Screening by anti-endomysium antibodies for celiac disease in Tunisian children with type 1 diabetes mellitus

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INTRODUCTION

Many disorders have been found in association with celiac disease (CD). Type 1 diabetes mellitus (DM1) and thyroid diseases [1, 2], as well as some other autoimmune conditions, occur more commonly than by chance alone. DM1 is the commonest and best documented associated condition [3, 4]. The co-occurrence of both diseases may be explained by a similar genetic background and similar mechanisms for the autoimmune process. More than 90% of patients with CD and approximately 60% to 70% of diabetics (type 1) carry the human leukocyte antigen heterodimer DQA1*0501DQB1*0201 [5-7].

Many screening studies have been carried out in children and the average prevalence of CD among children with DM1 in 26 reports is 4.5% (0.97-16.4%) [3]. Large differences have been found in the frequency of CD in different countries [8-10] and it is therefore not surprising that the coexistence of DM1 and CD shows a great regional variability as well. Indeed, differences in the type of antibody used for screening, the size of the cohort and duration of DM1 may also have influenced the variation of the results. In studies which used IgA class anti-endomysium antibodies (EMA) to...
screen for CD in diabetic children, the prevalence of biopsy-proven CD ranges between 1.4% and 10.4% [7, 11-18]. In most patients with DM1, the diagnosis of CD was made during screening studies. The reported rates are probably underestimated, because many patients with DM1 with positive antibody do not accept endoscopy and small bowel biopsy. Furthermore, serial screening of individuals with DM1 over a period of years has identified additional cases that initially had negative serological tests [17, 19].

The aim of our study was to determine the frequency of CD in children with DM1 in Tunisia (North Africa). For this purpose, we screened the patients with EMA.

Patients and methods

Study population

A prospective study was conducted on 205 children from four hospitals of the centre of Tunisia (92 girls, 113 boys, age range 6 months — 15 years, median: 11 years) with DM1. The median age at DM1 onset was 7 years (range: 3 months to 15 years). The median time elapsed from the onset of DM1 was 2 years (range: 0-14 years).

The study was approved by Local Ethics Committee. Informed consents were obtained from parents, patients or both.

Determination of anti-endomysium antibodies

We used an umbilical EMA technique as we described previously [20]. EMA titres were measured by indirect immunofluorescence using unfixed cryosections (4 µm thick) of human umbilical cord. The starting serum dilution was chosen as 1/10. The cryosections were incubated with the diluted serum samples for 30 min. Thereafter the slides were gently washed and rinsed in phosphate-buffered saline (pH=7.2) before incubation with fluorescein-labeled anti-human IgA antibodies (Bio-Rad®, Marnes La Coquette, France). A positive result of EMA was recorded if connective tissue surrounding the muscle cells fluoresced brightly in a honeycomb pattern. Any positive reading (+ to ++++) in immunofluorescence of a serum diluted 1:10 was considered positive.

Intestinal biopsy

Patients positive for EMA were informed of their high probability of being celiac and were offered gastrointestinal endoscopies with duodenal biopsy. Two or three mucosal biopsies were obtained. The specimens were read by two pathologists who were unaware of patient identity. The intestinal mucosa was classified in accordance with Marsh [21].

Statistical analysis

SPSS program was used to determine median age and sex ratio. The comparisons of median age were done by Students’ test. The comparisons of frequencies were studied with Fisher’s exact probability test, a p-value less than 0.05 was considered significant.

Results

The flow chart summarizes the results of screening (figure 1). EMA were positive in 17 out of 205 (8.3%) children with DM1. The frequency of EMA was higher in girls (9.8%, 9/92) than in boys (7%, 8/113) but the difference was not statistically significant (P=0.48). The median age at DM1 onset was significantly lower in patients with EMA (5 years) than those without EMA (7.2 years) (P<10⁻²) (table I).

In 13 of 17 EMA-positive patients, duodenal biopsies could be taken and a destructive type of CD was confirmed in 11 of them: 8 patients showed total villous atrophy, 3 patients showed a partial villous atrophy and all had elevated counts of intraepithelial lymphocytes. The other two patients showed a normal histological picture with normal number of intraepithelial lymphocytes. Parents of the remaining EMA-positive children refused endoscopy. Thus the prevalence of biopsy-proven CD was 5.3% (11/205). It was 7.6% (7/92) in girls and 3.5% (4/113) in boys but the difference was not statistically significant (P=0.226).

Only 3 of 11 patients (27%) with CD had signs or symptoms that could have been related to gluten intolerance in their clinical history (table II). One patient had mild gastrointestinal symptoms, 1 had anaemia and mild gastrointestinal symptoms, and one (a 15 years-old girl) had secondary amenorrhea since nine months, anaemia and weight loss. In one patient with biopsy-proven CD, the diagnosis of two other auto-immune diseases, systemic lupus erythematosus and systemic sclerosis, was made at the same time as CD screening.

All patients with biopsy-proven CD were put on gluten free diet (GFD). Six months after the introduction of GFD, the mild gastrointestinal symptoms resolved and the secondary amenorrhea disappeared.

