ORIGINAL ARTICLE

Mass screening for coeliac disease using antihuman transglutaminase antibody assay

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Aims: To determine coeliac disease prevalence by an anti-transglutaminase antibody assay in a large paediatric population; to evaluate acceptance of the screening programme, dietary compliance, and long term health effects.

Methods: Cross-sectional survey of 3188 schoolchildren (aged 6–12) and prospective follow up of diagnosed cases. Main outcome measures were: prevalence of coeliac disease defined by intestinal biopsy or positivity to both human tissue transglutaminase and anti-endomysium antibodies in HLA DQ2-8 positive subjects; percentage of children whose families accepted screening; dietary compliance as defined by negativity for anti-transglutaminase antibodies; and presence of clinical or laboratory abnormalities at 24 month follow up.

Results: The families of 3188/3665 children gave their consent (87%). Thirty biopsy proven coeliacs were identified (prevalence 1:106). Three other children testing positive for both coeliac related autoantibodies and HLA DQ2-8 but refusing biopsy were considered as having coeliac disease (prevalence 1:96). Of 33 cases, 12 had coeliac related symptoms. The 30 biopsy proven coeliacs followed a gluten-free diet. Of 28 subjects completing 18–24 months follow up, 20 (71.4%) were negative for anti-transglutaminase antibodies, while eight were slightly positive; symptoms resolved in all 12 symptomatic children.

Conclusions: Prevalence of coeliac disease is high in Italian schoolchildren. Two thirds of cases were asymptomatic. Acceptance of the programme was good, as was dietary compliance. Given the high prevalence and possible complications of untreated coeliac disease, the availability of a valid screening method, and evidence of willingness to comply with dietary treatment population mass screening deserves careful consideration.

Coeliac disease (CD) is an under-diagnosed gluten dependent enteropathy associated with increased risk of autoimmune disease.[1–3] Thanks to simple, accurate serological screening tests, population based studies have shown a high prevalence of CD (from 1:150 to 1:250).[4] Since few subjects have the classic gastroenterological symptoms, most cases go unnoticed, with a 1:7 ratio of diagnosed to undiagnosed cases.[4] The possible benefits of screening programmes are still being debated. Doubts have focused on compliance to gluten-free diets in asymptomatic subjects diagnosed by screening, and discussions about the most valid screening test are ongoing. Although the anti-gliadin antibody assay is highly reproducible, its sensitivity and specificity are much lower than that of the anti-endomysium antibody (AEA) assay. However, the AEA assay is costly and cannot guarantee reproducibility. Moreover, since only IgA-type AEA have sufficient specificity for use in every-day clinical practice, IgA deficient coeliac sufferers (2.8–8%) would elude diagnosis.[5] The antigen recognised by AEA has been identified as the enzyme tissue transglutaminase.[6] We have cloned and expressed the gene for human tissue transglutaminase and developed an ELISA test for IgA and IgG antihuman transglutaminase antibodies (h-tTG-ab).[7]

We performed a mass screening of a large population of schoolchildren using anti-h-tTG-ab ELISA to determine the prevalence of CD and assess the acceptance of the screening programme, and, in newly diagnosed cases, to evaluate compliance to the gluten-free diet and its effects on health.

SUBJECTS AND METHODS

Subjects

Between September 1999 and June 2000, we recruited 3665 primary school children (1655 girls, 2010 boys; median age 8 years, range 6–12) in Trieste, northeast Italy, into the screening programme, called "Buono come il Riso" (Good as Rice). Informed consent was obtained from parents invited by letter to school meetings. With the help of teachers and audiovisual aids, all children were informed about CD and the screening procedure. Two children known to be coeliacs were excluded from final calculations of prevalence.

Study design

During school time, samples of whole blood were drawn from a finger using a heparinated capillary tube. Samples were centrifuged and stored at −30°C. They were first tested for IgA and IgG anti-h-tTG-ab; an AEA assay was then performed on all samples testing positive for anti-h-tTG antibodies. Subjects with anti-h-tTG-ab values below cut-off limits were classified as not having CD. AEA were also tested in 200 children selected at random who were negative for antihuman transglutaminase antibodies. All children positive at the first test on the whole blood drop were recalled and a venous blood sample was taken to measure anti-h-tTG-ab and AEA in the serum. All subjects testing positive for anti-h-tTG-ab and AEA at the second screening step underwent intestinal biopsy. Subjects testing positive for anti-h-tTG-ab

Abbreviations: AEA, anti-endomysium antibody; CD, coeliac disease; GFD, gluten-free diet; h-tTG-ab, antihuman transglutaminase antibodies

but negative for AEA were tested for the CD associated HLA DQ2 and/or DQ8 molecules; if positive, they too underwent biopsy. Diagnosis of CD was confirmed following the revised criteria of ESPGHAN[8] and the intestinal biopsies were classified according to a modified version of Marsh's classification.[9]

