ORIGINAL ARTICLE

Do we need to measure total serum IgA to exclude IgA deficiency in coeliac disease?

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Background: Screening for IgA deficiency in patients with coeliac disease is essential because of the increased incidence of IgA deficiency associated with the disease, which usually relies on the estimation of IgA levels in each case.

Aim: To devise a method of excluding IgA deficiency without measuring total serum IgA in each case.

Materials and methods: The optical density readings on enzyme-linked immunosorbent assay (ELISA) of 608 routine samples received for tissue transglutaminase (TTG) antibody testing for coeliac disease were compared with their total IgA concentrations. Dilution experiments were also carried out to ensure linear relationships between optical density on ELISA and IgA concentrations and to compare the sensitivities for TTG and endomysium antibodies in TTG-positive samples.

Results and discussion: A clear relationship was shown between total IgA concentration and TTG optical density readings by ELISA. To ensure a positive TTG result if antibodies are present, it was possible to recommend an optical density level above which all samples have sufficient IgA. Samples with optical density <0.05 should be investigated further by estimating total IgA and, if low, samples should be subjected to immunofluorescence microscopy testing for IgA and IgG endomysium antibodies.

Conclusions: An easier, more cost-effective and practical way of excluding IgA deficiency in the investigation on coeliac disease is reported.

Coeliac disease is an autoimmune condition characterised by intolerance to dietary wheat (gluten) intake. It affects as many as 1:100 people in the UK and is believed to be considerably underestimated worldwide because of a complex combination of genetic and environmental factors added to a varied clinical presentation.^{1 2}

Diagnosis still relies on a suggestive duodenal or jejunal biopsy result, but has been helped in recent years by the observation that IgA gliadin, endomysium IgA antibody (EMA) and, more recently, tissue transglutaminase (TTG) antibodies have a high sensitivity and specificity for the disease. Recent work has shown that IgA TTG can be used as an initial serological screen for coeliac disease, with EMA used to confirm any positive samples. Many of these studies also recommend that total serum IgA is estimated in the same samples to exclude coexisting IgA deficiency,^{1 3 4} but if laboratories do not adopt this measure, we risk missing patients having coeliac disease with IgA deficiency. Selective IgA deficiency is 10–20 times more common in patients with coeliac disease than in the rest of the population;⁵ hence, to exclude this, laboratories have a choice of either including an IgG-specific assay for TTG or endomysium antibodies, or measuring total serum IgA in every screening test. The detection of IgG endomysium antibodies in people who are selectively IgA deficient has a sensitivity of 0.83, a specificity of 0.80 and a positive predictive value of 0.925 according to Cataldo *et al.*⁶ We are not aware of any other large studies proving that the diagnostic sensitivity and specificity of IgG antibodies is acceptable in such patients. At present, however, there are no other serological routes we can take in the absence of an IgA response.

At the time of this study, we measured TTG and total IgA on all requests for coeliac disease screening. On finding a serum sample with low levels of total IgA—that is, below the reference range for age, we investigated this more fully by looking for IgG endomysium antibodies with immunofluorescence instead of relying solely on TTG. Earlier work has,

however, shown that, with this approach, the number of patients testing positive for coeliac disease is low.⁷ Our own study on the raw TTG ELISA results and on those of others^{8 9} suggested that it may be possible to devise an optical density read-out cut-off value for ELISA, above which there was no IgA deficiency. If this proved possible, it would mean that only those samples with optical densities below a certain level would need to be investigated further for both IgA deficiency and the presence of gut-related IgG antibodies. This was therefore a retrospective study on a large consecutive number of routine coeliac disease screening samples that compared their optical density values on ELISA with their total IgA concentration in an attempt to devise a protocol that would allow us to detect IgA deficiency without actually estimating the total serum IgA separately.