Discussion

The present study confirms the high prevalence of EMA and biopsy-proven silent CD in young patients (<15 yrs) with DM1. Our results are concordant with those reported in other centres using EMA determination, along with small bowel biopsy, as the primary screening tool [7, 11-13]. The prevalence of EMA in our diabetic patients (8.3%) was significantly higher than that previously found in our group of 2500 healthy blood donors (0.28%) [8].

While the prevalence of EMA was 8.3%, the incomplete prospective histological assessment by small bowel biopsy precludes a final calculation of the prevalence of biopsy-proven CD in this patient group. In fact, in four patients with EMA, duodenal biopsy could not be performed, but a very high specificity has been reported for EMA using the human umbilical cord test [27, 28]. Our two patients with normal mucosal morphologic features may have latent CD [29]. It has recently been demonstrated that all patients with serum IgA EMA and intact duodenal villi will develop CD [29]. In another study, EMA found at diagnosis of DM1 were predictive of future development of CD [30]. Therefore, a “normal biopsy” never excludes the underlying presence of latent CD [31]. The overall potential prevalence of CD in the current cohort could be above 8%.

Autoantibodies to tissue transglutaminase (tTG) are sensitive serological parameters for detecting silent CD in patients with DM1 [32], but there may be false-positive results in this population [33]. While the specificity of EMA is considered to be virtually 100%, the tTG test does not achieve that degree of specificity [31].

We did not measure total IgA levels in sera of our diabetic patients. Although CD occurs with increased frequency in those with selective IgA deficiency, screening studies of the general population suggest that very few cases will be missed by not routinely measuring IgA levels as part of the screening regimen [34]. Nor is the frequency of selective IgA deficiency increased in those with DM1. Thus, the strategy of routinely determining serum IgA levels or adding IgG based serology as part of a
As in other studies [13-15, 35], in our cohort, females were more frequently positive for EMA, whereas in EMA-negative children with DM1 there was no female predominance, as it is normally found in children with diabetes. Like in other studies [13, 14, 16, 35], diabetics with EMA had an earlier onset of DM1 compared with the individuals with diabetes and without EMA. In contrast, DM1 did not occur at an earlier age in other studies [7, 17].

We have prescribed GFD for our diabetic patients with biopsy proven CD despite the fact that most of them seem to have silent CD. The number of patients with symptoms and signs differ widely. It is likely that some patients were regarded as asymptomatic when they were not. Patients with so called “silent” CD have often a decreased psychological well-being [36]. Improvement has been noted in symptoms, with a sense of improved well-being and vitality, growth in children, haemoglobin concentration and mean red cell volume [3]. It has been demonstrated that patients who had undiagnosed symptomatic CD during childhood exhibited higher prevalence of short stature compared to matched controls and to patients whose CD has been diagnosed during childhood [37]. Furthermore, after institution of GFD most patients exhibit catch-up growth [38].

In one of our diabetic patients with biopsy-proven CD, a 15-years-old girl, secondary amenorrhea disappeared thanks to GFD prescription. Health risks in CD include problems associated with reproduction. It has been observed that adolescents who were not compliant to GFD presented delayed menarche and secondary amenorrhea [39]. CD’s interference on reproduction is related to the multifactorial nature of the disease. CD induced malabsorption with consequent deficiencies of micronutrients such as iron, folic acid and vitamin K, might interfere with organogenesis, and fat-soluble vitamins important for spermatogenesis. Since these reproductive alterations are often reversible, it could be argued that the earlier the detection and treatment of CD, the better for the patient in this regard [40].

One of our diabetics had two other autoimmune diseases: a patient with systemic lupus erythematosus and a case of systemic sclerosis. An increased prevalence of autoimmune disorders occurs in patients with CD [41, 42]. It has been shown that autoimmune disorders involving organs other than the intestine could develop in unrecognized and/or untreated celiac subjects [41]. It has been found that the risk of another autoimmune disease is higher in type 1 diabetes patients with CD than in type 1 diabetes patients negative for EMA [42]. The higher prevalence of other autoimmune diseases in subjects with both CD and DM1 could be a consequence of a delayed diagnosis of CD [42]. The question whether the early diagnosis and treatment of CD might reduce the risk of development of other autoimmune diseases remains unanswered.

In conclusion, the prevalence of celiac disease in children with DM1 in Tunisia is at least 5.3% which is comparable with European and American studies. It can be therefore recommended to test asymptomatic children with DM1, and advice treatment for those proven to have intestinal changes of CD [37, 38]. Currently, the majority of risk-groups are not screened for CD by their clinicians. Implementation of this should be a major goal for the years to come [43]. In children with negative antibody titres at diagnosis of DM1, screening at regular intervals, e.g. 2-5 yrs, is recommended [30].

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Table II. – Characteristics of eleven patients with biopsy-confirmed celiac disease.

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<th>N</th>
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<th>Duration of DM1</th>
<th>Clinical features suggestive of CD</th>
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REFERENCES


