Clinical Findings and Anti-Neuronal Antibodies in Coeliac Disease with Neurological Disorders

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Background: Little is known about the clinical and immunological features of coeliac disease patients with neurological disorders. In a large series of adult coeliac disease patients, we investigated the prevalence of neurological disorders and anti-neuronal antibodies, along with the clinical course.

Methods: Neurological symptoms were investigated in 160 consecutive patients (120 F, 40 M) with biopsy-proven coeliac disease. Anti-neuronal antibodies to central/enteric nervous systems were investigated in all neurological patients, 20 unaffected ones and 20 controls.

Results: Thirteen (8%) patients had neurological disorders, including epilepsy (n = 3), attention/memory impairment (n = 3), cerebellar ataxia (n = 2), peripheral neuropathy (n = 2), multiple sclerosis (n = 1), Moyamoya disease (n = 1) and Steinert’s disease (n = 1). No significant demographic or clinical differences (gastrointestinal or other gluten-related signs) were found between patients with and without neurological involvement. In all but 2 of the 13 cases, the neurological disorder preceded diagnosis of coeliac disease. Neurological symptoms improved or disappeared in 7 patients who started a gluten-free diet within 6 months after neurological onset, and in none of 4 patients who began later. Prevalence of central nervous system anti-neuronal antibodies was significantly higher in neurological (61%) than in other patients (5%) (P = 0.0007) or controls (0%) (P = 0.00001). Conclusions: Coeliac disease can sometimes present in the guise of a neurological disorder, which may greatly improve when a gluten-free diet is started promptly. Therefore, the possible presence of coeliac disease needs to be carefully considered in patients with cerebellar ataxia, epilepsy, attention/memory impairment or peripheral neuropathy.

Key words: Anti-neuronal antibody; central nervous system diseases; coeliac disease; peripheral nervous system diseases

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Coeliac disease (CD) can be associated with a wide variety of central and peripheral nervous system (CNS and PNS) disorders, such as epilepsy, myoclonus, cerebellar ataxia, multifocal leukoencephalopathy, dementia and peripheral axonal and demyelinating neuropathies (1–4). Gastrointestinal symptoms often lead to the recognition of CD before the onset of neurological disorders (5). However, when neurological symptoms appear early in the course of CD, conditions such as epilepsy (with or without occipital calcifications) or cerebellar ataxia (6, 7) may provide the only clue for suspecting the presence of an occult gluten-sensitive enteropathy. In most cases, the course of the neurological dysfunction is relentless, and only a minority of epileptic and ataxic CD patients have shown improvement after the introduction of a gluten-free diet (6, 8, 9).

The data regarding circulating anti-neuronal antibodies in CD patients with neurological disorders are conflicting. Antibodies reacting with human brain vessel structures that were reported in the sera of untreated CD patients with neurological involvement significantly decreased following gluten withdrawal (10). Recently, however, some groups failed to confirm the existence of antibodies to monkey cerebellum in the same subset of CD patients (11, 12).

In a large series of consecutive adult CD patients, we investigated the prevalence of neurological disorders identified before or after diagnosis of CD. We examined the clinical course of the neurological disorder, particularly in relation to more or less prompt start of gluten-free diet, and anti-neuronal antibodies. We also investigated the prevalence of anti-neuronal antibodies to the CNS (i.e. cerebellar cortex neurons) and to the enteric nervous system in CD patients with and without neurological disorders (and controls) before and after gluten withdrawal.

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Methods

Patients

This study included 160 consecutive CD patients (120 F and 40 M; median age 37 years, range 18–79 years) diagnosed in our two Departments from January 1985 to June 2001, with a median follow-up of 6 years (range 3 months to 16 years). The majority of these subjects came from the Emilia-Romagna region (which is located in the north-east of the Italian peninsula), where the estimated prevalence of CD is 1 case out of 174 inhabitants (13). CD-related antibodies (anti-gliadin (AGA), anti-endomysial (EmA) and anti-human recombinant tissue transglutaminase (h-TTG)) were tested in all patients (14, 15). Diagnosis of CD was confirmed by endoscopic duodenal biopsy. Histological findings were graded according to Marsh’s revised criteria (16, 17). A formal neurological assessment was routinely performed in all CD patients on presentation, including analysis of the time of onset of any neurological symptoms with respect to diagnosis of CD. Periodical dietary interviews were routinely performed in all CD patients to assess compliance with gluten-free diet. All patients gave their informed consent to participate in the present study.

