A prospective study of the prevalence of undiagnosed coeliac disease in laboratory defined iron and folate deficiency

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Aims: To determine the prevalence of coeliac disease in a group of patients in the community who have been shown in the laboratory to have iron and/or folate deficiency. To assess the cost efficiency of this laboratory based case finding strategy.

Methods: The study was undertaken in a large general hospital in the UK serving a population of 300 000. Three hundred and thirty three eligible patients with iron and/or folate deficiency were identified and contacted over an 18 month period. Case finding was by testing for coeliac disease using serological methods and subsequent histological confirmation.

Results: Of the 333 eligible and contactable patients with iron and/or folate deficiency, 258 (77%) consented to coeliac disease antibody testing. Twenty eight patients (10.9%) were positive for coeliac disease antibodies. Of these, 24 patients proceeded to endoscopy and biopsy, resulting in 12 cases of histologically confirmed coeliac disease (4.7% (95% confidence interval, 2.1% to 6.8%) of patients tested for coeliac disease antibodies).

Conclusions: This laboratory based methodology detected a considerable number of new coeliac disease cases in the community. Many of these patients did not present with clinical findings suggestive of malabsorption and might not otherwise have been diagnosed. Laboratory based methodologies should be considered in conjunction with other strategies for the early identification and treatment of coeliac disease.

METHODS

Study design and participants

Over an 18 month period all patients in the community with iron and/or folate deficiency identified in the departments of haematology and biochemistry at York District Hospital were considered for inclusion in our study. Exclusion criteria were as follows:

1. Age less than 16 years or greater than 80 years.
2. Hospital outpatients or inpatients.
3. Previous investigation for coeliac disease.
4. Known coeliac disease.
5. Unavailability of an adequate laboratory sample for coeliac antibody testing.

Eligible patients were contacted to obtain consent for coeliac antibody testing on a stored blood sample used previously for ferritin and/or folate estimations. The request for consent was accompanied by an explanation of coeliac disease and the reason for screening. For consenting patients, laboratory testing for coeliac antibodies (as described below) was undertaken. For patients with positive coeliac disease antibody tests the general practitioner was contacted with a recommendation for referral to the hospital gastroenterology department for further investigations. All general practitioners had been contacted previously to inform them of our study and to emphasise that coeliac disease antibody testing should not deter other investigations for iron deficiency or folate deficiency of unknown cause. Patients who attended the gastroenterology department and gave consent proceeded to endoscopic duodenal biopsy and histological assessment. Patients with confirmed coeliac disease were counselled, started on a gluten free diet, and followed up in the gastroenterology outpatient department.
It is important to emphasise that case finding was not initiated in the community but was prompted in the laboratory by the finding of a low ferritin or serum folate value following a request to the laboratory for these tests to be performed.

Laboratory investigations
All investigations were performed in the laboratories at York District Hospital. All were performed in accordance with national and local quality assurance schemes using the following methodologies.

Serum ferritin
This was performed using a Nichols chemiluminescent assay. This is a double antibody sandwich assay with polyclonal goat antiferritin antibody. Normal ranges: male patients, 20–385 ng/ml; female patients: premenopause, 20–99 ng/ml; postmenopause, 20–345 ng/ml.

Serum folate
This was performed by radioimmunoassay (Simultrac; Becton-Dickinson). Normal range: > 2 ng/litre.

Coeliac disease antibody testing
Both IgA and IgG antigliadin and antiendomysial antibodies were measured to maximise the identification of coeliac disease cases.

IgA and IgG antigliadin antibodies
Antigliadin IgA and IgG antibodies were measured using a UniCAP™ 100 (Pharmacia, Milton Keynes, UK) analyser by fluoroenzymeimmunoassay. Purified gliadin covalently bound in the solid phase to immunoCAPs reacts with specific IgA or IgG antibodies in diluted patients' serum, which bind to the immobilised antigen. Unbound non-specific IgA/IgG is washed away and enzyme labelled antisera (rabbit anti-human IgA conjugated to β-glactosidase or mouse anti-human IgG conjugated to β-galactosidase) was added to each immunoCAP to form a complex. After incubation, unbound enzyme anti-IgA/IgG was washed away and the remaining enzyme activity was measured by incubating the bound complex with a developing agent (4-umbelliferyl-β-D galactoside). The reaction was stopped with sodium carbonate and the fluorescence eluted from the immunoCAPs was measured at 445 nm. The test response was compared directly with the response for calibrators and expressed in mg/litre by Rodbar 5-parameter calculation.

Cut off ranges for positivity: IgA, > 3.0 mg/litre; IgG, > 8 mg/litre.

Anti-endomysial antibodies
Antibodies (IgA/IgG) to endomysium were detected by indirect immunofluorescence. Diluted serum was applied to sections of monkey oesophagus (Biodiagnostics, Upton upon Severn, Worcestershire, UK). Endomysial antibodies in the serum bound to this specific antigen on the tissue. Immunoglobulin (rabbit anti-human IgA or IgG) conjugated to fluorescein isothiocyanate (FITC; Dako, Ely Cambridgeshire, UK) was used as a capture agent to detect the presence of bound antibodies. Antibodies were visualised using a fluorescent microscope fitted with a high pressure mercury bulb (Labophot-2; Nikon, Kingston upon Thames, Surrey, UK). FITC labelled substrate tissue was excited by light from the mercury lamp at a wavelength of 490 nm and emitted light at a wavelength of 525 nm. Two observers performed the microscopy.