MATERIALS AND METHODS

Levels of TTG antibodies were estimated with the Sweden Diagnostics Celikey kit (Sweden Diagnostics, Milton Keynes, UK). Optical density units were obtained from the ELISA worksheets and compared with the total serum IgA obtained from the same samples. We have previously validated the method for our own use by looking for interference from raised levels of total IgA in patients with liver disease and IgA myeloma, and we found no positive TTG or EMA antibodies (data not shown).

From a total of 608 routine consecutive samples tested for TTG antibodies in a 2.5-month period, we measured TTG optical density values ranging from 0.010 to 3.322. In all, 220 samples had optical density <0.07 units for TTG. This value was chosen because no patient with an optical density >0.07 had a total IgA concentration <1 g/l. We used 3 Celikey units/ml (equivalent to an optical density = 0.3 units) as a cut-off between negative and positive for IgA. This cut-off

Abbreviations: ELISA, enzyme-linked immunosorbent assay; EMA, epithelial membrane antigen; TTG, tissue transglutaminase

was different from that recommended by Sweden Diagnostics who quoted an equivocal range of 7–10 Celikey units, and values >10 units were considered to be positive. This was done after in-house studies to determine our own reference range. Many patients with Celikey units lower than the manufacturer's cut-off of 10 units had clearly positive endomysium antibodies, and we could not therefore classify them as normal. Any results of >3.0 Celikey units are checked for EMA before their release to the clinician.

The TTG tests were carried out using a BEST 2000 ELISA processor (Launch Diagnostics/Dynex, London, UK). Total serum IgA was estimated using a Behring BNII nephelometer (Dade Behring, Milton Keynes, UK). IgG and IgA endomysium antibodies were detected by indirect immunofluorescence using a 1:2 dilution of patient serum and either a 1:500 dilution (ie, 2 µl/ml phosphate-buffered saline pH 7.3) of anti-human IgG fluorescein isothiocyanate-conjugated anti-serum or a 1:142 dilution (ie, 7 µl/ml phosphate-buffered saline pH 7.3) of anti-human IgA fluorescein isothiocyanate-conjugated anti-serum and with monkey oesophagus as a substrate (all from The Binding Site, Birmingham, UK).

The department performs satisfactorily in the UK National External Quality Assessment Service scheme for Coeliac Disease. Internal quality assurance performance during this period conformed to the manufacturer's guidelines. Ethical committee approval was not required for this study as only the tests requested by the original clinician were carried out as part of a standard investigative protocol.

RESULTS

Of the 608 samples studied, four patients had IgA deficiency (IgA <0.05 g/l). One of these was a patient with known coeliac disease, who was compliant on a gluten-free diet and had no IgA or IgG endomysium antibodies; the remaining three had no IgA or IgG endomysium antibodies and were not investigated further. One other patient had low IgA for age (0.25 g/l; age-related reference range 0.3–1.2 g/l), no IgA endomysium antibodies but positive IgG antibodies, and a subsequent biopsy result suggestive of coeliac disease.

Figure 1 shows the relationship between optical density readings on ELISA from the 220 samples with optical density <0.07 units. A clear relationship was observed between optical density reading and total serum IgA. If optical density is > 0.050 units on ELISA, then all total serum IgA values are >0.5 g/l. If a gut-related specific IgA antibody is present and the total serum IgA is >0.5 g/l, there should be sufficient IgA present to be able to demonstrate a TTG-positive antibody.⁷ None of the patients with IgA <1 g/l had an optical density >0.07 (details not shown). This shows that when specific antibodies are not present, the background optical density is related to the underlying IgA level. Spearman's rank coefficient studies showed a correlation of $r = 0.7200$ with a two-tailed $p < 0.0001$.

Figure 1 IgA concentration v optical density (OD) on tissue transaminase (TTG) assay.

Figure 2 IgA concentration plotted against the difference in optical density (OD) between sample tissue transaminase (TTG) and zero standard (std).

