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DISTRIBUTION OF HLA SPECIFICITY IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

Aim: *to study the characteristics of the HLA phenotype in patients with acute lymphoblastic leukemia (ALL) in Kazakhstan, to search for the relationship between the development of ALL and the expression of HLA antigens.* ***Objects and methods:*** *3621 healthy donors and 261 patients with a diagnosis of ALL were examined. All patients were on treatment at the clinic of the National Scientific Center of Oncology and Transplantology in Astana and were diagnosed on the basis of the ALL-2013KZ protocol (Minutes No. 16 of August 16, 2013). DNA was isolated from peripheral blood leukocytes by a proteinase method using the PROTRANS DNA BOX reagent kit (Protrans, Germany). Typing of HLA antigens (HLA-A, B, C, DRB1, DQB1) was performed by polymerase chain reaction. The results were assessed using the descriptive statistics methods.* ***Results:*** *a study of the distribution of HLA antigens in patients with ALL and healthy donors suggested the existence of associative links between the presence of HLA-A*30, B*44, C*16, DRB1*07, *16 and the development of this pathology. Persons having phenotype antigens HLA-A*02, C*02, DQB1*06 are resistant to the onset of this disease.* ***Conclusion:*** *the obtained data can be used in the development of immunogenetic criteria for predicting the development of ALL and the study of various diseases associated with HLA antigens.*

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common type of tumor of hematopoietic tissue in children accounting for 30% of malignant tumors in childhood. Patients under the age of 15 are diagnosed with ALL in 75% of all acute leukemia cases. The highest incidence of ALL is at the age of 3–4, following that the frequency decreases [1, 2]. The incidence of ALL varies geographically, and on average accounts for 30–40 cases per 1 million of people per year.

As a result of many studies a considerable amount of evidence was obtained to prove the linkage between the predisposition to a number of human diseases and genes of the antigens of the main system of histocompatibility (human leukocyte antigen — HLA) which are located on the 6th chromosome and partly determine the structure of cell membrane glycoproteins. Most commonly the linkage between HLA and diseases becomes apparent in the form of associations. Disease-associated HLA antigens may be regarded as either the antigens of susceptibility to diseases or the markers of loci linked with the true antigens determining the predisposition to diseases, which enables early recognition of oncohematological diseases [3]. Early detection of a disease helps to prognosticate the course and the outcome of the disease, as well as to take preventive measures in order to decrease the risk of its occurrence [4].

Thus, a topical problem is the study of special features of HLA phenotype in patients with ALL in Kazakhstan as well as the search for the linkage between the development of this pathology and the HLA system antigens.

MATERIALS AND METHODS

Research was done on the incidence of class I and class II HLA antigens in patients with ALL living in Kazakhstan. 3882 people were examined: 3621 healthy blood donors and 261 patients with ALL diagnosed. Class I and class II HLA typing was performed using the molecular-genetic method and A, B, C, DRB1, DQB1 loci.

The average donor age (control group) was 41 years (within the range of 18 to 64). The average patient age (test group) was 25 years (within the range of 2 to 62). Sex-based distribution among the patients was as follows: male 166 (63.6%), female 95 (36.3%). Male donors predominated over the female ones, with 2136 (59.0%) and 1485 (41.0%), respectively.

HLA studies of the donors and the patients were carried out at the premises of the Science & Production Transfusiology Center, Astana. The genomic DNA for HLA antigen typing was taken from the leucocytes of peripheral blood using the proteinase method with columns having silica gel membrane and an assay kit — PROTRANS DNA BOX (Protrans, Germany). The method of polymerase chain reaction was used for typing patients (HLA-A, B, C, DRB1, DQB1) and blood donors (HLA-A, B, C, DRB1, DQB1), and the following commercial assay kits manufactured by Protrans were used — PROTRANS HLA-A*/B*/DRB1* Cyclerplate System, PROTRANS HLA-C* Cyclerplate System, PROTRANS HLA-DQB1* Cyclerplate System.

All patients were under medical treatment at the hospital of the National Scientific Center for Oncology

and Transplantology in Astana, and were diagnosed based on the ALL-2013KZ Record (Records of Diagnosis and Treatment of Hematological Diseases in Adults, Record No. 16 dated 16 August 2013).

The data were evaluated using the descriptive statistic methods, non-parametric χ^2-test, odds ratio (OR) and 95% confidence interval (CI 95%).

RESULTS AND COMMENTS

The results of investigation are given in Tables 1–5.