Serum antibody tests

Recombinant human transglutaminase was produced and purified as described earlier,[7] and serum anti-h-tTG-ab were determined by means of an ELISA assay with cut-off values for IgA (16%) and IgG (42%) calculated on a paediatric population as reported previously.[10] Serum IgA AEA was measured using sections of human umbilical cord as reported previously.[10] Organ specific auto-antibodies (thyreoperoxidase antibodies, islet cell antibodies, glutamic acid decarboxylase antibodies, and anti-insulin antibodies) were measured in all subjects testing positive for anti-h-tTG-ab, as described previously.[11]

Immunohistochemistry

Each intestinal specimen used to measure the density of $\gamma\delta TCR^+$ intraepithelial lymphocytes was processed as described previously by Savilahti and colleagues[12] with >3.5 cells/mm considered positive.

HLA-DQ typing

The susceptibility alleles for CD were determined by PCR with allele specific primers identifying DQ2 and DQ8, using a Dynal Classic SSP-DQ kit.

Diet and follow up

All newly diagnosed coeliacs were put on a gluten-free diet (GFD) and underwent routine clinical examination and laboratory testing (coeliac related antibodies and organ specific auto antibodies) at 9, 12, 18, and 24 months of the diet. Parents were interviewed to assess compliance, perceived health effects of the diet, and its implications for the child's wellbeing.

Statistical analysis

Sequential serum samples were compared using the Wilcoxon signed rank test; $p < 0.05$ was considered significant.

RESULTS

Informed consent was obtained from the families of 3188/3665 subjects (87%); the male:female ratio (1370 girls and 1818 boys) was in line with European demographic reports.[13] The h-tTG assay was positive in 48/3188 of the subjects tested (1.5%); 31/48 also tested positive for AEA. Of the 48 children testing positive on whole blood drop samples, 41 (85.4%) agreed to be re-screened and underwent venous blood puncture for antihuman transglutaminase and AEA tests. Parents of seven children refused to continue screening despite being informed of the risks of undiagnosed CD. Forty one (1.2% of the study population) retested positive for antihuman transglutaminase antibodies, and 31 were once again AEA positive (fig 1). All 200 serum samples randomly selected from children identified as being h-tTG-ab negative were confirmed as negative by AEA assay. Of the 31 subjects (24 girls, 7 boys; median age 8, range 7–12 years) testing positive for both anti-h-tTG-ab and AEA, 28 underwent intestinal biopsy. All biopsy specimens showed the typical CD lesions.

All three children (one boy aged 7 and two girls aged 8 and 9) who tested positive for both anti-h-tTG-ab and AEA but whose families refused intestinal biopsy were carriers of CD associated HLA DQ molecules; none had CD related clinical symptoms. Due to the extremely high positive predictive value (100%) of the co-presence of AEA, anti-h-tTG-ab, and CD-related HLA, these three children were considered as having CD.[4]

Of the 10 subjects testing positive for anti-h-tTG-ab but negative to AEA, four (one with total serum IgA deficiency) carried the DQ2 molecule. Informed consent for intestinal biopsy was obtained in three of these four cases. Two girls aged 7 and 9 had type 3a and 3b lesions respectively, confirming the diagnosis of CD. The third subject (a boy aged 10) had normal intestinal mucosa and a normal $\gamma\delta TCR+$ intraepithelial lymphocyte count, thus not fulfilling the diagnostic criteria for CD. Four of the 33 diagnosed subjects tested positive for anti-thyreoperoxidase antibodies.

Ultimately, we identified 30 biopsy proven coeliac subjects among 3188 screened children (prevalence 1:106). In addition, since those three children testing positive for AEA, anti-h-tTG-ab, and HLA DQ2-8 were considered to have CD, overall prevalence was 1:96 (10.3 per 1000; 95% CI 6.8 to 13.9). Two diagnosed cases (6%) were identified on the basis of an isolated response to IgG anti-h-tTG. However, if the two children with previously diagnosed CD are included, the prevalence increases to 1:91. Of our 33 new coeliacs, only 12 had symptoms related to gluten intolerance (table 1).

Follow up

Figure 2 shows the changes in AEA and anti-h-tTG-ab levels in 30 biopsy proven coeliacs, both at diagnosis and during follow up on GFD. In these patients, both IgA and IgG anti-h-tTG decreased significantly for the first 12 months. At follow up, all 30 coeliacs reported compliance with GFD (two for 12 months, six for 18 months, and 22 for 24 months); 20 (66.7%) were negative for anti-h-tTG-ab, while 10 (33.3%) were still positive, but with much lower levels than before GFD. During the diet, symptoms resolved in all 12 symptomatic children. Parents confirmed that dietary compliance was generally good (table 2), admitting suboptimal compliance on social occasions. This fact may explain the continuing (although low) positivity in 8/28 subjects after 18 months of GFD. Although benefits were most marked in previously symptomatic subjects, the parents of all our biopsy proven coeliacs approved of the screening initiative. Four asymptomatic children who tested positive for thyreoperoxidase antibodies at diagnosis, tested negative after one year of GFD.

