**AUTOANTIBODIES IN RELATIVES OF CELIAC DISEASE PATIENTS: a follow-up of 6-10 years**

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**ABSTRACT** – Context - Autoimmune diseases are 3 to 10 times more frequently in patients with celiac disease and their relatives than in the general population. Objective - To investigate a broad spectrum of autoantibodies in celiac disease relatives from Southern Brazil, in a serological follow-up of 6-10 years, aiming to associate with other autoimmune diseases, degree of parentage, demographic and clinical data. Methods - Serum samples of 233 relatives were analyzed in two different phases: n = 186 in phase I (1997-2000) and n = 138 (being 91 = follow-up group and 47 = newly tested) in phase II (2006-2007). As controls, 100 unrelated individuals were evaluated. Autoantibodies to smooth muscle, mitochondrial, liver-kidney microsome, parietal cell and thyroid microsome were tested by indirect immunofluorescence. Results - A significant increase of autoantibodies, in both phases, was observed in the relatives when compared to the non-relatives (\(P = 0.0064\)), specifically to anti-thyroid microsome and anti-parietal cell. In both phases, the female/male proportion of autoantibodies was of 4:1 to 3:1 (\(P\leq0.041\)). The frequency of autoantibodies amongst 1st and 2nd degree relatives was 11.8% and 9.68% in phase I and 4% and 6.67% in phase II. Conclusion - Celiac disease relatives presented other autoantibodies and serological screening is a useful instrument for identifying autoimmune diseases along the years.


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**INTRODUCTION**

Celiac disease (CD) is a life-long inflammatory autoimmune disease (AID) of the gastrointestinal tract affecting genetically susceptible individuals\(^{12}\). This enteropathy shows a well-known familial predisposition and a prevalence of 16% among first-degree relatives\(^{1,6}\). The risk is even greater in families with affected siblings carrying the human leukocyte antigen (HLA)-DQ2. Multiple cases in the same family are not rare and the risk of CD in second degree relatives is still significant\(^{11,19}\). Evidence for a familial risk in CD has been accumulated from many sources, including biopsy and serological studies in families with known CD, HLA-genotyping studies, genome-wide expression and linkage studies\(^{14,18}\).

Several AID are more prevalent among CD patients and their close relatives compared to the general population\(^{21}\). The coexistence of CD with other AID reinforces the involvement of common immune mechanisms and genetic factors in the pathophysiology of these disorders\(^{10}\). It is possible that chronic lymphocyte stimulation in the intestine of CD patients could result in increased autoantibody production and therefore, stimulate the development of other AID. The most frequently reported conditions associated with CD are type 1 diabetes mellitus and autoimmune thyroiditis\(^4,11\).

Considering that the delay in the diagnosis of CD can be associated to complications such as osteoporosis, anemia, infertility, malignancy, and more recently related to other AID\(^{15}\), the serological screening for autoantibodies (AAB) in relatives of celiac patients represents an important instrument for early detection of these diseases in these individuals. In this study, a broad spectrum of AAB was investigated in relatives of celiacs from southern Brazil, in a serological follow-up of 6-10 years, aiming to associate the data with occurrence of other AID, the degree of parentage, as well as demographic and clinical data of the individuals.

**METHODS**

The present study was approved by the local Ethics in Research Committee and written informed consent was obtained from all subjects prior to inclusion. A total of 333 individuals from Southern Brazil were included in the study. Table 1 displays demographic...
characteristics of these individuals.

Serum samples of 233 relatives (100♂, 133♀; 2-83 years) were analyzed, being 186 collected in phase I of the study (years 1997 to 2000) and 138 in phase II (years 2006 to 2007). Amongst the last one, 91 relatives were recovery from the phase I, and constitute the follow-up group; while 47 were relatives not evaluated in phase I (newly tested relatives). As control group, we studied 100 unrelated volunteers from the same geographical area, which reported no familial CD cases.

AAB to smooth muscle (SMA), mitochondrial (AMA), liver-kidney microsome (LKM), parietal cell (PCA) and thyroid microsome (ATM) were tested by indirect immunofluorescence (IFI), as previously described(7, 20), using FITC polyclonal anti-human globulin conjugate (Dako, Denmark). The substrate used in the tests were cryostatic tissue sections of mouse stomach (SMA, PCA), liver (LKM) and kidney (AMA, LKM), and human thyroid (ATM). The sample was considered positive if fluorescence was seen at dilution of 1:40 to SMA, 1:10 to ATM, and 1:20 to all the other AAB. All positive samples were titrated up to the end point. Positive and negative controls were used for each bath.

All positive serum for ATM was tested for anti-peroxidase antibody (anti-TPO) by quimioluminescence (kit DPC - Diagnostic Products Corporation, Los Angeles, CA, USA).

The data were compiled in frequency and contingency tables and statistical analyses were performed with Epi-Info, using Chi squared, Fisher and Proportion tests when indicated. The significance level was set at 0.05.