Anti-neuronal antibodies

The presence of anti-neuronal antibodies to CNS and enteric nervous system (ENS) was investigated in all CD patients with neurological involvement and in a group of CD patients without neurological dysfunction (n = 20). Sera from 20 patients with intestinal and autoimmune diseases (i.e. 10 with inflammatory bowel disease and 10 with autoimmune hepatitis) and 10 blood donors served as controls. Anti-neuronal antibodies were detected by indirect immunofluorescence on 5-μm cryostat sections of monkey and rat cerebellum as well as of rat ileum and colon (Medic, Turin, Italy). Patients’ sera were tested at the initial dilution of 1:10 and, when positive, were titrated up to the end-point. Rabbit anti-human IgG and IgA (Dako, Copenhagen, Denmark) were used as secondary antibody at the appropriate working dilution (1:60 and 1:100 on rat and monkey tissue, respectively).

Statistics

The Fisher exact test, two-tailed, was used to compare clinical findings and prevalence of anti-neuronal antibodies in CD patients with and without neurological disorders.

Results

A neurological syndrome was recorded in 13 of the 160 (8%) CD patients (11 F and 2 M; median age at diagnosis of CD, 36 years, range 19–56 years). In 11 of the 13 cases, the neurological dysfunction presented before diagnosis of CD. Ten patients had disorders of the CNS, including epilepsy (n = 3), attention/memory impairment (n = 3), cerebellar ataxia (n = 2), multiple sclerosis (n = 1) and acute cerebrovascular disease (Moyamoya disease) (n = 1). The remaining three patients had PNS disorders, namely peripheral neuropathy (n = 2) and myotonic dystrophy (Steinert’s disease) (n = 1) (Table I).

CD patients with CNS disorders

In the three patients with epilepsy (all females), CD was diagnosed 6, 9 and 264 months after discovery of the neurological dysfunction (Table I). These patients had been

Table I. Clinical, immunological and histological features of the 13 CD patients with associated neurological disorders

