Screening for coeliac disease using anti-tissue transglutaminase antibody assays, and prevalence of the disease in an Australian community

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ABSTRACT

Objectives: To determine (i) the prevalence of positive results of anti-tissue transglutaminase (anti-tTG) antibody assays and coeliac disease (CD) in a rural Australian community; and (ii) whether confirmatory testing of a positive assay result with an alternative anti-tTG assay improved the positive predictive value of the test in population screening for CD.

Design: Retrospective analysis in December 2004 of stored serum samples taken in 1994–1995 from 3011 subjects in the Busselton Health Study follow-up. Assays for IgA and IgG anti-tTG antibodies were performed, and positive or equivocal samples were retested with a different commercial anti-tTG assay. Available subjects with one or more positive assay results were interviewed, had serum collected for repeat anti-tTG assays and for HLA-DQ2 and HLA-DQ8 haplotyping and, if appropriate, gastroscopy and duodenal biopsy were performed. In unavailable subjects, HLA-DQ2 and -DQ8 haplotyping was performed on stored sera. Total serum IgA levels were assessed in subjects with initially negative assay results.

Main outcome measure: Prevalence of anti-tTG positivity and biopsy-proven CD.

Results: In 47 of 3011 serum samples (1.56%), at least one anti-tTG assay gave positive results: 31 of the subjects who provided these sera were available for clinical review, and 21 were able to have a gastroscopy. Seventeen subjects (0.56%) were diagnosed with definite CD (14 were confirmed at gastroscopy, and three unavailable subjects had three positive results of anti-tTG assays and an HLA haplotype consistent with CD); in a further 12 unavailable subjects, CD status was considered equivocal, with one or more positive anti-tTG assay results and an HLA haplotype consistent with CD. If these subjects were regarded as having CD, the prevalence of CD would be 0.96%. The positive predictive value when all three anti-tTG assays gave positive results was 94%, but fell to 45.2% with only one positive result.

Conclusions: The prevalence of anti-tTG antibodies in this population is 1.56%; the prevalence of CD is at least 0.56%. The utility of a single, positive result of an anti-tTG assay in screening for CD in the community is poor, and repeat and/or collateral assessment with different assays may decrease the need for gastroscopy and distal duodenal biopsy.

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Coeliac disease (CD) is an autoimmune disease, triggered by the ingestion of gluten in genetically susceptible individuals, and causing an inflammatory reaction and damage to the small intestine. The diagnostic "gold standard" is the normalisation of the small intestinal mucosa when gluten is eliminated from the diet. Population studies have shown that CD is relatively common in white populations, with about 0.5%–1% estimated to have undetected CD.[1-3]

In 2001, our group published the results of anti-endomysial antibody (anti-EMA) testing of 3011 adults in the Busselton (Western Australia) population, finding a prevalence of CD of about 0.4% (1 : 250).[4] The Busselton Health Study comprises a series of cross-sectional general population surveys conducted every 3 years from 1966 to 1981, with a follow-up study of all available previous participants in 1994–1995. At this follow-up, serum samples suitable for serological and HLA testing were taken for long-term storage. These samples were used in our 2001 study.[4]

Studies of CD prevalence with anti-EMA testing have shown a wide variation in the sensitivity (74%–100%) and specificity (64%–100%) of this test, which may have arisen from differences in the laboratory techniques employed.[5-11] These variations suggest that anti-EMA testing may not be suitable as a screening tool. Testing for human anti-tissue transglutaminase (anti-tTG) antibodies appears to give more consistent results and is likely to be the best test for CD; initial reports have shown a sensitivity of 95%–98% and a specificity of 94%.[12,13] An even higher sensitivity (99.5%) and specificity (99.6%) have been achieved using recombinant human anti-tTG in a radioligand assay,[14] confirming the reliability of anti-tTG testing.[15]

The aims of our study were, first, to determine the prevalence of anti-tTG antibodies and CD in a predominantly Anglo-Celtic Australian rural community (the Busselton population) using a screening anti-tTG assay; and, second, to determine whether confirmatory testing of a positive screen result with an alternative anti-tTG assay improved the positive predictive value of the test in population screening for CD.