**RESULTS**

Significant increase of AAB was observed in relatives (10.3%; 24/233) compared to the non-relatives (1%; 1/100; P = 0.0064), being raised and constant so in phase I (10.75%; 20/186) as in phase II of research (8.7%; 12/138). Amongst the evaluated AAB, an increased frequency for the anti-ATM (6.45%; 12/186; P≤0.0050 and 5.8%; 8/138; P≤0.0117) and anti-PCA (3.76%; 7/186; P≤0.0117 and 3.62%; 5/138; P≤0.0635) was detected in the two phases, respectively. AMA and anti-LKM were not detected. SMA was positive in two relatives in phase I (1.08%; 2/186) and in one subject in the control group (Table 2).

The female/male proportion of AAB was of 4:1 (16♂; 4♀; P<0.0001) to 3:1 (9♂; 3♀; P = 0.041) in each phase. The frequency of AAB in 1st and 2nd degree relatives was 11.8% (19/161) and 4% (1/25) in phase I and 9.68% (9/93) and 6.67% (3/45) in phase II, without significant difference in both. Among different groups of relatives, it was observed increased and constant frequency of positive AAB in siblings.

### TABLE 1. Demographic data of the relatives of celiac disease patients and control group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number</th>
<th>Sex*</th>
<th>Age mean (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I – Relatives Phase I</td>
<td>186</td>
<td>108F 78M</td>
<td>33.68 (2-79)</td>
</tr>
<tr>
<td>Group II – Relatives Phase II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-A Follow-up</td>
<td>91</td>
<td>55F 36M</td>
<td>40.64 (10-82)</td>
</tr>
<tr>
<td>II-B newly tested relatives</td>
<td>47</td>
<td>25F 22M</td>
<td>24.85 (2-83)</td>
</tr>
<tr>
<td>Group III – Non relatives</td>
<td>100</td>
<td>63F 37M</td>
<td>33.05 (2-78)</td>
</tr>
</tbody>
</table>

*F=Females; M=Males

### TABLE 2. Frequency of autoantibodies in relatives of celiac and non-relatives

<table>
<thead>
<tr>
<th>Autoantibodies</th>
<th>Phase I (n = 186)</th>
<th>Phase II (n = 91)</th>
<th>Non-relatives (n=100)</th>
<th>P* Relatives phase I x Non relatives</th>
<th>P* Relatives phase II x Non relatives</th>
<th>P* Relatives phase I x Relatives phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM</td>
<td>12 (6.45)</td>
<td>6 (6.59)</td>
<td>2 (4.26)</td>
<td>0.0050</td>
<td>0.0117</td>
<td>NS</td>
</tr>
<tr>
<td>PCA</td>
<td>7 (3.76)</td>
<td>3 (3.30)</td>
<td>2 (4.26)</td>
<td>0 (0)</td>
<td>0.0473</td>
<td>0.0635</td>
</tr>
<tr>
<td>SMA</td>
<td>2 (1.08)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (1)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LKM</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AMA</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TOTAL</td>
<td>20 (10.75)</td>
<td>8 (8.79)</td>
<td>4 (8.51)</td>
<td>1 (1)</td>
<td>0.0055</td>
<td>0.0220</td>
</tr>
</tbody>
</table>

ATM = anti-thyroid microsome
PCA = anti-parietal cell
SMA = anti-smooth muscle
AMA = anti-mitochondrial
LKM = anti-liver-kidney microsome
NS = non significant

Fisher
Chi squared
in both phases (9.43%; 6.25%, respectively) (Figure 1). No statistical difference was reached between AAB distribution by age, with trend to the significance ($P = 0.083$) in relatives older than 60 years compared to younger individuals in phase II (Figure 2).

The serological follow-up in 91 relatives showed positivity of 7.69% to AAB in phase I, being 2.2% (2/91) to anti-ATM, 4.4% (4/91) to anti-PCA and 1.1% (1/91) to anti-SMA. In a second occasion, after 6-10 years, the total positivity to AAB in the same ones was 8.79% (8/91), with 6.59% (6/91) to anti-ATM and 3.3% (3/91) to anti-PCA, with one relative positive concomitantly to these both antibodies in phase II. After 6-10 years, 12.09% (11/91) of the family members reevaluated showed changes in their serological profile, or remained positive for the same AAB. At this, amongst the seven positive relatives in first testing, four remained positive (three to anti-PCA; one to anti-ATM) and three became negative, whereas four previously negative individuals became positive to anti-ATM and one relative positive to anti-PCA since phase I demonstrated seroconversion for anti-ATM either ($P = \text{NS}$). Until moment, clinical diagnosis of auto-immune gastritis was confirmed in one followed-up relative.

**DISCUSSION**

Even though untreated celiac patients, as well as their relatives, present high prevalence of AAB, it has been not yet clarified if CD is an inflammatory disease with secondary auto-immune reactions, or if it is a primary AID induced by known environmental factors. The present study shows an increased prevalence of AAB in relatives of celiac patients, which was significantly higher in relation to non-relatives ($P = 0.0064$; 10.3% and 1% respectively). These data are in concordance with previous reports.