The total cost for each test including reagent costs and staff time (Welcan units) was £2.87 for IgA and IgG anti-endomysial antibody testing and £11.48 for IgA and IgG antigliadin antibody testing. Trust overheads were not included.

Clinical and histological investigations
It was recommended that patients with possible coeliac disease on the basis of the detection of coeliac autoantibodies were referred for investigation to the department of gastroenterology, subject to the consent of the patient and their general practitioner. In the absence of contraindications, further investigation for coeliac disease was undertaken with endoscopy and multiple duodenal biopsies. The histological diagnosis of coeliac disease was made on accepted morphological criteria. All patients with confirmed coeliac disease were counselled, started on a gluten free diet, and followed up according to normal practice in the department of gastroenterology.

Consent and ethical considerations
The study was approved by the local research ethics committee. General practitioners consented to the entry of their patients into the study and all patients were antibody tested and referred for further investigations only after their consent. The study did not interfere with the normal investigation and management of iron or folate deficiency. Information sent to patients made it clear that they could potentially benefit from early diagnosis of coeliac disease as the cause of their iron or folate deficiency, but that non-participation would not otherwise affect their management.

RESULTS
Study participants
Over an 18 month period, 345 eligible patients with iron and/or folate deficiency were identified. Twelve patients were non-contactable and the remaining 333 were asked for consent for coeliac disease antibody testing. Two hundred and fifty eight (77%) of the contacted patients gave consent for coeliac disease antibody testing (26 male and 232 female patients; median age, 47 years; age range, 16–80). Two hundred and forty seven patients had iron deficiency alone, 10 folate deficiency alone, and one combined iron and folate deficiency.

Coeliac disease antibody testing
Table 1 summarises the results of coeliac antibody testing. Of the 258 tested patients, 28 (10.9%) were positive for at least one coeliac disease associated antibody.

Further investigation of coeliac disease antibody positive cases
Twenty four of the 28 patients identified as having positive coeliac disease antibodies proceeded to endoscopy and biopsy (table 1). The reasons for not proceeding to endoscopy were as follows:

• Significant coexistent disease: two patients.
• Warfarin treatment contraindicating biopsy: one patient.
• Patient unwilling to proceed to endoscopy: one patient.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>n (%)</th>
<th>Endoscopy (n)</th>
<th>Histologically confirmed CD (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>End + GL</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>GL only</td>
<td>16</td>
<td>28 (10.9%)</td>
<td>13</td>
</tr>
<tr>
<td>End only</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>None</td>
<td>230</td>
<td>89.1%</td>
<td>12</td>
</tr>
</tbody>
</table>

Total 258 24 12

CD, coeliac disease; End, endomysial; GL, gliadin.
Twelve of the 24 patients who underwent endoscopy were found to have histologically confirmed coeliac disease. The relation to coeliac disease antibody positivity is illustrated in Table 1. All 11 patients with endomysial antibody positivity who proceeded to endoscopy and biopsy were found to have coeliac disease. In contrast, of the 13 patients with gliadin only antibody positivity who proceeded to endoscopy only one was found to have histologically confirmed coeliac disease. One patient with iron deficiency and endomysial antibody positivity declined endoscopy.

Clinical and laboratory characteristics of patients with histologically confirmed coeliac disease

Table 2 summarises the clinical and laboratory characteristics of the 12 patients with histologically confirmed coeliac disease. The clinical details accompanying the initial laboratory request were variable and in no case were they specifically suggestive of coeliac disease. Most patients were women (10 of the 12 cases) and the age range for the group was wide (32–74 years). Eleven patients were iron deficient and only one was folate deficient. Nine of the 12 patients had at least one blood count parameter outside the reference range (either haemoglobin concentration, mean corpuscular volume, or red cell distribution width). However, only seven had a haemoglobin concentration below the normal reference range and only one was severely anaemic (haemoglobin, < 100 g/litre). The number of cases of histologically confirmed coeliac disease represented 4.7% (95% confidence interval, 2.1% to 6.8%) of the patients consenting for coeliac disease antibody testing (4.4% for iron deficiency, 9% for folate deficiency).

DISCUSSION

It has been suggested that patients with isolated iron or folate deficiency should be referred directly for endoscopic duodenal biopsy. However, at present many of these patients are not referred at all for investigation for coeliac disease. Endomysial antibody testing may offer a more acceptable and less invasive alternative request at all for investigation for coeliac disease. Endomysial antibody testing may offer a more acceptable and less invasive alternative request for coeliac disease. The clinical details accompanying the initial laboratory request were variable and in no case were they specifically suggestive of coeliac disease. Most patients were women (10 of the 12 cases) and the age range for the group was wide (32–74 years). Eleven patients were iron deficient and only one was folate deficient. Nine of the 12 patients had at least one blood count parameter outside the reference range (either haemoglobin concentration, mean corpuscular volume, or red cell distribution width). However, only seven had a haemoglobin concentration below the normal reference range and only one was severely anaemic (haemoglobin, < 100 g/litre). The number of cases of histologically confirmed coeliac disease represented 4.7% (95% confidence interval, 2.1% to 6.8%) of the patients consenting for coeliac disease antibody testing (4.4% for iron deficiency, 9% for folate deficiency).

