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Coeliac disease profile in Down syndrome patients

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Abstract
Introduction and objective. Individuals with Down syndrome (DS) are a major risk group for coeliac disease (CD). The aim of this study is to find differences in the CD profile in this group in order to take a different medical approach.
Patients and methods. This observational, descriptive and comparative study included 81 patients aged under 15 years monitored between January 1999 and December 2008. Patients were divided into two groups, a first group including 28 children with CD and DS, and a second age—and sex-matched group of 53 children with CD and no DS. Retrospective data from medical records were analyzed.
Results. There were no statistically significant differences in age at diagnosis, clinical presentation, symptoms at diagnosis, body measurements, serological markers and histological data. Members of the DS group were significantly likelier to have no family history of CD or an association with autoimmune thyroiditis. Breastfeeding was initiated less frequently in the DS group, and the introduction of gluten was significantly delayed. The genetic study showed a significantly high frequency of the DQ8 heterodimer in patients with SD.
Conclusions. The clinical profile of CD in children with DS appears to be similar to that for children without this condition. The risk heterodimer distribution in DS individuals in this series differs from published data. Some nutritional features in this population could entail new risk factors that might trigger the onset of CD.

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PALABRAS CLAVE
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Trisomía 21

Perfil de la enfermedad celíaca en los pacientes con síndrome de Down

Resumen
Introducción. El colectivo de personas con síndrome de Down (SD) es uno de los más importantes dentro de los grupos de riesgo de enfermedad celíaca (EC). Nuestro objetivo es encontrar diferencias en el perfil de la EC en este colectivo que permitan un manejo médico diferente.
Pacientes y método. Estudio observacional, descriptivo y comparativo que incluyó a 81 pacientes menores de 15 años controlados entre enero de 1999 y diciembre de 2008. Se establecieron dos grupos; el primero incluyó a 28 niños con EC y SD y el segundo incluyó a 53 niños con EC y sin SD, ajustados por edad y sexo. Se analizaron retrospectivamente los datos procedentes de las historias clínicas.
Resultados. No se encontraron diferencias estadísticamente significativas en cuanto a la edad de diagnóstico, la presentación clínica, la sintomatología al diagnóstico, la somatometría, los marcadores serológicos o los datos histológicos. Se observaron diferencias estadísticamente significativas en el grupo SD en relación con la ausencia de antecedentes familiares de EC y en la asociación con tiroiditis autoinmune. Este grupo inició menos frecuentemente la lactancia materna y la introducción del gluten fue significativamente más tardía. El estudio genético mostró una importante frecuencia de heterodímeros DQ8 en el grupo de pacientes con SD.
Conclusiones. El perfil clínico de la EC en el niño con SD parece similar al del niño sin esta condición. La distribución de los heterodímeros de riesgo en los individuos con SD de nuestra serie difiere de los datos publicados. Existen peculiaridades nutricionales en este colectivo que podrían determinar la presencia de nuevos factores de riesgo que precipiten la aparición de una EC.

Introduction

Coeliac disease (CD) is a permanent intolerance to gluten proteins in people with a genetic predisposition and is characterised by immune inflammation which affects the lining of the intestine. It affects both children and adults and the male/female ratio is 2:1. The worldwide prevalence is estimated at 1/266[1] and in Spain it ranges between 1/118 in children and 1/389 in adults[2]. The known prevalence of CD is thought to be the tip of the iceberg since there is a high percentage of undetected cases[3]. There are people who are much more likely to develop coeliac disease, such as those with Down syndrome (DS). There is a far greater frequency of digestive abnormalities in people with DS than in the general population, with the most common being oesophageal atresia, duodenal atresia or stenosis, anorectal malformations and coeliac disease[4].

Since DS is the most common chromosomal disorder and one of the most important risk groups for CD, a fundamental aim is to find differences which make it possible to provide the DS group of patients with different clinical approach.

Patients and method

This is a descriptive, comparative observational study of 81 patients under 15 years old, who were monitored in our centre between January 1999 and December 2008. An initial group was established including 28 children with CD and DS and a second one of 53 children with CD and without DS, matched by age and sex. A retrospective analysis was carried out of data from their medical records.

- **Inclusion criteria.** Having DS in group 1, under 15 years old at time of diagnosis, diagnosis by endoscopic biopsy, signing informed consent.
- **Exclusion criteria.** Having a chromosomal disorder or polymalformation syndrome different to DS, diagnosis with Watson-Crosby capsule or lack of biopsy, and refusal to participate in the study.