<table>
<thead>
<tr>
<th>Cases</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Neurological disease (ND)</th>
<th>Time of ND before or after CD diagnosis</th>
<th>Gastrointestinal and other CD-related signs</th>
<th>IgA AGA &gt; 1:10</th>
<th>IgA EmA &gt; 1:5</th>
<th>IgA h-TTG &gt; 7AU</th>
<th>Duodenal histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>F</td>
<td>Multiple sclerosis</td>
<td>48 months after</td>
<td>Absent</td>
<td>1:40</td>
<td>1:80</td>
<td>15 AU</td>
<td>3a</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>F</td>
<td>Epilepsy</td>
<td>6 months before</td>
<td>Constipation</td>
<td>1:160</td>
<td>1:160</td>
<td>&gt;20 AU</td>
<td>3b</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>F</td>
<td>Epilepsy</td>
<td>264 months before</td>
<td>Absent</td>
<td>1:80</td>
<td>1:160</td>
<td>18 AU</td>
<td>3b</td>
</tr>
<tr>
<td>4</td>
<td>47</td>
<td>F</td>
<td>Epilepsy</td>
<td>6 months before</td>
<td>Iron-deficiency anaemia</td>
<td>1:20</td>
<td>1:80</td>
<td>12 AU</td>
<td>3b</td>
</tr>
<tr>
<td>5</td>
<td>37</td>
<td>F</td>
<td>Cerebellar ataxia</td>
<td>24 months before</td>
<td>Iron-deficiency anaemia</td>
<td>1:160</td>
<td>1:320</td>
<td>&gt;20 AU</td>
<td>3c</td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>F</td>
<td>Cerebellar ataxia</td>
<td>3 months before</td>
<td>Absent</td>
<td>1:10</td>
<td>1:20</td>
<td>8 AU</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>36</td>
<td>M</td>
<td>Attention/memory impairment</td>
<td>4 months before</td>
<td>Raised serum transaminases</td>
<td>1:20</td>
<td>1:160</td>
<td>&gt;20 AU</td>
<td>3b</td>
</tr>
<tr>
<td>8</td>
<td>32</td>
<td>F</td>
<td>Attention/memory impairment</td>
<td>5 months before</td>
<td>Constipation</td>
<td>1:80</td>
<td>1:160</td>
<td>&gt;20 AU</td>
<td>3b</td>
</tr>
<tr>
<td>9</td>
<td>34</td>
<td>F</td>
<td>Attention/memory impairment</td>
<td>6 months before</td>
<td>Absent</td>
<td>1:10</td>
<td>1:10</td>
<td>9 AU</td>
<td>3b</td>
</tr>
<tr>
<td>10</td>
<td>19</td>
<td>F</td>
<td>Moyamoya disease</td>
<td>120 months after</td>
<td>Diarrhoea</td>
<td>1:80</td>
<td>1:160</td>
<td>14 AU</td>
<td>3b</td>
</tr>
<tr>
<td>11</td>
<td>56</td>
<td>M</td>
<td>Steinert’s disease</td>
<td>36 months before</td>
<td>Diarrhoea</td>
<td>1:10</td>
<td>1:20</td>
<td>9 AU</td>
<td>3a</td>
</tr>
<tr>
<td>12</td>
<td>38</td>
<td>F</td>
<td>Peripheral neuropathy</td>
<td>4 months before</td>
<td>Absent</td>
<td>1:10</td>
<td>1:80</td>
<td>16 AU</td>
<td>3b</td>
</tr>
<tr>
<td>13</td>
<td>46</td>
<td>F</td>
<td>Peripheral neuropathy</td>
<td>10 months before</td>
<td>Absent</td>
<td>1:40</td>
<td>1:80</td>
<td>11 AU</td>
<td>3b</td>
</tr>
</tbody>
</table>

CD = coeliac disease; AGA = antigliadin; EmA = antiendomysial; h-TTG = human recombinant tissue transglutaminase antibodies; AU = arbitrary units; duodenal histology: Marsh’s revised classification 1 († intraepithelial lymphocyte count -IEL), 2 († IEL and crypt hyperplasia), 3a, 3b, 3c (mild, marked, total villous atrophy).
classified as having, respectively, complex partial temporal lobe seizures at the age of 20 years, simple partial seizures (with motor signs) at the age of 46 years and primary generalized seizures with pure absence (petit mal) at the age of 3 years. Brain computerized tomography (CT) scan was normal in all of them and showed no occipital calcification. Symptoms suggesting CD (severe constipation and iron-deficiency anaemia) were present in two patients, whereas the patient with long-standing epilepsy completely lacked non-neurological symptoms. A gluten-free diet stopped the seizures in the two patients with symptomatic CD, whereas it did not affect the seizures of the third patient.

Of the two patients (both female) with cerebellar ataxia, a 39-year-old woman had a neurological onset characterized by muscle weakness and pain associated with leg and arm paraesthesias. After a few months the patient began to complain of impaired balance. A muscle biopsy showed a type-2 fibre hypotrophy. Despite the absence of gastrointestinal and other CD-related signs, the patient underwent a wide array laboratory screening revealing IgA EmA positivity. The following duodenal biopsy, performed only 3 months after the neurological onset, proved the presence of CD. This patient had a striking response to gluten-free diet with complete resolution of the neurological symptoms. By contrast, the clinical outcome of the other patient who presented with cerebellar ataxia was unfavourable. She was a 37-year-old woman with severe gait ataxia, slurred speech and dysphagia. Her symptoms progressed rapidly, so that she needed bilateral support and later a wheelchair to ambulate. CD, which was suspected due to the finding of a severe iron-deficiency anaemia and autoimmune thyroiditis, was diagnosed only 24 months after the onset of neurological impairment. Gluten-free diet associated with high-dose vitamin E treatment was completely unhelpful, as was prednisolone and azathioprine treatment.