Table 2 Characteristics of 12 patients with histologically confirmed coeliac disease

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex (M/F)</th>
<th>Age (years)</th>
<th>Deficiency (Fe/Fol)</th>
<th>Hb (g/l)</th>
<th>MCV (fl)</th>
<th>RDW</th>
<th>Clinical details</th>
<th>CD antibodies positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>60</td>
<td>Fe</td>
<td>105*</td>
<td>91</td>
<td>16.4*</td>
<td>Post viral infection</td>
<td>End, GL</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>54</td>
<td>Fe</td>
<td>124</td>
<td>88</td>
<td>13.9</td>
<td>Past history of anaemia</td>
<td>End, GL</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>57</td>
<td>Fe</td>
<td>90*</td>
<td>69*</td>
<td>18.9*</td>
<td>Iron deficiency, cause unknown</td>
<td>GL</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>64</td>
<td>Fe</td>
<td>127*</td>
<td>86</td>
<td>13.4</td>
<td>Malaise, cough</td>
<td>End, GL</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>39</td>
<td>Fe</td>
<td>127</td>
<td>89</td>
<td>18.4*</td>
<td>Iron deficiency, cause unknown</td>
<td>End, GL</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>64</td>
<td>Fe</td>
<td>114*</td>
<td>87</td>
<td>14.2</td>
<td>Fatigue</td>
<td>End, GL</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>32</td>
<td>Fe</td>
<td>102*</td>
<td>76*</td>
<td>14.9</td>
<td>Cold intolerance</td>
<td>End, GL</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>56</td>
<td>Folate</td>
<td>136</td>
<td>103*</td>
<td>16.2*</td>
<td>Previous anaemia</td>
<td>End, GL</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>47</td>
<td>Fe</td>
<td>112*</td>
<td>89</td>
<td>12.3</td>
<td>History of iron deficiency</td>
<td>End, GL</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>40</td>
<td>Fe</td>
<td>106*</td>
<td>74*</td>
<td>15.8</td>
<td>Iron deficiency, cause unknown</td>
<td>End, GL</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>74</td>
<td>Fe</td>
<td>122</td>
<td>93</td>
<td>15.1</td>
<td>Previous borderline anaemia</td>
<td>End, GL</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>54</td>
<td>Fe</td>
<td>142</td>
<td>89</td>
<td>13.6</td>
<td>Fatigue</td>
<td>End, GL</td>
</tr>
</tbody>
</table>

*Abnormal result.
CD, coeliac disease; End, endomysial; Fe, iron; Fol, folate; GL, gliadin; Hb, haemoglobin; MCV, mean corpuscular volume; RDW, red blood cell distribution width.

Take home messages

- Twelve (4.7%) of the 258 patients with iron and/or folate deficiency who consented to coeliac disease antibody testing had histologically confirmed coeliac disease
- Many of these patients did not present with clinical findings suggestive of malabsorption and might not otherwise have been diagnosed
- Laboratory based methodologies such as this should be considered in conjunction with other strategies for the early identification and treatment of coeliac disease

One disadvantage of this methodology is that it presumes a relatively high rate of detection of iron and folate deficiency in the community. In our study, many of the patients with iron deficiency had a normal haemoglobin concentration. It must be presumed that ferritin estimations were undertaken on the
basis of a presumptive diagnosis of anaemia on clinical grounds or perhaps because of a suspicion of nutritional deficiency. Many of the patients who were found to have coeliac disease on the basis of laboratory defined deficiency had no clinical symptoms suggestive of the disease.

This appears to be a highly cost effective method of detecting coeliac disease. The total laboratory cost of combined endomysial and gliadin antibody testing was £3733, giving a laboratory cost of approximately £300 for each case of coeliac disease detected. Gastroenterology referral, endoscopy, and biopsy were additional to this. Half of the endoscopies performed led to a diagnosis of coeliac disease. If coeliac disease serological testing had been limited to endomysial antibody testing then coeliac disease would have been detected in all patients in whom endoscopies were performed but one case would have been missed. The administrative costs of obtaining consent for coeliac antibody screening should not be underestimated. This is the case for all types of screening. A distinct advantage of this method compared with other screening protocols is that the blood sample for coeliac disease antibody testing is already available in the laboratory, allowing the rapid identification of patients at high risk of the disease. However, implementing routine testing for coeliac disease in all iron and folate deficient samples has important ethical implications, which would have to be explored carefully before adopting this strategy in routine clinical practice. Larger studies are needed to define the particular role of coeliac disease testing in folate deficiency and to determine whether the inclusion of gliadin antibody testing is justified.

ACKNOWLEDGEMENTS

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REFERENCES