Before withdrawing gluten from the patients' diet, intestinal villus atrophy and/or cryptic hyperplasia together with positive serum markers were considered compatible with CD. Compatible findings after gluten withdrawal were a fast, clear and unmistakable improvement in symptoms, and negative markers.

The following data were recorded: sex, chromosomal study in group 1, family history, age at diagnosis, form of clinical presentation, symptoms, associated conditions, somatometry, serum markers, histological lesions, HLA typing and nutritional history.

The karyotype study was performed in a lymphocyte culture using the low and medium resolution GTG band technique (trypsin using giemsa). The karyotypes were interpreted following international recommendations,

analysing at least 20 metaphases. Nephelometric analysis (Dade Behring, Marburg, Germany) determined the serum IgA concentrations. Anti-endomysial, anti-gliadin and antitransglutaminase antibodies were studied with ELISA (InmunoCAP Phadia, Uppsala, Sweden). When IgA deficiency was detected, the IgG isotope was studied with a specific conjugate. The histological identification was performed by gastroscopy, and multiple duodenal biopsies were taken using paediatric gastroscopes (Olympus Spain, Barcelona). The histological analysis of the biopsies was carried out following the Marsh criteria[5]. HLA typing was performed using genomic DNA extracted from mononuclear blood cells using DNAzol (Talron Biotech, Israel). The HLA-DRB1 and DQB1 alleles were detected using PCR-SSO and PCR-RFLP reverse dot-blot hybridization, respectively. The somatometric study included weight and length measurements using scales and a height measure if the patient was under 3 years old and a Carpenter stadiometre if older. Standardised growth charts were used for weight and length in both groups.

The statistical analysis was performed using version 16.0.1 of the SPSS computer program (SPSS Ibérica, Madrid, Spain). Quantitative variables are expressed as mean ± standard deviation or medians [p25-p75] and qualitative variables as percentages. A simple or bivariate analysis was performed to study statistical associations. The Pearson χ^2 test was used to analyse the qualitative variables and the student T test to compare quantitative variables between independent groups. $P<0.05$ was taken to be statistically significant.

Results

A group of 28 subjects (group 1) was obtained from 255 children with DS who were monitored. The control group (group 2) included 53 children, matched by age and sex.

The age at diagnosis in group 1 ranged from 17 to 133 months with a mean of 50.8 ± 34.3 months. In group 2, it ranged from 12 to 132 months, with a mean of 57.7 ± 38.6 months.

The results of the chromosomal study were obtained for 16 patients in group 1: 15 had a regular trisomy and 1 had a translocation.

In group 1, 7.7% of the subjects had a family history of CD and 14.8% had a family history of another kind of autoimmune disorder. Eighteen point five percent were linked with recurrent miscarriage. In group 2, 26.9% of the subjects had a family history of CD and 18.9% had a family history of another kind of autoimmune disorder. Nine point four percent of the patients had a family history of recurrent miscarriage. No significant differences were found with regard to the history of autoimmune disorders ($p = 0.652$) or miscarriages ($p = 0.245$), but the family history of CD did reveal significant differences ($p = 0.047$).

In group 1, 15 of the 28 patients started breast feeding (BF), which was continued during a median of 2 [1-9] months (mean 5.5 ± 6.5). In group 2, 28 of the 35 patients who knew this information started BF, which was continued during a median of 3.5 [2-6] months (mean 6.2 ± 10.9). The difference in the number of patients who started BF reached statistical significance ($p = 0.038$). The median age for the introduction of gluten in group 1 was 8.5 [8-11.6] months (mean 10.7 ± 6.5) and in group 2 it was 8 [8-8] months (mean 8.2 ± 1.1); this difference was also statistically significant ($p = 0.028$).

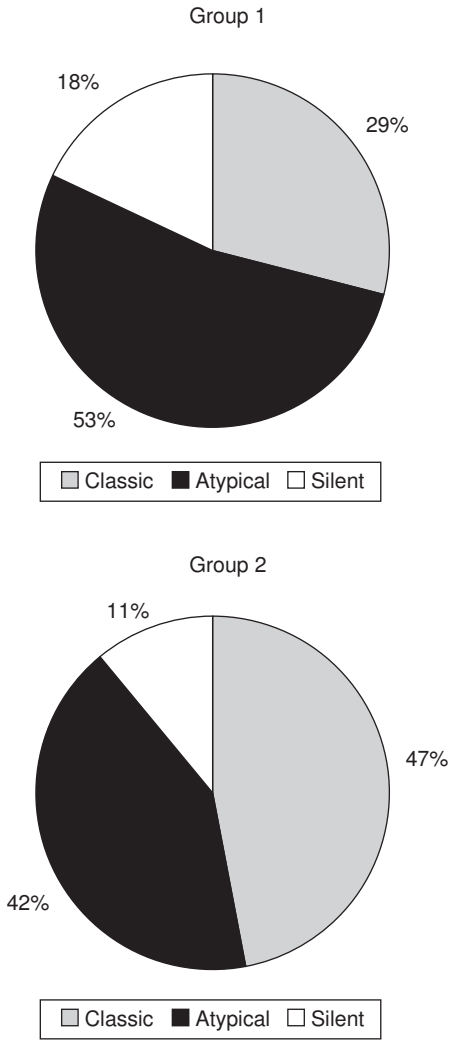

Figure 1 Forms of clinical presentation.