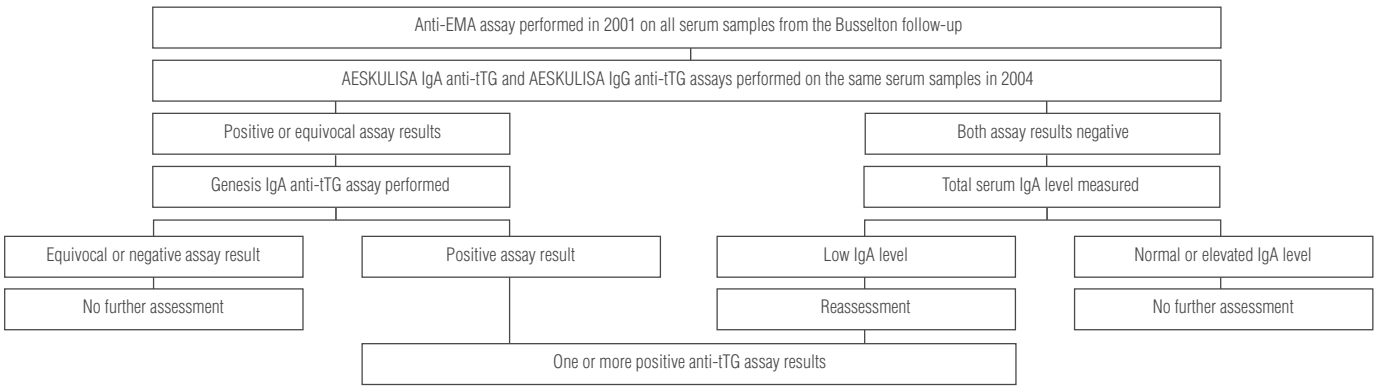
METHODS

Anti-tTG antibody assays

In December 2004, we performed AESKULISA IgA and IgG anti-tTG assays (AESKU.DIAGNOSTICS, Wendelsheim, Germany) on the 3011 stored serum samples of subjects attending the 1994–1995 Busselton Health Study follow-up (the same samples tested for anti-EMA in our 2001 study).[4]

Serum samples giving at least one positive result, or equivocal results, of the AESKULISA IgA anti-tTG assay (cut-off, > 10 U/mL) and the AESKULISA IgG anti-tTG assay (cut-off, > 15 U/mL) were tested with the Genesis IgA anti-tTG assay (cut-off > 7 U/mL; Genesis Diagnostics Ltd, Cambridge, UK). In samples with negative results of the anti-tTG assays, serum IgA levels were measured. The procedure is summarised in Box 1.

1 Flow diagram of procedure for screening stored serum samples* for anti-tissue transglutaminase (anti-tTG) antibodies



EMA = endomysial antibody. * Stored serum from subjects attending the 1994–1995 Busselton Health Study follow-up. ◆

Clinical review

Attempts were made to contact all subjects whose serum samples returned one or more positive results of three anti-tTG assays.

Subjects available for clinical review

Available subjects were reviewed by a gastroenterologist between December 2004 and July 2005, and a symptom questionnaire was completed. Blood was taken for repeat AESKULISA IgA/IgG anti-tTG and Genesis IgA anti-tTG testing; HLA-DQ2 and HLA-DQ8 haplotyping; full blood examination; measurement of serum ferritin, vitamin B_{12}, vitamin D and blood sugar levels; and liver function tests.

If there were no specific contraindications to gastroscopy (and no previous histological proof of CD status), subjects were advised to have a gastroscopy and distal duodenal biopsy.

Subjects not available for clinical review

In unavailable subjects, HLA-DQ2 and HLA-DQ8 haplotyping was performed on their stored sera.

Subjects with low IgA levels

If available for clinical review, subjects with low IgA levels were clinically assessed, had a repeat AESKULISA IgG anti-tTG assay and haplotyping performed. If appropriate, they were offered gastroscopy and distal duodenal biopsy. In unavailable subjects haplotyping was performed.

