THE IMPORTANCE OF DETERMINING HUMAN LEUCOCYTE ANTIGENS IN PREVENTING INTESTINAL LYMPHOMA IN PATIENTS WITH CELIAC DISEASE

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Keywords: prevention, intestinal lymphoma, genetic tests, human leucocyte antigens, celiac disease
Abstract: Identification of celiac disease, by determining human leucocyte antigens DQ2/DQ8, is important since recent long-term studies have shown that the mortality of celiac disease is increased, if it is unrecognized and untreated. In this sense, we wanted to see the usefulness of genetic tests in celiac disease diagnosis and screening. Material and methods. During 2010 we determined by PCR, DQ2/DQ8 haplotype, in a group of 27 children with celiac disease and 9 of their brothers, serologically negative for celiac disease. Results. 22 children and 7 of their brothers confirmed the diagnosis of celiac disease, DR3-DQ2 haplotype was predominant in children with celiac disease and DR4-DQ8 to their brothers. Conclusions. Genetic testing to determine human lecocyte antigens remain the most reliable test in the diagnosis of celiac disease but also in identifying family risk for people with celiac disease.

INTRODUCTION

Human leukocyte antigens (HLA) are glycoproteins present on the cell membranes. These proteins are responsible for the recognition of self and non-self structures, for the immune response to antigenic stimuli, as well as for the coordination of humoral and cellular immune response[1]. Celiac disease is an autoimmune enteropathy affecting patients with a genetic predisposition[2] who are exposed to gluten, a protein that is dominant in wheat and similar gramineae: rye, oats. The only treatment for patients with celiac disease is the exclusion of gluten from diet.

Unidentified and untreated, celiac disease presents a risk of intestinal lymphoma[3]. An increased mortality and the risk of cancer have been found particularly in adults with celiac disease, within three years of diagnosis. When the diagnosis of celiac disease is made in childhood, the risk is lower and consequently, the role of laboratory tests increases significantly in the screening of the disease. The maintenance of a gluten-free diet may reduce the risk. Some studies suggest that undiagnosed persons are the most exposed[4].

In celiac disease, the genetic risk factor is the HLA-DQ2/DQ8 haplotype[5]. HLA markers have been used for a long time in clinical studies on celiac disease. In 2005, Butterworth et al.[6] found in South Asian residents from Great Britain differences in HLA alleles compared to Caucasian patients with celiac disease, and they concluded that non-HLA regions can play a stronger role in the presentation of the disease. In 2006, Eller et al.[7] did not find any evidence of a high risk genotype in celiac disease in Arabian Bedouin communities (isolated consanguineous populations). In 2007, Crovella et al.[8] found in Brazil, the Recife area, a low HLA-DQ2 prevalence of 0.84%. In 2008, Díaz de Entresotos Villazán et al.[9] reported in Spain, the Cantabria area, a 71% prevalence of HLA-DQ2, which is low compared to other regions of Spain. In 2009, Cummins et al.[10] noted in the Asia-Pacific region: Iran, Syria, Australia, Israel, New Zealand, Turkey, a low genetic predisposition of celiac disease, several rare genotypes belonging to HLA-DQ2: HLA-DQB1*02 (*0201 or *0202) being identified. In 2010, Alarida et al.[11] found in Libya a high genetic predisposition of celiac disease, the following genotypes being identified in children with celiac disease: hetero DQ2 (n=15), DQ2 with homo beta2 (10), DQ8 and beta2 positive (3), DQ8 (2), and hetero beta2 (1), and in controls, hetero DQ2 (n=36), hetero beta2 (30), DQ2/DQ8 negative (23), DQ8 (19), alpha5 (14), DQ2 with homo beta (12), homo beta2 (10), DQ8 and beta2 positive (7), and DQ2/DQ8 (5).

Aim of the study

The principal objective of this study was the identification of the main genetic haplotypes in children with celiac disease from the Cluj and surrounding areas. As a secondary objective, the study aimed to identify the potential genetic risk factors, the HLA-DQ2/DQ8 haplotype, in the relatives of these children, i.e. siblings negative for celiac disease, by screening using conventional serological tests.

MATERIAL AND METHOD

Design of the study

The study was performed during the course of 2010, at the Clinical Laboratory of Immunology and Gastroenterology of the Cluj Regional Center for the Management of Celiac Disease, organized within the structure of the Clinical Emergency Pediatric Hospital of Cluj-Napoca, Clinic of Pediatrics II, where serological testing for celiac disease

and DNA extraction were performed, in collaboration with the Department of Diagnosis Molecular Biology, Immunology, HLA and Virusology of the Fundeni Clinical Institute, where genetic HLA typing was performed. This was an analytical observational study.