In group 1, 28.6% had a classic presentation of the disease and in 53.6% of the cases it was atypical or there was a paucity of symptoms. In group 2, 47.2% had a classic presentation and in 41.5% of the cases it was atypical. The differences observed did not reach statistical significance ($p = 0.257$) (Figure 1). The most common atypical symptoms in both groups were mild digestive symptoms (constipation and stomach pain in 40% and 59.1%, respectively) and iron deficiency with or without anaemia (in 40% and 54.5%). Short stature was less frequent (26.7% and 31.8%), as were hypertransaminasaemia, enamel defects and personality changes. No statistically significant differences were observed in the clinical symptoms. In group 1, 17.9% had silent symptoms, while in group 2 it was 11.3%, these differences not reaching statistical significance ($p = 0.257$).

Thirty-five point seven percent of the subjects in the group with DS had a thyroid pathology, in the form of

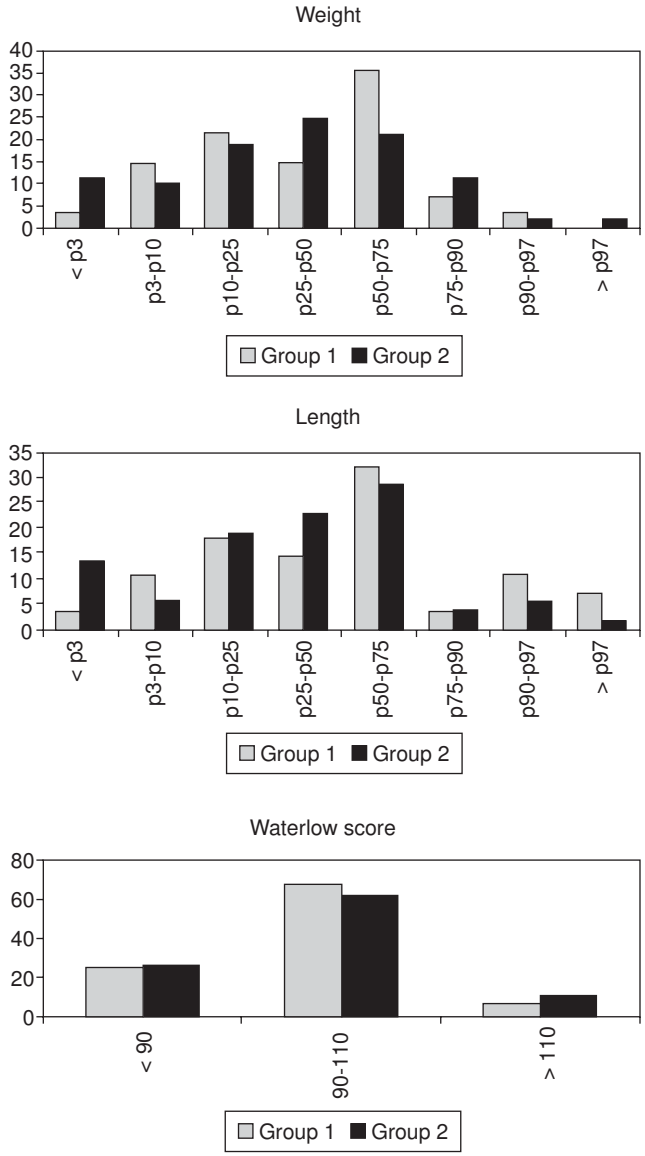

Figure 2 Distribution of weight and length percentiles (p) and Waterlow scores.

autoimmune thyroiditis. In the control group, other autoimmune phenomena (diabetes mellitus, vitiligo) were also observed, but with lower frequency.

With regard to nutritional conditions, the differences observed in the distribution of weight and length percentiles and Waterlow scores did not reach statistical significance (Figure 2).

In the case study group, no cases of IgA deficit were detected. In the control group there were 2 cases. In both groups, anti-gliadin, anti-endomysial and antitransglutaminase antibodies were used for CD screening (Table 1). The differences observed with regard to the positivity of the different tests did not reach statistical significance.