Definitions

Definite CD

- *Clinically reviewed and able to have a gastroscopy*

At least one positive result of an anti-tTG assay and confirmed duodenal changes of Marsh Classification type I or greater.[16]

- *Unable to be clinically reviewed*

Three positive results of antibody tests (IgA/IgG AESKULISA, Genesis or anti-EMA) and an HLA haplotype consistent with CD.

Equivocal CD

- *Unable to be clinically reviewed*

One or more positive results of anti-tTG assays and an HLA haplotype consistent with CD.

Ethics approval

Our study was approved by the Human Research Ethics Committee at Fremantle Hospital and the Busselton Population Medical Research Foundation.

RESULTS

Demographic characteristics

The 3011 serum samples came from 1491 women and 1520 men, with an age range of 20–79 years; 99% of subjects were of Anglo-Celtic origin.

Anti-tTG assay results

Forty-seven serum samples gave positive results for at least one anti-tTG assay, a prevalence of 1.56%. The serological and duodenal biopsy results of the 47 subjects with at least one positive result of anti-tTG assays are summarised in Box 2.

Clinical review

Sixteen of the 47 subjects whose serum samples gave positive results of anti-tTG assays were unable to be clinically reviewed: five had died, 10 had changed their address (ie, could not be contacted), and one was too ill to participate in our study.

The remaining 31 subjects had a clinical review, and 21 subjects were able to have a gastroscopy and distal duodenal biopsy. A further six subjects had already had a distal duodenal biopsy. Thus, in 27 subjects, the presence or absence of CD was proved by biopsy.

Definite CD

Seventeen subjects met our criteria for definite CD, giving a prevalence of at least 0.56%. Of these, six subjects had histologically proven CD diagnosed outside our study; eight were diagnosed with histologically proven CD during our study; and three had three positive results of antibody assays, and an HLA haplotype consistent with CD, but were not available for clinical review or gastroscopy. By our definition, they were also considered to have definite CD.

Equivocal CD

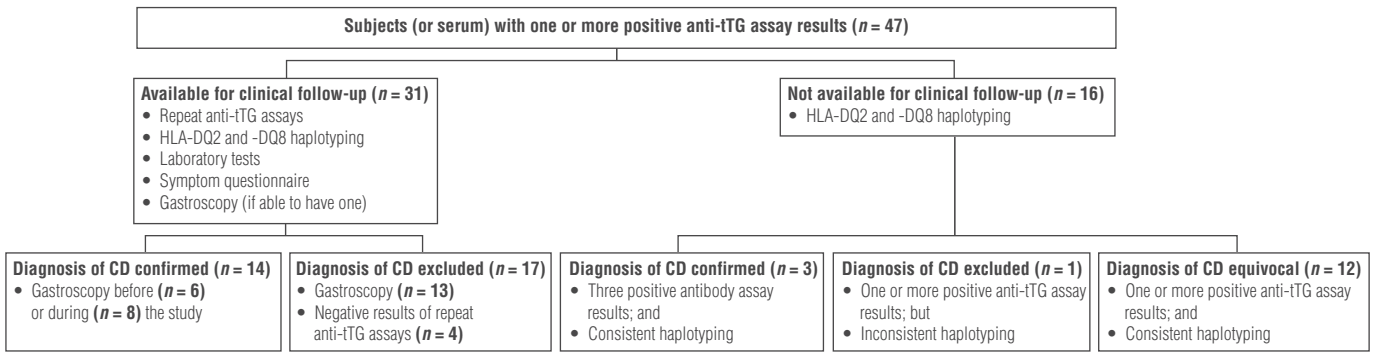
Twelve subjects who had one or more positive results of anti-tTG assays, and an HLA haplotype consistent with CD, but were not available for clinical review or gastroscopy, were defined by our study to be of equivocal status.

If these subjects were included in our estimated prevalence of CD in this population, it would increase to 0.96%.

No CD

In 17 subjects with one or more positive results of anti-tTG assays, CD was excluded: 13 subjects had normal results of a distal duodenal biopsy; and four subjects were unable to have a gastroscopy and biopsy and were diagnosed as not having CD on the basis of negative results of repeat anti-tTG assays.