Patients

The study group included 27 children diagnosed with celiac disease, 21 girls and 6 boys. The study also included 9 relatives, siblings of these, who were found serologically negative for celiac disease. The testing consisted of the immunoenzymatic serological determination by ELISA of IgA anti-tissue transglutaminase antibodies (TgA-IgA), the determination by indirect immunofluorescence of IgA antiendomysial antibodies (EmA-IgA) for the monitoring of the gluten-free diet, and the genetic determination of histocompatibility antigens HLA-DQ2/DQ8.

Method

The serological tests were performed using *in vitro* diagnostic (IVD) kits, produced by Inova Diagnostics Inc. (USA) for the determination of TgA-IgA and EmA-IgA. The immunoenzymatic ELISA tests were performed on the automated analyzer ChemWell 2910 Awareness Technology Inc., and indirect immunofluorescence tests were read on the Olympus CX31 fluorescence microscope.

The genetic tests were performed using DNA extraction kits and HLA typing kits, by polymerase chain reaction (PCR), produced by the Inno-Train Diagnostik company, Germany. PROTRANS Domino System HLA kits were used, which determine the following haplotypes specific for celiac disease:

1.*pattern 1* DR3-DQ2- and *pattern 5* DR4-DQ8 as single haplotypes;

2.*pattern 2* DR3-DQ2/DR3-DQ2 as two identical homozygous haplotypes;

3.*pattern 3* DR3-DQ2/DR7-DQ2 and *pattern 4* DR5-DQ7/DR7-DQ2 as combinations of two haplotypes.

Statistical analysis

Statistical analysis was performed using the SPSS software version 16.0, and data were collected and analyzed using Microsoft Excel.

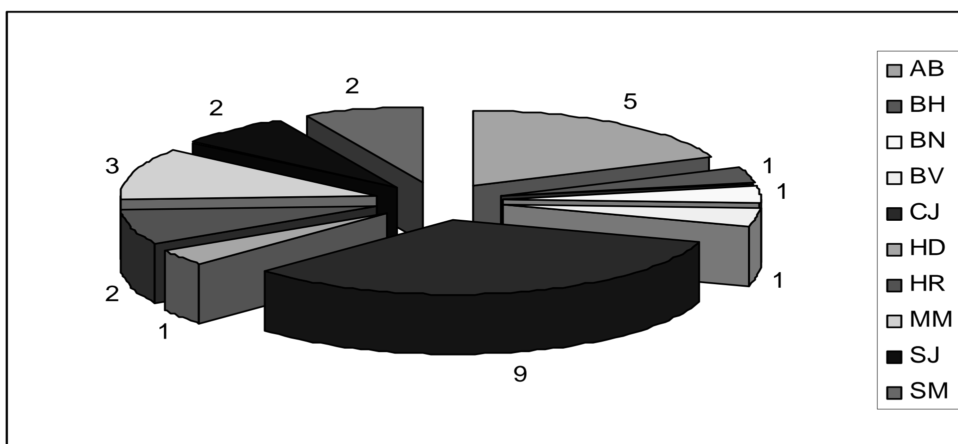
RESULTS AND DISCUSSION

I. Demographic and clinical characteristics of the studied subjects

The geographic area of the investigated patients included Cluj county and the neighboring areas: Alba, Bihor, Bistriţa-Năsăud, Maramureş, Sălaj, Satu Mare (*Figure 1*).

Figure 1. The geographical distribution of patients

The clinical characteristics of our group included patients with old celiac disease under a gluten-free diet, who were serologically monitored for the maintenance of the gluten-free diet.



II. Results obtained in the group of patients included in the study

Pattern 1 DR3-DQ2/- was identified in 7 patients. *Pattern 2* DR3-DQ2/DR3-DQ2 was identified in 6 patients. *Pattern 3* DR3-DQ2/DR7-DQ2 was identified in 1 patient. *Pattern 4* DR5-DQ7/DR7-DQ2 was identified in 4 patients. *Pattern 5* DR4-DQ8/- was identified in 2

patients. Also, 2 patients presented a combination of *pattern 1* and *pattern 5,* DR3-DQ2/DR4-DQ8, which oriented us towards a diagnosis of celiac disease, 1 patient had the DR5-DQ7/- *pattern*, which excluded the diagnosis of celiac disease, and in 4 patients, no *pattern* specific for celiac disease was identified, the patients being considered negative.