Table 1

HLA-A locus antigens in patients with ALL

HLA Antigens	Patients		Donors		χ^2	p	OR [CI 95%]
	n	%	n	%			
A*01	54	20.7	706	19.5	0.22	> 0.05	1.08 [0.79–1.47]
A*02	113	43.3	1802	49.8	4.08	< 0.05	0.77 [0.60–0.99]
A*03	44	16.9	703	19.4	1.02	> 0.05	0.84 [0.60–1.18]
A*11	33	12.6	522	14.4	0.62	> 0.05	0.86 [0.59–1.25]
A*23	13	5.0	148	4.1	0.49	> 0.05	1.23 [0.69–2.20]
A*24	78	29.9	1036	28.6	0.19	> 0.05	1.06 [0.81–1.40]
A*25	8	3.1	158	4.4	1.003	> 0.05	0.69 [0.34–1.43]
A*26	23	8.8	325	9.0	0.01	> 0.05	0.98 [0.63–1.53]
A*29	4	1.5	77	2.1	0.42	> 0.05	0.72 [0.26–1.97]
A*30	26	10.0	219	6.0	6.31	< 0.05	1.72 [1.12–2.64]
A*31	23	8.8	286	7.9	0.28	> 0.05	1.13 [0.72–1.76]
A*32	13	5.0	168	4.6	0.06	> 0.05	1.08 [0.60–1.92]
A*33	24	9.2	317	8.8	0.06	> 0.05	1.06 [0.68–1.63]
A*34	1	0.4	6	0.2	0.63	> 0.05	2.32 [0.28–19.32]
A*36	0	0.0	0	0.0	NS	> 0.05	0.00 [0.00–0.00]
A*43	0	0.0	1	0.0	0.07	> 0.05	0.00 [0.00–0.00]
A*66	1	0.4	26	0.7	0.39	> 0.05	0.53 [0.07–3.93]
A*68	10	3.8	234	6.5	2.86	> 0.05	0.58 [0.30–1.10]
A*69	0	0.0	6	0.2	0.43	> 0.05	0.00 [0.00–0.00]
A*74	0	0.0	4	0.1	0.28	> 0.05	0.00 [0.00–0.00]
A*80	0	0.0	1	0.0	0.07	> 0.05	0.00 [0.00–0.00]

NS – non-significant.

An increase in the incidence of HLA-A*30 antigen allows making a statement about the association of ALL with this antigen. Similar publications established the effect of HLA-A*03, *11, *32, *33 antigens predisposing to ALL development, which was not revealed in our population [5, 6].

Negative associative links in this locus were noted for HLA-A*02. Significantly, our study revealed no A*36, *43, *69, *74, *80 locus antigen (see Table 1).

As can be seen from the Table 2 HLA-B*44 is associated with ALL. No negative associative links were observed in this locus. The antigens that were not detected among our population include HLA-B*42, *45, *47, *59, *67, *78, *81, *82, *83.

Fernandez-Torres et al showed HLA-B*40 antigen as having the predisposing effect for the development of ALL in Mexican patients. E.S. Cameron and co-authors in West India arrived at a similar conclusion [7, 8]. A contrary regularity for HLA-B*40 ($p = 0.002$) was detected in Turkey: it predominated among healthy population [5]. Other foreign publications highlight the HLA-B22, *07, 17, *45, *56, *67 antigens as predisposing to the development of ALL, which was not established in our study [6, 9]. Protective effect of HLA-B*13 antigen was observed in Turkish population [5].

A considerable increase in the incidence of HLA-C*16 antigen within the group of patients with ALL offers the possibility to regard it as the gene having a predisposing effect on the development of this pathology. The presence of HLA-C*02 antigen is more commonly established in the control group, which makes it possible to assume that there is a protective effect of this antigen on the development of ALL (see Table 3).

