that of *HLA DRB1* gene alleles, **17:01* and **04:01* were more frequently found in patients with cystic echinococcosis. The association of allele **04:01* with increased risk of the disease has been described in China, but the Chinese publication refers to alveolar echinococcosis (Mosayebi *et al*., 2013). Alleles **01:01* and **15:01* rarely occur among the patients in the cystic echinococcosis group. Similar observations on the protective action of allele **01:01* are mentioned in studies from Russia (Mosayebi *et al*., 2013), Lebanon (Aydinli *et al*., 2007), Saudi Arabia (Chakhtoura *et al*., 2007) and Turkey (Yalcin *et al*., 2010). Alleles **17:01* and **11:01* occur more frequently in patients with alveolar echinococcosis. Contradictory data also exist: allele **11:01* in a study conducted in Germany was considered to be protective in the cases of alveolar echinococcosis (Eiermann *et al*., 1998), similar data have been obtained in Turkey (Yalcin *et al*., 2010), and studies conducted in different European countries suggest that it has a protective character in case of cystic echinococcosis (Lukmanova *et al*., 2011). Allele **01:01* was identified as protective in the case of alveolar echinococcosis. It must be added that results similar to those in our study have been obtained from the nearest regions, for example, Europe or Russia, while partially differing data originate from Asian countries. These differences are potentially due to genetic differences in race.

Our study of *HLA-DQB1* alleles indicated that allele **03:02* is significantly more frequent among cystic echinococcosis patients, and allele **03:01* in the alveolar echinococcosis patient group. These results contradict those in literature from Iran, where this allele was directly associated with a lower risk of cystic echinococcosis development (Mosayebi *et al*., 2013), which could be explained by genetic differences.

The analysis of *HLA-DQA1* alleles showed that allele **04:01* was significantly more frequent among cystic echinococcosis patients, while allele **01:01* was less common. No significant differences in allele frequency was found in the patient group with alveolar echinococcosis. The available literature lacked information on the association of alleles *HLA-DQA1* with echinococcosis. The data obtained by us here could be used in similar studies in other Baltic States, as well as European Union countries, which could expand the data base used for the detection of similarities and differences.

In-depth analysis in the patient groups examined showed that the following haplotypes were associated with a more severe course of disease in the cystic echinococcosis group: *HLA-DRB1*04:01/-DQB1*03:01/-DQA1*03:01*, and *HLA-DRB1*11:01/-DQB1*03:01/-DQA1*05:03*. The following genotypes were more frequent in the alveolar echinococcosis group: *HLA- DRB1*17:01/ -DQB1*02:01-2/-DQA1*01:01, HLA-DRB1*11:01/ -DQB1*03:01/-DQA1*01:03* and *HLA- DRB1*11:01/ -DQB1*03:01/-DQA1*03:01* gene frequency of occurrence 0.63, OR = 165.0, p = 0.000.

The following haplotypes were identified as protective in all patient groups: *HLA-DRB1*15:01/-DQĀ1*06:02-8/ -DQA1*03:01* and *HLA-DRB1*13:01/ -DQB1*02:01-2/ -DQA1*05:01*.

Immunogenetic data could prove significant for therapy planning in accordance with the individual characteristics of a patient, because no data on the optimum duration of therapy and whether the therapy can be terminated without facilitating the relapse of the infection are currently available. It is known that there are patients, in whom the disease is progressing slowly and does not create serious complications, but it must be kept in mind that there is a group of patients, in whom even a brief suspension of therapy can lead to a rapid increase in the activity of parasitic process.

CONCLUSIONS

In the event of cystic echinococcosis a more severe course of a disease can be anticipated in the presence of *HLA-DRB1* alleles **17:01* and **07:01*, *-DQB1 *03:01* and *03:02, -DQA1*04:01* and haplotypes *HLA-DRB1*04:01/ -DQB1*03:01/-DQA1*03:01, HLA-DRB1*11:01/ -DQB1*03:01/-DQA1*05:01*.

In the event of alveolar echinococcosis a more severe course of a disease can be anticipated in the presence of *HLA-DRB1* alleles **17:01* and **07:01*, *-DQB1 *05:01* and haplotypes *HLA-DRB1*17:01/-DQB1*02:01-2/ -DQA1*01:01, HLA-DRB1*11:01/-DQB1*03:01/ -DQA1*01:03* and *HLA-DRB1*11:01/-DQB1*03:01/ -DQA1*03:01*.

In the event of cystic echinococcosis *HLA-DRB1* alleles **15:01,-DQB1*05:01* and **06:02-8*, *-DQA1 *01:02* can be considered as protective. In the event of alveolar echinococcosis *HLA-DRB1* alleles **01:01*, **15:01* and **13:01*, *HLA-DQB1*06:02-8, HLA-DQA1*01:02* and **05:01* can be considered to be protective, but these differences were not significant. The following haplotypes were identified as protective in all patient groups: *HLA-DRB1*15:01/ -DQĀ1*06:02-8/-DQA1*03:01* and *HLA-DRB1*13:01/ -DQB1*02:01-2/-DQA1*05:01*.

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HLA ALĒĻU IZPLATĪBA PACIENTIEM AR CISTISKO UN ALVEOLĀRO EHINOKOKOZI LATVIJĀ

Ir zināms, ka ehinokokozes attīstība ir saistīta ar dažādiem faktoriem, kas ir gan saimniekorganisma, tostarp imūnģenētiski, gan parazīta. Balstoties uz pieejamajiem datiem, var secināt, ka cilvēku vidū uzņēmība pret šo parazitozi ir dažāda, kā arī slimības gaita ir atšķirīga. Šī pētījuma mērķis bija izpētīt sakarības starp HLA II klases alēlēm divās pacientu grupās — ar cistisko un alveolāro ehinokokozi, dati tika salīdzināti ar veselām kontroles grupas personām. Darba rezultātā secinājām, ka cistiskās ehinokokozes gadījumā smagāka slimības gaita ir saistāma ar *HLA-DRB1* alēlēm **17:01* un **07:01*, *-DQB1 *03:01* un **03:02, -DQA1*04:01* un sekojošiem haplotipiem *HLA-DRB1*04:01/-DQB1*03:01/-DQA1*03:01, HLA-DRB1*11:01/-DQB1*03:01/-DQA1*05:01.* Alveolārās ehinokokozes gadījumā tās bija *HLA-DRB1* alēles **17:01* un **07:01*, *-DQB1*05:01* un haplotipi *HLA-DRB1*17:01/-DQB1*02:01-2/-DQA1*01:01, HLA-DRB1*11:01/-DQB1*03:01/-DQA1*01:03* un *HLA-DRB1*11:01/-DQB1*03:01/ -DQA1*03:01.* Šādi haplotipi var tikt uzskatīti par protektīviem abās slimības grupās *HLA-DRB1*15:01/-DQĀ1*06:02-8/-DQA1*05:01* un *HLA-DRB1*13:01/ -DQB1*02:01-2/-DQA1*05:01.* Jāpiebilst, ka imūnģenētiskie dati var būt nozīmīgi, plānojot terapijas veidu un ilgumu, kas nākotnē varētu būt individualizēts.