The overall cost of the screening programme included materials and equipment (€17 000), and time spent by health professionals on sampling (€10 500), on introductory meetings with teachers, pupils, and parents (€2000), on dietary instruction before and during follow up (€2000), and on data processing (€500), plus costs of further diagnostic work-ups (intestinal biopsies, €8000), giving a total of €46 000, equivalent to €1400 per case diagnosed.

DISCUSSION

This is the first large scale application of anti-h-tTG-ab dosage as a screening test in a general paediatric population. It confirms the high prevalence of CD (1:96 or 1:91 if the two subjects already known as coeliacs are included) reported in other recent studies;[14 15] the ratio of symptomatic to asymptomatic cases diagnosed was 1:2.

The anti-h-tTG-ab assay is reported as having similar sensitivity to the AEA assay for screening of CD.[4] In our experience,[10] however, the anti-h-tTG-ab test is cheaper and simpler, and more sensitive for mass screening than the AEA assay. A novel element of this study is the inclusion of specific IgG measurement, allowing identification of coeliacs with IgA deficiency as well as those with normal levels of total IgA who produce only specific IgG.[16]

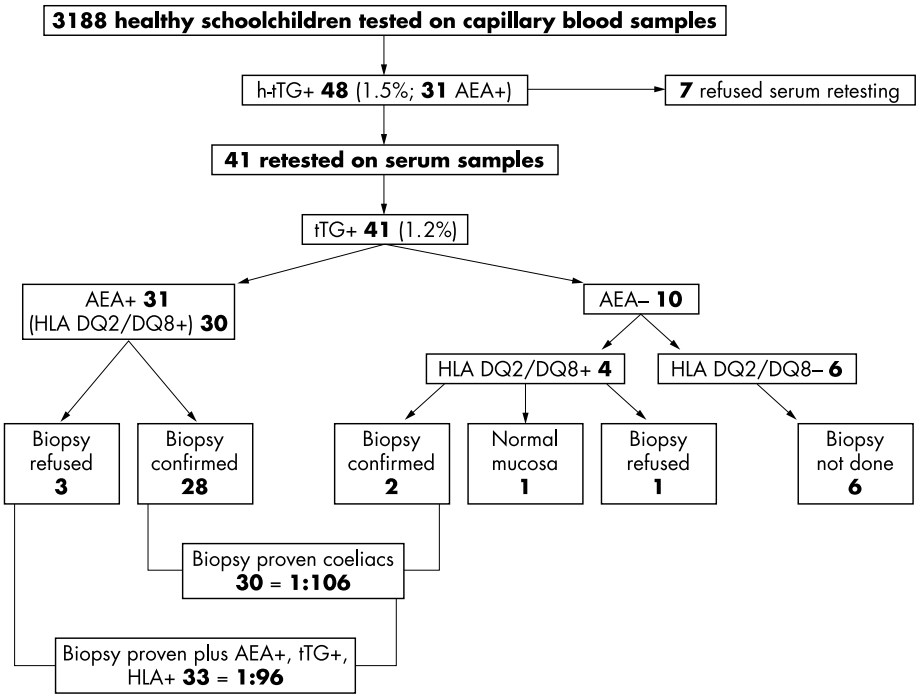

Figure 1 Flow chart and results of screening for coeliac disease.

Of our 12 symptomatic subjects, only six had gastrointestinal symptoms. The other 21 were asymptomatic, confirming that gastrointestinal complaints are present only in a minority of CD subjects. Recent clinical and epidemiological studies in children[17] and adults[18] confirm our findings and show that if CD is suspected only in the presence of gastrointestinal symptoms, many cases are bound to pass undiagnosed.

All our biopsy diagnosed subjects were told what treatment was necessary and given dietary advice. Nearly three quarters of our coeliacs who complied with the diet for more than 18 months tested negative for anti-h-tTG-ab and symptoms resolved in all symptomatic subjects. This is encouraging, given previous reports of unsatisfactory compliance in subjects diagnosed by screening compared with subjects diagnosed on the basis of clinical symptoms.[19] Furthermore, the disappearance of other auto-antibodies (anti-thyreoperoxidase) from four asymptomatic patients on GFD suggests that potential autoimmune problems may have been averted. This mirrors a previous finding[11 20] and indicates that mass screening and treatment for CD could also prevent autoimmune disorders.[1–3] Knowledge of long term risks associated with symptomatic and silent CD underpins the information given to families, and this can enhance active participation in screening projects. In fact, 87% of our families consented to screening and most said they would repeat the experience.