Table 2

HLA-B locus antigens in patients with ALL

HLA Antigens	Patients		Donors		χ^2	p	OR [CI 95%]
	n	%	n	%			
B*07	47	18.0	519	14.4	2.55	> 0.05	1.31 [0.94–1.82]
B*08	24	9.2	310	8.6	0.11	> 0.05	1.08 [0.70–1.67]
B*13	47	18.0	505	14.0	3.19	> 0.05	1.35 [0.97–1.88]
B*14	9	3.4	131	3.6	0.02	> 0.05	0.95 [0.48–1.88]
B*15	28	10.7	475	13.2	1.28	> 0.05	0.79 [0.53–1.19]
B*18	17	6.5	202	5.6	0.38	> 0.05	1.17 [0.70–1.96]
B*27	24	9.2	306	8.5	0.16	> 0.05	1.09 [0.71–1.69]
B*35	43	16.5	721	20.0	1.90	> 0.05	0.79 [0.56–1.11]
B*37	9	3.4	123	3.4	0.001	> 0.05	1.01 [0.51–2.01]
B*38	13	5.0	191	5.3	0.05	> 0.05	0.94 [0.53–1.67]
B*39	10	3.8	115	3.2	0.32	> 0.05	1.21 [0.63–2.34]
B*40	41	15.7	571	15.8	0.003	> 0.05	0.99 [0.70–1.40]
B*41	9	3.4	78	2.2	1.83	> 0.05	1.62 [0.80–3.20]
B*42	0	0.0	0	0	NS	> 0.05	0.00 [0.00–0.00]
B*44	55	21.1	510	14.1	9.38	< 0.01	1.62 [1.19–2.21]
B*45	0	0.0	11	0.3	0.79	> 0.05	0.00 [0.00–0.00]
B*46	5	1.9	133	3.7	2.22	> 0.05	0.51 [0.21–1.26]
B*47	0	0.0	9	0.2	0.65	> 0.05	0.00 [0.00–0.00]
B*48	10	3.8	178	4.9	0.64	> 0.05	0.77 [0.40–1.47]
B*49	5	1.9	128	3.5	1.96	> 0.05	0.53 [0.22–1.31]
B*50	13	5.0	193	5.4	0.07	> 0.05	0.93 [0.52–1.65]
B*51	40	15.3	507	14.1	0.32	> 0.05	1.11 [0.78–1.57]
B*52	10	3.8	204	5.7	1.55	> 0.05	0.66 [0.35–1.27]
B*53	2	0.8	18	0.5	0.34	> 0.05	1.54 [0.36–6.67]
B*54	9	3.4	87	2.4	1.08	> 0.05	1.44 [0.72–2.90]
B*55	7	2.7	118	3.3	0.27	> 0.05	0.81 [0.38–1.77]
B*56	3	1.1	45	1.2	0.02	> 0.05	0.92 [0.28–2.98]
B*57	9	3.4	193	5.4	1.78	> 0.05	0.63 [0.32–1.25]
B*58	13	5.0	265	7.3	2.04	> 0.05	0.66 [0.37–1.17]
B*59	0	0.0	0	0.0	NS	> 0.05	0.00 [0.00–0.00]
B*67	0	0.0	10	0.3	0.72	> 0.05	0.00 [0.00–0.00]
B*73	1	0.4	12	0.3	0.01	> 0.05	1.15 [0.15–8.90]
B*78	0	0.0	0	0.0	NS	> 0.05	0.00 [0.00–0.00]
B*81	0	0.0	0	0.0	NS	> 0.05	0.00 [0.00–0.00]
B*82	0	0.0	0	0.0	NS	> 0.05	0.00 [0.00–0.00]
B*83	0	0.0	0	0.0	NS	> 0.05	0.00 [0.00–0.00]

NS – non-significant.

Table 3

HLA-C locus antigens in patients with ALL

HLA Antigens	Patients		Donors		χ^2	p	OR [CI 95%]
	n	%	n	%			
C*01	30	11.5	492	13.7	1.04	> 0.05	0.82 [0.55–1.21]
C*02	18	6.9	512	14.3	11.20	< 0.01	0.44 [0.27–0.72]
C*03	71	27.2	1058	29.5	0.64	> 0.05	0.89 [0.67–1.18]
C*04	47	18.0	689	19.2	0.24	> 0.05	0.92 [0.67–1.28]
C*05	21	8.0	200	5.6	2.72	> 0.05	1.48 [0.93–2.36]
C*06	74	28.4	934	26.1	0.65	> 0.05	1.12 [0.85–1.48]
C*07	93	35.6	1356	37.9	0.51	> 0.05	0.91 [0.7–1.18]
C*08	26	10.0	414	11.6	0.61	> 0.05	0.85 [0.56–1.29]
C*12	37	14.2	676	18.9	3.55	> 0.05	0.71 [0.5–1.02]
C*14	11	4.2	134	3.7	0.15	> 0.05	1.13 [0.6–2.12]
C*15	26	10.0	356	9.9	0.00	> 0.05	1.00 [0.66–1.53]
C*16	16	6.1	116	3.2	6.13	< 0.05	1.95 [1.14–3.34]
C*17	7	2.7	89	2.5	0.04	> 0.05	1.08 [0.5–2.36]
C*18	0	0.0	1	0.0	0.052	> 0.05	0.00 [0.00–0.00]

In Germany the protective function of the HLA-Cw7 antigen in the development of ALL was shown, while in Spain the function was shown by the Cw3 gene [8, 10].