This is a pioneer follow-up for AAB in relatives of celiac patients, and the percentage of individuals recovered during 6-10 years was relevant (48.9%; 91/186) compared to previous studies, where the maximum of recovery samples has been 30.3% (40/132) ($P = 0.002$).

Our findings suggest that one time testing is insufficient to identify all AAB positive individuals, and reinforce the need to re-evaluate family members of celiac patients who have been negative on a first serological screening. In addition, a persistent risk of developing AAB was seen in adults and children in both, first and second degree relatives. These data suggest that CD relatives should be screened not only for CD but also for other AID, even in the absence of symptoms.

Although the association of thyroid’s AID and CD has been shown to be frequent, the reports on thyroid’s AID in CD relatives are scarce. In both phases of this study a high prevalence to anti-ATM in relatives ($P = 0.0050$; $P = 0.0117$), followed for anti-PCA ($P = 0.0473$; $P = 0.0635$) was observed in CD relatives when compared with the control group. Ansaldi et al. showed that 26% of Italian CD patients had positive autoimmune thyroid serology compared to 10% in the control subjects. Ventura el al. demonstrated high titers of serum TPO antibodies present in 14.4% of CD patients from Italy. Similar results for parietal cell auto-antibodies were obtained by Utiyama et al. in a Brazilian study that found 3.6% of positivity in CD patients and 3.4% in first degree relatives.

The concurrently occurrence of CD and pernicious anemia and/or atrophic gastritis is rare. However, in this study,
one female 52 years old, that was amongst the five relatives positive to anti-PCA in phase II (Table 2), had clinical and endoscopic diagnosis of auto-immune gastritis, while the others relatives still await for the results.

A highly significant positivity for AAB was seen in the women when compared to males in both phases ($P<0.0001$; $P = 0.041$, respectively). These results corroborate with the higher predisposition of female including celiac relatives to the development of different AID$^{8,9}$.

The positivity of AAB between relatives > 60 years (17.65%) demonstrated a trend to the significance ($P = 0.083$) in relation to other individuals, suggesting that the persistent consumption of gluten can represent a trigger to these positivity in genetically susceptible individuals, such as celiac relatives. Although CD occurs at any age and with a great variety of manifestations, there is a marked increase in the incidence rates of CD among adults > 60 years$^{10}$. It is important to systematically include serological screening tests for CD and other AID in the evaluation of these susceptible adults. Atypical manifestations and low suspicion can delay diagnosis even during years$^{13,17}$.

The findings of parentage were interesting. The value of this serological study between siblings of celiac patients was emphasized, since AAB positivity were similar after 6-10 years, in contrast to the frequency of AAB in the other relatives. This issue highlights the increased risk to the development of other AID in these individuals (Figure 1), similarly to the higher risk to develop CD$^{13,14}$.

**CONCLUSION**

In conclusion, our data suggest that first and second degree relatives of celiac patients are a group of individuals that shows positive serological test results for AID, hence, this population need to be evaluated for the presence of AAB more than once, irrespective to the presence of symptoms. Serological screening is a useful instrument for identifying these affections along the years.

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**RESUMO** – **Contexto** - Doenças autoimunes são 3 a 10 vezes mais frequentes em pacientes com doença celiaca e em seus familiares que na população em geral. **Objetivos** - Realizar amplo perfil de autoanticorpos em familiares de celiacos do sul do Brasil, em seguimento sorológico de 6-10 anos, visando associá-lo com outras doenças autoimunes, grau de parentesco, dados demográficos e clínicos desses indivíduos. **Métodos** - Foram analisadas amostras de 233 familiares em duas etapas diferentes: $n = 186$ na etapa I (1997-2000) e $n = 138$ (91 recoleta e 47 novos familiares testados) na etapa II (2006-2007). Como controle foram avaliadas amostras de 100 não-familiares. Anticorpos antimúsculo liso, antimitocondrial, anticélula gástrica parietal, antimicrossomal de fígado e rim e antimicrossomal tireoidiano foram testados por imunofluorescência indireta. **Resultados** - Foi observado um aumento significativo de positividade para os autoanticorpos em familiares de celiacos, quando comparados aos não-familiares ($P = 0.0064$), especificamente para o antimicrossomal tireoidiano e anticélula gástrica parietal. Entre os indivíduos com autoanticorpos positivos, a proporção do sexo feminino para o masculino foi de 4:1 e 3:1 em ambas as etapas ($P<0.041$). A frequência de autoanticorpos detectada entre familiares de primeiro e segundo graus foi de 11,8% e 9,68% na etapa I e 4% e 6,67% na etapa II. **Conclusão** - Familiares de pacientes celiacos apresentam autoanticorpos positivos e o acompanhamento sorológico desses indivíduos é utilizado como instrumento na identificação de doenças autoimunes ao longo dos anos.

REFERENCES