It is regrettable that despite all our efforts, 10 families whose children tested positive to anti-h-tTG-ab refused to complete the screening. However, increasing public awareness of the long term risks of CD, and policies to make

Table 1 Clinical findings in the 33 screening detected coeliac patients (30 biopsy proven patients and three positive for HLA, AEA, and anti-h-tTG-ab)

Clinical findings	No.
No problems (silent subjects)	21
Atopic dermatitis and recurrent arthralgia	3
Chronic tiredness and anaemia	2
RAP and recurrent aphthous stomatitis	2
RAP and diarrhoea	2
RAP, constipation, and anaemia	1
RAP, chronic urticaria, and constipation	1
Constipation	1

RAP, recurrent abdominal pain.

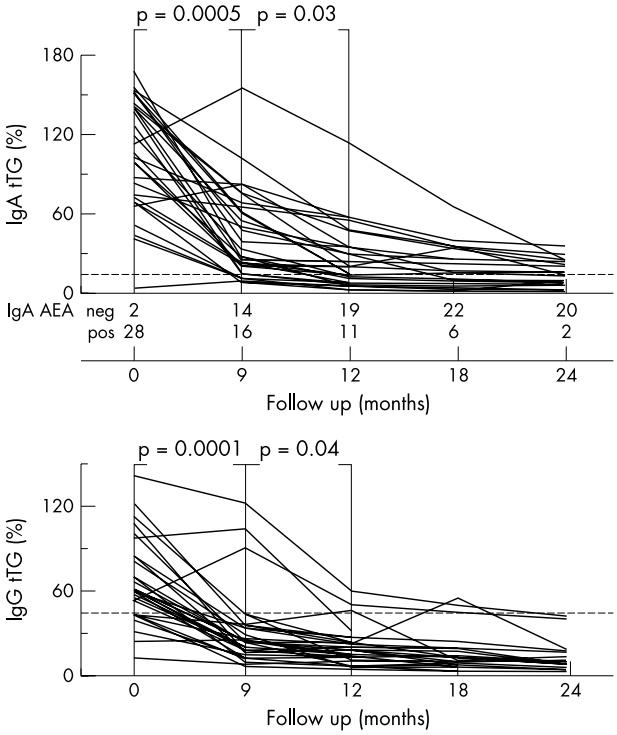

Figure 2 IgA and IgG antihuman transglutaminase values in 30 biopsy proven coeliacs at time of diagnosis and during follow up on a gluten-free diet. The dashed lines indicate the cut-off limits. In the middle, the IgA anti-endomysium results are indicated.

Table 2 Follow up interview with parents of children found to have CD

Questions	34 parents of biopsy proven coeliacs	4 parents that refused the intestinal biopsy
Is your child following the diet properly?		
Yes	30	1
No	4	3
Main problems		
Practical and culinary	6	1
Social occasion	21	2
None	7	0
Overall effect of the diet		
Positive	13	0
Negative	2	0
None	19	2
Are you glad you allowed your child to be screened?		
Yes	30	0
I really regret it	0	3
I do not know	4	1
Would you give your permission again?		
Yes	34	1
No	0	3
Did you tell your first degree relatives about the screening programme?		
Yes	15	3
No	19	1

Thirty biopsy proven coeliacs and three children tested positive for IgA-AEA, anti-transglutaminase antibodies, and HLA DQ2 or HLA DQ8).

gluten-free products cheaper and more acceptable should improve adherence and compliance.

Without screening, 21 asymptomatic coeliacs would almost certainly have eluded diagnosis. Without considering the personal costs in terms of suffering, the cost to the Italian health service of delayed/complicated diagnosis of CD is about €8700 per case, including both symptomatic and asymptomatic cases.[21]

This study also raises the question of management guidelines for asymptomatic individuals having to undergo lifelong dietary modification. The potential benefits to the asymptomatic patient are reduction of long term mortality and morbidity.[22 23] Given people's common tendency to engage in risky behaviour, an asymptomatic CD patient should be made aware of the probable additional risk he/she runs by not following a GFD. The additional risk for autoimmune diseases is currently estimated as 1.1% for each year without diagnosis.[1] We believe in providing patients with the information they need to make an informed decision. In theory, identifying and treating 1500 unidentified CD patients per year in the Italian population[21] could prevent 100 cases of autoimmune diseases (mostly type 1 diabetes and autoimmune thyroiditis),[1] and perhaps as many as 20 cases of fatal non-Hodgkin's lymphoma.[22–24]

Given the high prevalence of undiagnosed CD and the possible complications of untreated CD, we are convinced of the need for further studies to evaluate the real cost:benefit ratios of screening programmes like ours. In conclusion, as well as showing the feasibility of mass screening for CD using a simple anti-tTG assay, our study also points to the need for appropriate management policies should such screening become widespread.

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