Table 4

HLA-DRB1 locus antigens in patients with ALL

HLA Antigens	Patients		Donors		χ^2	p	OR [CI 95%]
	n	%	n	%			
DRB1*01	40	15.3	553	15.4	0.001	> 0.05	1.00 [0.70–1.41]
DRB1*03	37	14.2	665	18.5	3.05	> 0.05	0.73 [0.51–1.04]
DRB1*04	77	29.5	960	26.7	0.97	> 0.05	1.15 [0.87–1.51]
DRB1*07	79	30.3	887	24.7	4.06	< 0.05	1.33 [1.01–1.74]
DRB1*08	24	9.2	365	10.2	0.25	> 0.05	0.90 [0.58–1.38]
DRB1*09	12	4.6	208	5.8	0.64	> 0.05	0.78 [0.43–1.42]
DRB1*10	9	3.4	133	3.7	0.04	> 0.05	0.93 [0.47–1.85]
DRB1*11	54	20.7	643	17.9	1.29	> 0.05	1.20 [0.88–1.64]
DRB1*12	20	7.7	288	8.0	0.04	> 0.05	0.95 [0.59–1.53]
DRB1*13	44	16.9	738	20.5	2.03	> 0.05	0.78 [0.56–1.10]
DRB1*14	24	9.2	410	11.4	1.19	> 0.05	0.79 [0.51–1.21]
DRB1*15	49	18.8	861	23.9	3.62	> 0.05	0.73 [0.53–1.01]
DRB1*16	17	6.5	140	3.9	4.27	< 0.05	1.72 [1.02–2.89]

During the study of peculiarities of HLA-DRB1 distribution an increased incidence of HLA-DRB1*07, *16 was observed among patients, which makes it possible to assume that there is an association of the above antigens with ALL (see Table 4).

According to the data of large foreign research the HLA-DRB1*01, *03, *04, *14 antigens have a resistant effect on the development of ALL [5, 11–16]; F. Yari and co-authors also established the protective effect of the HLA-DRB1*13 antigen [11, 12].

Table 5

HLA-DQB1 locus antigens in patients with ALL

HLA Antigens	Patients		Donors		χ^2	p	OR [CI 95%]
	n	%	n	%			
DQB1*02	104	39.8	1340	37.5	0.58	> 0.05	1.11 [0.85–1.43]
DQB1*03	154	59.0	2319	64.8	3.63	> 0.05	0.78 [0.60–1.01]
DQB1*04	23	8.8	282	7.9	0.29	> 0.05	1.13 [0.72–1.76]
DQB1*05	79	30.3	1128	31.5	0.18	> 0.05	0.94 [0.72–1.24]
DQB1*06	95	36.4	1555	43.5	4.98	< 0.05	0.74 [0.57–0.97]

The presence of HLA-DQB1*06 antigens is more commonly established in the control group, which enables consideration of its protective effect on the development of ALL (see Table 5). In foreign publications HLA-DQB1*04, *05 is described as the antigen having protective effect on the development of this pathology [11, 17, 18].

CONCLUSION

The study of the distribution of HLA system antigens in patients with ALL offered the possibility to assume that there are associative links between the presence of HLA-A*30, B*44, C*16, DRB1*07, *16 and the development of this pathology. It was also presumably established that the individuals having HLA-A*02, C*02, DQB1*06 in their phenotype are resistant to this disease.

The acquired data may be used in the development of immune-genetic criteria for prognostication of ALL progression and in the study of various diseases associated with HLA antigens.

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РОЗПОДІЛЕННЯ СПЕЦИФІЧНОСТЕЙ HLA У ПАЦІЄНТІВ З ГОСТРИМ ЛІМФОБЛАСТНИМ ЛЕЙКОЗОМ

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Резюме. ***Мета:*** *дослідження особливостей HLA-фенотипу у пацієнтів із гострим лімфобластним лейкозом (ГЛЛ) в Казахстані, вивчення зв'язку між роз-*

витком ГЛЛ та експресією HLA-антигенів. ***Об'єкт і методи:*** *обстежені 3621 здоровий донор та 261 хворий на ГЛЛ. Усі пацієнти перебували на лікуванні в клініці Національного наукового центру онкології і трансплантології м. Астана та були діагностовані за протоколом ГЛЛ-2013KZ (Протокол № 16 від 16.08.2013 р). ДНК виділяли з лейкоцитів периферичної крові протеїназним методом за допомогою набору реагентів PROTRANS DNA BOX (Protrans, Німеччина). HLA антигени (HLA-A, B, C, DRB1, DQB1) визначали за допомогою методу полімеразної ланцюгової реакції. Для оцінки результатів дослідження використовували методи дескриптивної статистики.* ***Результати:*** *дослідження розподілу антигенів системи HLA у фенотипі пацієнтів із ГЛЛ та здорових донорів показало існування асоціативних зв'язків між наявністю HLA-A*30, B*44, C*16, DRB1*07, *16 та розвитком цієї патології. Особи, в фенотипі яких наявні антигени HLA-A*02, C*02, DQB1*06, виявилися стійкими до розвитку ГЛЛ.* ***Висновок:*** *отримані дані можуть бути використані при розробці імуногенетичних критеріїв прогнозування розвитку ГЛЛ та інших захворювань, асоційованих із HLA-антигенами.*

Ключові слова: антигени HLA, фенотип, гострий лімфобластний лейкоз, онкогематологічні захворювання.

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