Three patients (1 M and 2 F) presented a neurological syndrome characterized by loss of concentration along with attention/memory impairment, which started 4–6 months before diagnosis of CD. The assessment of attention/memory impairment was carefully evaluated through a thorough neuropsychological examination before and after a gluten-free diet (1). All three patients had a normal brain CT or magnetic resonance imaging (MRI) scan. In one of them (a 36-year-old man), CD was suspected owing to raised serum transaminase levels of unknown origin and in another one (a 32-year-old woman) owing to a severe constipation. The third patient was affected by a potential CD, characterized by positivity for EmA and tTG at low titre and mild histological lesions (increased number of intraepithelial lymphocytes) (16, 17). The gluten-free diet brought a substantial improvement in neurological symptoms in all 3 patients within 1 year.

In the remaining two patients with CNS disorders, diagnosis of CD preceded the onset of neurological symptoms. A 24-year-old woman was identified as having CD during a screening programme of first-degree relatives. She had no gastrointestinal sign and her biochemical parameters were all within the normal range. Her compliance with the gluten-free diet was poor due to the asymptomatic state. Four years later, she developed diplopia, upper motor neuron dysfunction (right hand weakness and spasticity) and posterior column lesion (tingling or tightness of the extremities and band-like sensation along the trunk). MRI showed demyelinating multifocal white matter lesions in the brainstem and spinal cord. The patient became compliant with the gluten-free diet without any improvement in her neurological disease, which later partially responded to high-dose steroids and β-interferon treatment. The other patient was found to have CD at the age of 19 years due to a malabsorption syndrome. Ten years later, she developed a cerebral infarction with a transient left hemiplegia. Brain CT and MRI scans showed a large right fronto-temporal ischaemic area and small ischaemic lesions in the left hemisphere. Brain angiography led to a diagnosis of Moyamoya disease based on the typical ‘puff of smoke’ image indicating collateral flow circulation around the middle cerebral occlusive lesion. During the following 12 years, she strictly adhered to a gluten-free diet in association with salicylic acid, and had no recurrence of acute cerebrovascular disease.

**CD patients with PNS disorders**

Two of the three patients with PNS impairment were women. They were aged 38 and 46 years at the onset of neurological dysfunction, which preceded diagnosis of CD by 4 and 10 months, respectively (Table 1). Neither of them complained of gastrointestinal nor other CD-related signs. The younger woman complained of muscle pain and leg paraesthesias. Although electroneuromyography was normal, neurological assessment led to a diagnosis of small fibre neuropathy, and neurological symptoms significantly improved 1 year after gluten withdrawal. In the older woman, a distal axonopathy was diagnosed based on clinical symptoms (i.e. intense leg paraesthesias, numbness and hyporeflexia) and electroneuromyography results. A gluten-free diet did not affect the neurological symptoms. In the remaining patient (a male), Steinert’s myotonic dystrophy was diagnosed at the age of 53 years. The clinical picture was characterized by weakness of eyelid, facial and neck flexor muscles, and of distal extremity muscles. Myotonia was demonstrable in hand grip. CD was diagnosed later because of a malabsorption syndrome. A gluten-free diet did not improve the neurological picture.