The duodenal biopsies of both groups were revised. Thirty-nine percent of the patients in group 1 had MARSH-IIIa versus 17% in group 2; 25% of group 1 had MARSH-IIIb versus 39.6% in group 2; and 36% of group 1 had MARSH-IIIc versus 41.5% of group 2. The differences observed between both groups were not statistically significant ($p = 0.138$).

Table 1 Serological study results

Serological test	Group 1 (n = 28)	Group 2 (n = 53)	p
Anti-gliadin IgA	25/28 (89.3%)	44/51 (86.3%)	0.700
Antitransglutaminase IgA	14/17 (82%)	30/33 (90.9%)	0.371
Anti-endomysial antibody	10/13 (77%)	25/26 (96.2%)	0.062
Anti-gliadin IgG	0	2/2 (100%)	—
Antitransglutaminase IgG	0	2/2 (100%)	—

As part of the genomic typing, the type II HLA of 17 of the 28 patients in group 1 was analysed, in addition to 24 of the 53 subjects in group 2. The DQB1 and DRB1 alleles of all the patients were analysed. Their distribution is shown in Table 2.

Discussion

It is known that autoimmune conditions are more common in children with DS than in those without it. The relationship between DS and CD was described over 30 years ago with the case of an adolescent with DS who, as well as having CD, also had retinoblastoma[6].

Our results confirm the high prevalence of CD in children with DS. Out of a total of 225 DS children studied, 28 cases of CD were confirmed, giving a prevalence of 11%. This is somewhat higher than that observed in most series, and clearly higher than that described in the general population[1,2] (Table 3).

Regarding prevalence by sex, a male-female ratio of 2 to 1 has been described in the general population. The different series in patients with DS have provided disparate results[9,18]. In our series, we found a similar ratio to that described in the general population.

CD is more common among relatives of patients, with figures ranging between 2.6 and 16%[9]. There are very few studies performed with the DS population to establish if this relationship is maintained within this group. In his study, Trier reported that the prevalence in first degree relatives of people with DS is similar to that in the general population[20]. However, in our series we observed a stronger association between the relatives and patients in group 2. This could be due to bias from the patient selection procedure or the study design, so it should be contrasted with further studies.

There are no conclusive studies regarding the age of diagnosis of CD in patients with DS. In patients without this condition, the age of diagnosis varies, depending on the series. Several European studies[10,11,15,17] have reported a later diagnosis of people with DS. In our study, the mean

Table 2 Distribution of HLA alleles in both groups

HLA II	DQ2	DQ2/DQ2	DQ8	DQ8/DQ8	DQ2/DQ8	Others
Group 1 (n = 17)	10 (58.8%) DR3 = 7 (70%) DR1 = 2 (20%) DR7 = 1 (10%) DR5DQ7 = 3 (30%)	0	3 (17.6%) DR4 = 3 (100%)	1 (5.9%) DR4 = 1 (100%)	1 (5.9%) DR3/DR4 = 1 (100%)	2 (11.8%)
Group 2 (n = 24)	14 (58.3%) DR3 = 9 (64.3%) DR7 = 5 (35.7%) DR5DQ7 = 3 (21.4%)	8 (33.4%) DR3 = 5 (62.5%) DR3DR7 = 3 (37.5%)	0	0	0	2 (8.3%)

Table 3 Studies of prevalence of CD in DS

Author	n	Location	Prevalence (%)
Rumbo et al (2001)[7]	56	Argentina	3.6
Shamaly et al (2007)[8]	52	Israel	3.8
Hansson et al (1999)[9]	76	Switzerland	3.9
Zubillaga et al (1993)[10]	70	Spain	4.3
Castro et al (1993)[11]	155	Italy	4.5
Bonamico et al (2001)[12]	1,202	Italy	4.6
Wouters et al (2009)[13]	155	Germany	5.2
Carnicer et al (2001)[14]	284	Spain	6.3
George et al (1996)[15]	115	Denmark	7
Czismadia et al (2000)[16]	137	Holland	8
Jansson et al (1995)[17]	54	Sweden	16.9
Agardh et al (2002)[18]	48	USA	19