2 Results of clinical review (with or without gastroscopy) and haplotyping in subjects (or serum) with one or more positive results of anti-tissue transglutaminase (anti-tTG) antibody assays — diagnosis of coeliac disease (CD)



Other test results and clinical symptoms

All the subjects assessed had normal haemoglobin levels, but three subjects with confirmed CD were iron deficient. If CD was confirmed histologically, bone densitometry was recommended. Four of those with CD were diagnosed with osteoporosis.

Of the 11 subjects diagnosed with definite CD during our study (eight of whom were clinically assessed), four had symptoms of diarrhoea, four suffered from fatigue, five complained of excessive flatulence and bloating, and one complained of nausea and cramping.

Positive predictive value of a positive result of an anti-tTG assay

Based only on subjects with definite CD (diagnosed on biopsy criteria), the positive predictive value of at least one positive anti-tTG assay result was 45.2%. This was low compared with the positive predictive value of three positive assay results, which was 94%.

DISCUSSION

We found that the prevalence of anti-tTG antibodies in a predominantly Anglo-Celtic Australian population was 1.56%. This is about three times higher than the prevalence of anti-EMA that we reported in the same population in 2001.[4] To our knowledge, this is the largest study published to date of the prevalence of anti-tTG antibodies and CD in a general healthy population. About two-thirds of the subjects with positive results of at least one anti-tTG assay were reviewed clinically, and just over two-thirds of the reviewed group underwent upper gastrointestinal gastroscopy, and distal duodenal biopsy.

We estimate the prevalence of CD in an Anglo-Celtic Australian population to be at least 0.56% (1 : 177) and it may be as high as 0.96% (1 : 103). Thus, use of the anti-tTG assay, as compared with serological testing for IgA anti-EMA, increased the detection of CD by at least 40%, suggesting that CD is more common in Australia than previously thought. As antibody-based screening studies usually underestimate the prevalence of CD by not diagnosing seronegative disease, our findings should be considered as the minimum prevalence of CD in Australia.

Five of the eight subjects with newly diagnosed CD had clinical symptoms of CD, and three were iron deficient. The high prevalence of positive results of anti-tTG assays and CD, the non-specific high symptom rate in the population, the serious health ramifications of CD, and the reproducibility of the test, all support consideration of anti-tTG testing as part of a screening program for CD. Although our study did not assess the cost-effectiveness of such screening, the early detection of CD and the prevention of the many serious complications, including osteoporosis, iron-deficiency anaemia and malignancy, support its role as a screening tool.

Although the anti-tTG assay is reported to be a highly sensitive and specific test, we found the clinical utility of a single positive anti-tTG assay (positive predictive value) to be only 45.2%. This has serious ramifications if anti-tTG assays are to be used by primary care physicians for testing patients with non-specific ailments which may be attributable to CD. By combining the AESKULISA IgA/IgG and the Genesis IgA anti-tTG assays, we found that the positive predictive value of three positive tests increased to 94%. We would recommend that primary care physicians assess patients for possible CD first with an AESKULISA IgA/IgG anti-tTG assay. If positive results are obtained, further evaluation with the Genesis assay and haplotyping may prevent unnecessary gastroscopy. An inconsistent haplotype, because of its specificity, effectively rules out the diagnosis.

This cohort of subjects has been followed up for up to 40 years as part of the Busselton Health Study. In 27 subjects, positive anti-tTG assay results were obtained in two serum samples taken with a gap of 12 years between them. Despite this long period of stable seropositivity, we were able to definitely refute the diagnosis of CD in 17 of these subjects by gastroscopy or negative results of repeat anti-tTG assays. Latent or potential CD is an entity that is sometimes used to describe patients with few or no symptoms, abnormal results of serological tests for CD and normal histological findings.[17] Although there is debate and controversy about how to manage these patients,[18] we have shown in our study that a significant proportion of them do not go on to develop active CD.

In conclusion, CD is a common, underdiagnosed and often unsuspected medical condition in Australia today. We have shown that screening subjects with an AESKULISA anti-tTG assay increases the detection rate compared with serological screening for IgA anti-EMA. Confirming the result using a different commercial anti-tTG assay (eg, Genesis) may help to decrease the need for referral for gastroscopy.

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COMPETING INTERESTS

None identified.

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