**Anti-neuronal antibodies**

Anti-neuronal antibodies to CNS were detected in 8 of the 13 (61%) CD patients with neurological involvement (1 with multiple sclerosis, 2 with epilepsy, 2 with attention/memory impairment, 2 with cerebellar ataxia, and 1 with myotonic dystrophy), as compared with only 1 of 20 (5%) patients without neurological symptoms. No positive staining was found in control subjects. The prevalence of anti-neuronal
antibodies to CNS was significantly higher in patients with neurological symptoms than in those without neurological symptoms ($P = 0.0007$) or controls ($P = 0.00001$) (Table II). All the positive cases belonged to the IgG class (associated with IgA in 2 patients) with antibody titres ranging from 1:50 to 1:200. The anti-neuronal antibody pattern was characterized by nuclear and cytoplasmic positivity of Purkinje cells (Fig. 1A and B) and nuclear staining of granular layer neurons (Fig. 1A) of both monkey and rat cerebellar cortex. No immunolabelling was visualized around blood vessels. In 6 of the 8 positive neurological patients, anti-neuronal antibodies to CNS disappeared after 1 year of a strict gluten-free diet; in 5 out of 6 cases, this was accompanied by improvement/disappearance of neurological symptoms (Fig. 2).

No significant difference was found in the prevalence of anti-neuronal antibodies to the ENS in patients and controls (Table II). These antibodies were found in two CD patients with neurological symptoms (one with epilepsy and one with attention/memory impairment), in one patient without neurological dysfunction, and in one patient with Crohn disease. The three positive CD patients all complained of severe constipation. The antibody pattern was characterized by a

<table>
<thead>
<tr>
<th>Cases</th>
<th>NA to central nervous system (CNS) (%)</th>
<th>NA to enteric nervous system (ENS) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A - Coeliac disease with neurological dysfunction</td>
<td>13</td>
<td>61</td>
</tr>
<tr>
<td>B - Coeliac disease without neurological dysfunction</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>C - Autoimmune hepatitis</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>D - Inflammatory bowel disease</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>E - Blood donors</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

NA to CNS in A versus B: $P = 0.000686$; A versus C + D + E: $P = 0.000009$.
NA to ENS in A versus B and in A versus C + D + E: $P =$ ns (Fisher exact test).

Table II. Prevalence of anti-neuronal antibodies (NA) in CD with and without neurological disorders and in controls

Fig. 1. Representative photomicrographs showing anti-neuronal antibody immunoreactivity in the rat cerebellum (A, B) and enteric nervous system (C) of patients with CD and neurological involvement. Picture A shows intense immunostaining in the nucleus and cytoplasm of Purkinje cells (arrow) along with positivity of granular layer neurons (arrowhead) in a CD patient with cerebellar ataxia (patient no. 5 listed in Table I). Picture B displays immunolabelling of Purkinje cell cytoplasm in a CD patient with multiple sclerosis (patient no. 1). Picture C illustrates a bright staining in the myenteric plexus (m.p.) of the rat ileum observed in a CD patient with attention/memory impairment and severe constipation (patient no. 8). Picture D indicates negative anti-neuronal antibody pattern in a control (blood donor) subject; arrow points to an unlabelled Purkinje cell. Magnification $\times40$ in A and D; $\times80$ in B and C.
bright cytoplasmic fluorescence detectable in ganglion cell bodies of the myenteric (Auerbach’s) (Fig. 1C) and sub-mucous (Meissner’s) plexus.

Discussion

Growing evidence indicates that CNS and PNS diseases can be strictly associated with CD (1–9), and that neurological symptoms are sometimes the only clinical clue of an occult CD (18, 19). Our study sheds light on the clinical and immunological features of CD with neurological involvement. We found that among 160 CD patients, 13 (8%) had neurological disorders. The neurological dysfunction comprised a wide variety of conditions including epilepsy, loss of concentration and attention/memory impairment, cerebellar ataxia, peripheral neuropathy, multiple sclerosis, Steinert’s myotonic dystrophy and acute cerebrovascular (i.e. Moyamoya) disease. Previous studies already established close associations of CD with epilepsy (most frequently with complex partial seizures) (5, 6), cerebellar ataxia (7, 9, 12, 19), memory impairment (1) and peripheral neuropathy (18). An association with multiple sclerosis has been documented only in sporadic case reports (20–22). In the present paper, we also describe associations with Steinert’s myotonic dystrophy and Moyamoya disease. Although clinically relevant, the occurrence of these two rare disorders in our CD patients may have been casual. Therefore, the significance of these associations with CD requires further investigation.