III. The geographical distribution of the haplotypes obtained

Pattern 1 DR3-DQ2/- was identified in patients from Cluj, Bistriţa-Năsăud and Braşov counties. *Pattern 2* DR3-DQ2/DR3-DQ2 was identified in patients from Alba, Bihor, Cluj, Satu-Mare counties. *Pattern 3* DR3-DQ2/DR7-DQ2 was identified in patients from Hunedoara county. *Pattern 4* DR5-DQ7/DR7-DQ2 was identified in patients from Alba, Cluj, Sălaj, Satu-Mare counties. *Pattern 5* DR4-DQ8/- was identified in patients from Maramureş and Sălaj counties. A combination of *pattern 1* and *pattern 5,* DR3-DQ2/DR4-DQ8, was identified in patients from Harghita county (*Figure 2*).

Figure 2. The geographical distribution of the haplotypes obtained

IV. The sex distribution of the haplotypes obtained

Pattern 1 DR3-DQ2/- was identified in 5 girls and 2 boys. *Pattern 2* DR3-DQ2/DR3-DQ2 was identified in 6 girls. *Pattern 3* DR3-DQ2/DR7-DQ2 was identified in 1 girl. *Pattern 4* DR5-DQ7/DR7-DQ2 was identified in 3 girls and 1 boy. *Pattern 5* DR4-DQ8/- was identified in 1 girl and 1 boy. A combination of *pattern 1* and *pattern 5,* DR3-DQ2/DR4-DQ8, was identified in 2 girls.

V. Correlations with serological tests

TgA-IgA positivity was identified in 2 patients with *pattern 1* DR3-DQ2/-, in 2 patients with *pattern 2* DR3-DQ2/DR3-DQ2, in no patient with *pattern 3* DR3-DQ2/DR7-DQ2, in 2 patients with *pattern 4* DR5-DQ7/DR7-DQ2, and in 2 patients with *pattern 5* DR4-DQ8/-. (*Table 1, Table 2*). EmA-IgA positivity was identified in 1 patient with *pattern 1* DR3-DQ2/-, in 2 patients with *pattern 2* DR3-DQ2/DR3-DQ2, in no patient with *pattern 3* DR3-DQ2/DR7-DQ2, in 2 patients with *pattern 4* DR5-DQ7/DR7-DQ2, and in 2 patients with *pattern 5* DR4-DQ8/-. (*Table 3, Table 4*)

Table 1. Distribution of positive and negative values of TgA-IgA

TgA-IgA	Pattern	1	2	3	4	5	1/5	Total
negative	**Number of patients**	5	4	1	2	0	2	14
	% of all pacients with TgA-IgA	35.71	28.57	7.14	14.29	0.00	14.29	100.0
	% of total pacients sample	22.73	18.18	4.55	9.09	0.00	9.09	63.64
positive	**Number of patients**	2.00	2.00	0.00	2.00	2.00	0.00	8.00
	% of all pacients with TgA-IgA	25.00	25.00	0.00	25.00	25.00	0.00	100.0
	% of total pacients sample	9.09	9.09	0.00	9.09	9.09	0.00	36.36
Total	**Number of patients**	7.00	6.00	1.00	4.00	2.00	2.00	22.00
	% of all pacients with TgA-IgA	31.82	27.27	4.55	18.18	9.09	9.09	100.0
	% of total pacients sample	31.82	27.27	4.55	18.18	9.09	9.09	100.0

Table 2. Correlation of TgA-IgA with celiac disease patterns

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	5.74	5	0.33	0.38
Fisher's Exact Test	4.97			0.44
12 cells (100.0%) have expected count less than 5. The minimum expected count is .36.				

Since the observed significance level of Fisher's exact test p = 0.44> 0.05, statistically insignificant, there is no association between pattern type and TgA-IgA.

Table 3. Distribution of positive and negative values of EmA-IgA,

EmA-IgA	Pattern	1	2	3	4	5	1/5	Total
Negative	**Number of patients**	6	4	1	2	0	2	15
	% of all pacients with EmA-IgA	40.00	26.67	6.67	13.33	0.00	13.33	100.00
	% of total pacients sample	27.27	18.18	4.55	9.09	0.00	9.09	68.18
positive	**Number of patients**	1.00	2.00	0.00	2.00	2.00	0.00	7.00
	% of all pacients	14.29	28.57	0.00	28.57	28.57	0.00	100.00

	with EmA-IgA							
	% of total pacients sample	4.55	9.09	0.00	9.09	9.09	0.00	31.82
Total	**Number of patients**	7.00	6.00	1.00	4.00	2.00	2.00	22.00
	% of all pacients with EmA-IgA	31.82	27.27	4.55	18.18	9.09	9.09	100.00
	% of total pacients sample	31.82	27.27	4.55	18.18	9.09	9.09	100.00

Table 4. Correlation of EmA-IgA with celiac disease patterns

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	7.29	5	0.20	0.21
Fisher's Exact Test	6.23			0.25
12 cells (100.0%) have expected count less than 5. The minimum expected count is .36.				