age of diagnosis was similar in both groups. Neither did we find differences in the forms of clinical presentation. In the general population a ratio of 1:8[21] is reported between symptomatic and silent forms. This ratio becomes 4:1 in favour of symptomatic forms in some series of patients with DS[2]. In our series, a ratio of around 8-10:1 is maintained between symptomatic and silent forms in both groups. These differences could be explained by considering that the information about clinical symptoms was collected retrospectively. Thus, symptoms which could have been omitted from consideration after patients began a diet without gluten have been taken into account, increasing the number of symptomatic forms of the disease. In the study of associated conditions, there is a strong association in group 1 with autoimmune thyroid disease. There are numerous studies describing the increased prevalence of autoimmune diseases among patients with CD. These have tried to associate gluten exposure time in CD patients with these diseases, with contradictory results. In contrast to Ventura et al[23], Hakanen et al[22] showed that gluten exposure time in coeliac disease patients seems unrelated to the onset of autoimmune thyroid disease. It is not known if the link between DS, CD and the different autoimmune diseases depends exclusively on HLA genetic association or if there are non-HLA genes which may contribute to the development of both entities. Also unknown is whether gliadin can lead to the formation of autoreactive lymphocytes that affect endocrine organs (pancreas, thyroids), and more importantly, if it is possible to prevent autoimmunity from developing with early detection and treatment of CD in DS.

According to the recommendations of the Spanish Health Programme for People with DS, an adequate screening method is the determination of anti-endomysial or antitransglutaminase antibodies at 3-4 and 6-7 years of age. In our centre the determination of anti-gliadin and antitransglutaminase antibodies is performed at 3-4 years of age and then every two years if the results are negative. In this study anti-endomysial antibodies were also used at a time when the determination of antitransglutaminase antibodies was not used. Anti-gliadin antibodies were the first to be used for the diagnosis of CD, but in the DS population they were thought to be less useful. Later, first anti-endomysial antibodies, and then antitransglutaminase antibodies were considered to have greater sensitivity and specificity[24]. With regard to the data in our series, taken in isolation it might seem that the most valuable test was the determination of anti-gliadin IgA antibodies. However, in only 5 of the 28 cases studied was endoscopy indicated based on high levels of anti-gliadin IgA antibodies in the absence of other positive tests. In the remaining cases, a positive test for anti-gliadin IgA antibodies was accompanied by a positive antitransglutaminase IgA test or, less frequently, positive anti-endomysial antibodies. Thus, it still has to be ascertained if the greater value of the determination of the antitransglutaminase IgA antibodies would improve if other serum tests were associated with it, as happens in our centre.

There are no articles linking a specific mutation in Down syndrome (classic trisomy, translocation or mosaicism) with the onset of CD. In fact, loci associated with coeliac disease have not been detected on chromosome 21. The genetic explanation for the association between CD and DS is still unknown. However, CD has one of the strongest known associations with the class II HLA region. Over 90% of coeliac disease patients are carriers of the DQ2 heterodimer (DQA1*0501 DQB1*02) or the DQ8 heterodimer (DQA1*03 DQB1*0302)[25]. These data can be extrapolated to the DS

population[9,15]. In our series the DQB1 and DRB1 alleles were studied. Statistical relationships were not established but the high frequency of DQ8 heterodimers in group 1 is very striking. We could not find any similar results in the literature. On the other hand, it is strange that CD develops in the absence of any high risk heterodimer. In this study, two patients in each group were diagnosed with CD without having the classic heterodimers. This finding suggests that the HLA typing strategy as a screening measure might not be totally effective. Both Czismadia et al[16] and Wouters et al[13] propose HLA typing as a screening test, followed by regular determination of antibodies in the carrier population only. However, there have been recent studies which have reached similar results to ours[25]. This is a matter of discussion, based on criteria of effectiveness and cost-effectiveness[4,9,15].

There has been speculation about the importance of BF as a determining environmental factor in the development of CD. In addition, the early introduction of gluten has been associated with an increase in the prevalence of CD. Furthermore BF seems to exert a protective effect against CD, but the data available in the literature are not conclusive[26]. In this study, we analysed the number of patients who began and maintained BF, and its duration. The time when gluten was introduced into the diet was also analysed. The differences observed in the number of patients who began BF and the age at which gluten was introduced reached statistical significance. These differences are probably due to external factors which make it difficult to start BF in the group of patients with DS.

Conclusion

Although this paper has methodological limitations, it confirms the high prevalence of CD in the DS population in our country. Generally speaking, the profile of CD in children with DS seems similar to that in other children. However, the distribution of the risk heterodimers in the people with DS in our series differs to that published in other series. In this study, it is seen that in the group of children with DS, BF was less common and the gluten was introduced into their diets significantly later. These factors could lead to the presence of new risk factors in this population, which in turn are responsible for the onset of CD. Further in-depth studies are necessary in order to establish recommendations for these patients about starting feeding and the introduction of gluten.

Conflict of interest

The authors declare they have no conflicts of interest.

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