Figure 2 shows the relationship between optical density <0.07 on ELISA minus the zero standard optical density value and serum IgA. The coefficients of variation for these immunoassays were particularly poor, and this exercise was carried out to account for the effect of any batch-to-batch variation in the optical density of zero standard, and thus in the background optical density of a patient sample, and to assess whether this approach could detect low serum IgA concentrations better. In this case, a cut-off of optical density = 0.04 units could be used to identify patients with low IgA, but this approach included a larger number of people with normal IgA levels. These patients would have to be investigated further; however, as this approach does not increase the sensitivity to patients with low IgA, it does not seem justified.

Figure 3 shows the results of a study on a sample with normal IgA concentration = 1.05 g/l but negative IgA TTG or endomysium antibodies, which was diluted with serum from a patient with IgA deficiency (IgA <0.05 g/l). This further shows that the background optical density is related to the overall IgA concentration and appears linear below 1.0 g/l.

Table 1 shows the results of a study on five samples with positive IgA TTG antibody titre, which were diluted with serum from a patient with IgA deficiency (IgA concentration <0.05 g/l). This shows that the endomysium antibody is visible at dilutions below the threshold for detection of TTG antibody. Thus, in case of a specific IgA response to TTG, particularly in an IgA-deficient person, levels below the manufacturer's cut-off may be relevant. The contribution of baseline IgA to the overall optical density is reduced or absent in these patients compared with people with normal levels of IgA and in whom TTG results are typically used to determine reference ranges.

Figure 3 IgA concentration v optical density (OD) of anti-tissue transaminase (TTG).

Table 1 Five samples with positive tissue transaminase (TTG) antibodies diluted with IgA-deficient serum

TTG theory (Celikey units)	Measured TTG antibody (Celikey units)	TTG optical density (OD units)	EMA
10	10.2	0.936	Moderate
5	4.9	0.529	Moderate-weak
3	2.8	0.31	Weak
2	1.7	0.199	Weak
1	0.9	0.108	Very weak
18	17.4	1.58	Moderate
9	9.1	0.95	Moderate
4.5	4.4	0.52	Moderate
2	1.8	0.24	Weak
1	1	0.13	Very weak
26	26.3	1.99	Moderate
13	13.8	1.06	Moderate
5.2	5.1	0.58	Moderate
2.6	2.4	0.3	Weak
1.3	1.6	0.21	Very weak
5.8	5.8	0.64	Moderate
2.9	2.8	0.33	Weak
1.9	1.6	0.21	Weak
0.95	0.9	0.12	Very weak
0.47	0.5	0.08	Negative
8.1	8.1	0.84	Moderate
4	4.1	0.44	Moderate
2.1	2.2	0.29	Weak
1.0	1.1	0.15	Very weak
0.5	0.6	0.08	Negative

EMA, endomysium IgA antibody; OD, optical density; TTG, tissue transglutaminase.

DISCUSSION

Our study has shown that it is possible to predict those routine screens for people with coeliac disease that are likely to require further investigation on IgA deficiency by using the optical density values obtained by TTG ELISA. This removes the need to test for IgA deficiency by estimating the total serum IgA in every case.

The need to exclude IgA deficiency in patients for routine screening of coeliac disease is not in question, with the incidence being much higher (2.6%) in this population than in a normal population,¹⁻³ and various UK, Australian and US guidelines recommend the need to investigate the possibility.^{1-3,4} Both serum IgA estimation and the inclusion of an IgG TTG test are feasible options for this purpose, but these have to be carried out in addition to the routine IgA TTG estimation. The other possibility would be to measure IgG endomysium antibodies directly as a routine screen for patients with coeliac disease. Conflicting results on the diagnostic validity of IgG antibodies against gliadin, endomysium and TTG, however, have been reported.¹⁰⁻¹¹ These are expensive tests, and if there is a reliable predictive method using a single test that will detect patient samples which need further investigation, then this represents a more efficient strategy without adversely affecting patient care. The incidence of coeliac disease is such that some have suggested screening the population for latent or subclinical forms of the disease;¹² hence any measure that cuts the cost of this has to be taken seriously. The counter argument here is that screening for patients with latent disease requires asymptomatic patients who are less likely to adhere to a gluten-free diet, and therefore, why look for it in the first place? Whatever test or combination of tests is used, it must be reliable, especially with the suggestion that positive endomysium antibodies could obviate the need for duodenal biopsy in selected paediatric populations;¹³ this is not a view held by all, however.¹⁴