Since the observed significance level of Fisher's exact test p = 0.25> 0.05, statistically insignificant, there is no association between pattern type and EmA-IgA.

VI. Results obtained in the relatives of serologically negative patients for celiac disease

Pattern 1 DR3-DQ2/- was identified in 1 patient. *Pattern 2* DR3-DQ2/DR3-DQ2 was identified in 2 patients. *Pattern 3* DR3-DQ2/DR7-DQ2 was identified in no patient. *Pattern 4* DR5-DQ7/DR7-DQ2 was identified in 1 patient. *Pattern 5* DR4-DQ8/- was identified in 3 patients. One patient also presented *pattern* DR5-DQ7/-, which excluded the diagnosis of celiac disease, and in 1 patient, no *pattern* specific for celiac disease was identified, the patient being considered negative.

VII. Discussion

In the studied group, the dominant *patterns* were those corresponding to HLA-DQ2, which were present in 18 patients (7 single haplotypes, 6 homozygotes, 5 heterozygotes) and the *patterns* corresponding to HLA-DQ8 were present in 2 patients. In 2 patients, we identified a new *pattern*, which has not been described before, a combination of HLA-DQ2 and HLA-DQ8, and in 5 patients, genetic tests excluded the diagnosis of celiac disease, other celiac-like syndromes being present. The geographical distribution of the studied patients showed the presence of *patterns* corresponding to HLA-DQ2 in patients from Cluj, Bistriţa-Năsăud, Alba, Bihor, Satu-Mare, Sălaj, Braşov, Hunedoara counties, while the *patterns* corresponding to HLA-DQ8 were present in patients from Maramureş and Sălaj counties. In central Romania, in Harghita county, we identified a new *pattern*, which has not been described before, a combination of HLA-DQ2 and HLA-DQ8. The sex distribution of the haplotypes obtained showed the presence of *patterns* corresponding to HLA-DQ2 in 15 girls and 3 boys, while the *patterns* corresponding to HLA-DQ8 were present in 1 girl and 1 boy. A combination of *pattern 1* and *pattern 5,* DR3-DQ2/DR4-DQ8, was also identified in 2 girls.

In the majority of the relatives of serologically negative patients for celiac disease, the *patterns* corresponding to HLA-DQ2 were unevenly present in 4 patients (1 single haplotype, 2

homozygotes and 1 heterozygote), and the *patterns* corresponding to HLA-DQ8 were present in 3 patients. In 2 patients, genetic tests did not confirm the diagnosis of celiac disease.

Megiorni et al.[12] found that in patients with celiac disease, the DQ2/DQ8 haplotype was more frequent in women than in men, while in negative controls, this was more frequent in men, suggesting a possible transmission of the DQ2 haplotype in the paternal line to daughters. Srivastava et al.[13], by evaluating the prevalence and the role of HLA DQ2/DQ8 testing in first degree relatives of children with celiac disease, found that 85% of the first degree relatives were HLA DQ2 positive and thus, presented the risk of developing celiac disease. Hadithi M et al.[14] state that TgA and EmA tests are the most sensitive serological tests in celiac disease, but a negative HLA-DQ result excludes the diagnosis. Thomas et al.[15] conclude that the proportion of EmA positive patients is higher in subjects with HLA-DQ2 than in those with HLA-DQ8, and is increased in HLA-DQ2 homozygotes. The authors also find a correlation between HLA-DQ frequency and the severity of celiac disease.

As a possible future practical approach, we propose the application of a screening program for the relatives of serologically negative patients for celiac disease, a desirable objective given the long term risks and, implicitly, the need for an early diagnosis of the disease.

CONCLUSIONS

This study confirms the diagnostic and prognostic role of genetic tests, of histocompatibility antigens HLA DQ2/DQ8, in the diagnosis of celiac disease in children and in the identification of persons at risk for celiac disease. The determination of HLA DQ2/DQ8 is required for the identification of new haplotypes specific for celiac disease.

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