Clinically, no significant difference was found between CD patients with and without neurological involvement. Both groups showed a similar median age at diagnosis of CD and a higher prevalence of female gender. Gastrointestinal and other CD-related signs, such as iron-deficiency anaemia, were absent in about half of both the neurological and non-neurological CD patients. In all but two cases, who developed multiple sclerosis and Moyamoya disease during gluten-free diet, the diagnosis of neurological impairment preceded the identification of CD.

Our data confirm the importance of early recognition of CD, since a gluten-free diet, started promptly on appearance of the neurological dysfunction can produce a significant improvement or even complete resolution of neurological symptoms (8, 9). This was observed in 7 patients (2 with epilepsy, 3 with memory impairment, 1 with cerebellar ataxia and 1 with peripheral neuropathy) who started gluten restriction within 6 months of the neurological onset. In contrast, four patients with long-standing neurological disorders (one with epilepsy, one with cerebellar ataxia, one with Steinert’s disease, and one with peripheral neuropathy) showed no response to gluten withdrawal.

The pathophysiology of neurological dysfunction in CD is still unclear. One hypothesis regards an overt or even subclinical malabsorption causing deficiencies of nutrients (folic acid and vitamin B12) known to exert neurotrophic and neuroprotective effects (23). However, only a minority of our neurological patients (2 out of 13) had an overt malabsorption syndrome. Selective vitamin E deficiency has also been suggested as a potential cause of neurological complications of CD (24), although administration of large doses of this vitamin in CD ataxic patients did not improve the clinical picture (25). Neurological involvement in CD might arise as a consequence of an autoimmune mechanism mediated by AGA (7, 26). In this respect, CD can be considered an autoimmune disease where, unusually, several pathogenetic factors are well known, i.e. the extrinsic trigger (gliadin), a close genetic background (HLA-DQ2 or -DQ8), and a highly specific immune response directed to a well-characterized autoantigen (tissue transglutaminase). Our data on the presence of anti-neuronal CNS antibodies provide further support for the autoimmune hypothesis of neurological dysfunction in CD patients. In our CD patients, the presence of these antibodies was strongly associated with CNS involvement. Furthermore, the antibodies most often (in 6 out of 8 cases) disappeared within a year of starting a gluten-free diet, and their disappearance was frequently (in 5 out of 6 cases) accompanied by improvement or disappearance of the neurological picture. Anti-neuronal antibodies are not specific for neurological disorders associated with CD, since they have been described in a wide array of CNS and autonomic nervous system disorders, such as cerebellar ataxia, subacute peripheral sensory-motor neuropathy, and others (27). The actual pathogenetic significance of these anti-neuronal antibodies is currently unknown. Specifically designed in vitro studies aimed at testing the effects evoked by anti-neuronal antibodies on brain cell tissue culture should clarify whether these antibodies may play a direct role in producing neurological damage. Neuropathological findings in CD patients, including cerebellar lymphocytic infiltration, Purkinje cell loss and posterior columns damage, provide further

Fig. 2. Titre of anti-neuronal CNS antibodies before and after gluten-free diet in CD patient responders (improvement of neurological symptoms) or non-responders to gluten withdrawal (persistence of neurological symptoms). Positive cases were defined at dilution titre >1:10 (broken line). CNS = central nervous system; GFD = gluten free diet.
evidence of an immune-mediated mechanism underlying neural dysfunction in CD (2, 9, 23).

In conclusion, the present study strongly reinforces the concept that CD can sometimes present in the guise of a neurological disorder. Our findings suggest that in such cases the neurological picture can greatly improve when a gluten-free diet is started promptly. Closer interaction between neurologists and gastroenterologists should facilitate earlier identification of an underlying CD. Therefore, the possible presence of CD needs to be carefully considered in patients with cerebellar ataxia, epilepsy, attention/memory impairment or peripheral neuropathy.

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