One patient in our series had an optical density = 0.014—that is, a negative TTG—and, after follow-up testing, showed

a detectable but low serum IgA = 0.25 g/l (age-related reference range 0.3–1.2 g/l). On the basis of the low optical density read-out, we looked for an IgG response and found a moderately positive IgG endomysium antibody. The results of the patient's jejunal biopsy showed characteristic villous flattening, and she is being treated for coeliac disease. Clearly though, it is not only patients who are truly IgA deficient who need to be investigated more fully; a low, but detectable IgA cannot exclude a false-negative coeliac screen. Therefore, patients with total IgA results lower than the age-related reference range should also be investigated with a stepwise approach, as advocated by Lock and Unsworth,⁷ of further serological testing before considering to proceed to more invasive procedures. This patient was the only one in this group to have undergone further investigation after this study. We have subsequently identified two more patients who were TTG or EMA negative but IgG endomysium antibody positive and were diagnosed with coeliac disease.

We found that if optical density values are >0.050 on ELISA, then all total serum IgA values are >0.5 g/l and this concentration is 10 times >0.05 g/l, which defines IgA deficiency.¹⁵ The idea of using IgA TTG antibodies as a surrogate screen for IgA deficiency has been suggested in two poster presentations.⁸⁻⁹ Data are often obtained from studies conducted in a research setting, and it is possible the tests will be less accurate when used in the routine clinical setting.¹⁶ Our study considers this with sequential samples taken from a routine clinical laboratory setting. One particularly useful study is that by Fernandez *et al*,¹⁷ who studied several clearly defined patient groups (IgA deficient, low serum IgA, healthy controls and a large cohort of pregnant women enrolled in a study on coeliac disease) and showed that a human IgA anti-TTG ELISA could not only detect cases of coeliac disease but could effectively screen for people with IgA deficiency. Our study shows that these findings also apply to an unscreened routine population, and those patients with subnormal IgA can also be identified and should be further investigated.

We suggest that the following algorithm be considered. If TTG optical density <0.05, a total serum IgA should be estimated. If total IgA is within the age-related reference range, the negative TTG antibody can be reported. If IgA is below the reference range, endomysium IgA and IgG should be assayed.

Take-home messages

- Screening for IgA deficiency in coeliac disease is essential because of the increased incidence of IgA deficiency associated with the disease.
- This is often done by measuring the total serum IgA concentration. A clear relationship was shown between total IgA concentration and tissue transglutaminase (TTG) antibody optical density by enzyme-linked immunosorbent assay and it was possible to recommend an optical density (OD) level above which all samples have sufficient IgA to ensure a positive TTG result if antibodies are present.
- Samples with OD <0.05 should be investigated further by estimating total IgA and, if low, immunofluorescence microscopy testing for IgA and IgG endomysium antibodies should be carried out.
- This represents a more efficient way of excluding IgA deficiency in coeliac disease.

Although it may seem illogical to measure an IgA endomysium antibody, such patients may still have a sufficiently large specific antibody response to produce detectable endomysial antibodies by indirect immunofluorescence, as our study has shown that these IgA are still detectable even if TTG is negative, unless patients are completely IgA deficient. Further, a false-positive TTG should be excluded, as shown by Lock *et al.*¹⁸ Our serial dilution studies have shown that if IgA endomysial antibodies are present, the EMA test is positive at levels as low as 1 unit/ml anti-TTG.

Laboratories should conduct an exercise to determine the optical density cut-off to use for their own population and, most certainly, if another kit manufacturer is used.

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