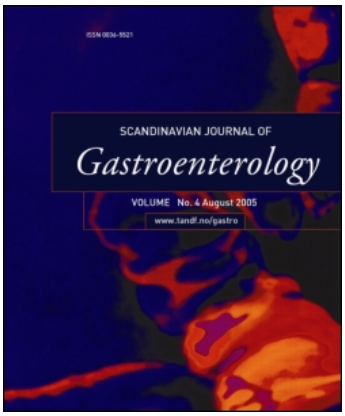

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Coeliac disease-specific tissue transglutaminase autoantibodies are associated with osteoporosis and related fractures in middle-aged women

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ORIGINAL ARTICLE

Coeliac disease-specific tissue transglutaminase autoantibodies are associated with osteoporosis and related fractures in middle-aged women

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Abstract

Objective. To investigate whether the serological marker for coeliac disease, tissue transglutaminase autoantibody (tTGAb), is associated with decreased bone mass density (BMD) and increased frequency of fractures in middle-aged women screened for osteoporosis. ***Material and methods.*** The study comprised 6480 women (mean age 56 years, range 50–64) who answered a number of questionnaires and who underwent dual X-ray absorptiometry of the wrist bone. Serum samples were analysed for tTGAb using radioligand binding assays. A tTGAb level of >4 U/ml was used to determine a positive value and a level of >17 U/ml was used as an alternative discrimination of high levels. ***Results.*** A tTGAb level >4 U/ml was found among 90/6480 (1.4%) women and correlated with lower BMD (multiple linear regression coefficient -382.1; 95% CI $= -673.6$–90.7, $p=0.011$) and with fracture frequency ($r=0.18$, $p=0.023$). The 59 women with tTGAb levels ≥ 17 U/ml had a lower BMD (0.41 ± 0.08 g/cm^2 versus 0.44 ± 0.08 g/cm^2, $p=0.001$) and a lower T-score (-1.40 ± 1.28 versus -0.90 ± 1.40, $p=0.003$) as well as a higher prevalence of osteoporosis (13.4% versus 6.5%, $p=0.008$) compared with the remaining 6421 women with tTGAb levels <17 U/ml. Furthermore, fracture frequency was more pronounced in women with tTGAb levels ≥ 17 U/ml, among whom 19/59 (32.2%) had fractures during the study period compared with 1204/6421 (18.8%) among women with tTGAb levels <17 U/ml ($p=0.009$). ***Conclusions.*** High levels of tTGAb indicating coeliac disease are associated with lower BMD and higher fracture frequency in women between 50 and 64 years of age. Osteometry is therefore warranted in middle-aged women detected with tTGAb.

Key Words: *Coeliac disease, fracture, osteoporosis, screening, tissue transglutaminase, women*

Introduction

Osteoporosis affects 7% of Scandinavian women aged 50 to 60 years [1,2] and Sweden is among the countries in the Western world with the highest incidence of osteoporotic fractures [2,3]. Several risk factors for osteoporosis have been identified, among which old age, female gender, loss of ovarian function, low physical activity, low body mass index (BMI), smoking and low calcium intake are common [4]. Negative effects on the mineralization of the skeleton can also be caused by failure of calcium and vitamin D uptake due to gastrointestinal malabsorptive disorders [5].

Coeliac disease is a chronic small-bowel disorder caused by dietary gluten and occurs in more than 1% of the Caucasian population [6]. The disease often leads to gastrointestinal symptoms, but the spectrum of clinical presentation is wide and tends to be more diffuse with increasing age [7]. Atypical symptoms and clinical signs are common and screening for coeliac disease has demonstrated that the majority of individuals in the general population with small-intestinal villous atrophy remain unrecognized [6]. The only signs of underlying asymptomatic coeliac disease may therefore be secondary to the effects of prolonged malabsorption [8–10].

Moreover, coeliac disease is strongly associated with the formation of autoantibodies against tissue transglutaminase (tTG) [11]. However, the role of tTG autoimmunity in the pathogenesis of coeliac disease is a matter of debate [12–14]. The presence

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of tTG autoantibodies (tTGAbs) is a striking feature of coeliac disease with a sensitivity and specificity reaching 98% and 99%, respectively [15], making these autoantibodies useful as diagnostic tests in clinical practice or in screening programs.

A number of previous studies have already shown a slight increase in the prevalence of asymptomatic coeliac disease in middle-aged women with reduced bone mineral density (BMD) using either antigliadin antibodies [16] or tTGAbs [17], whereas another study using endomysial autoantibodies (EMAs) showed no association [18]. It was recently demonstrated that tTGAbs are associated with lower BMD in the general population, suggesting that conditions such as osteoporosis might be identified by screening for coeliac disease using specific autoantibodies [19]. Although there are conflicting results concerning the risk for patients with coeliac disease of developing osteoporosis and its related fractures [20–23], there is still no general recommendation for BMD screening in newly diagnosed patients with coeliac disease today [24]. However, larger studies on fracture risk in the general population screened for coeliac disease have not yet been published.

The present study of a geographically defined total population of nearly 6500 middle-aged or older women is numerically by far the most extensive study of women screened for osteoporosis [1]. Osteoporosis and associated fragility fractures have a major impact on the quality of life of the individual, as well as on the costs to society in general, which is why further detection of individuals at high risk may be important in order to initiate preventive measures and, vice versa, screening for coeliac disease may be beneficial when osteoporosis or fragility fractures are identified.

The aim of the present study was to analyse tTGAbs in a geographically defined extensive population of women aged 50 to 64 years without previously known osteoporosis and to investigate any association with fractures and impairment of quality of life. All participants underwent physiological and anthropometric measures including serology testing for tTGAbs and osteometry. We hypothesized that the presence of tTGAbs indicating coeliac disease was associated with lower BMD as well as a higher frequency of fractures in middle-aged and older women.

Materials and methods

Subjects

A total of 10,766 women living in a defined area of Southern Sweden were identified through a population register and were asked to participate in the Women's Health in the Lund Area (WHILA) Study between 1 December 1995 and 3 February 2000; the inclusion criteria are described elsewhere [25]. Screening for osteoporosis was carried out in 6917 women (mean age 56 years, range 50–64), blood samples being available from 6481 of them for purposes of this study. Of these 6481 women, a total of 595 (9.2%) were premenopausal (PM), 3454 (53.2%) were postmenopausal without hormone therapy (PMO) and the remaining 2432 (37.5%) were postmenopausal but receiving hormone therapy (PMT).

Physical examination and questionnaires

Each participant underwent a baseline physical examination including body-weight, height, BMI (kg/m^2) and minimal waist and maximal hip circumference ratio (WHR). BMD of the non-dominant wrist was measured (at the 8-mm distal position) using dual-energy X-ray absorptiometry (DXT 200; Osteometer MediTech Inc., Hawthorne, Calif., USA). A phantom for daily calibration of the instrument was used, and one technician performed all the measurements. The measured BMD was automatically compared with a "reference" population furnished by the instrument supplier, giving T-scores, defined as $(BMD_o–BMD_m)/SD$, where BMD_o is the obtained BMD, BMD_m is the mean value for 20-year-old Danish female controls and SD is the standard deviation in the same reference population. Osteopenia was defined as $-2.5 <$T-score< -1.0, and osteoporosis as T-score< -2.5, according to the World Health Organization (WHO), based on measurement of the proximal femur [4]. Questionnaires were used to determine medical history, menopausal status, pharmacological treatment, leisure-time exercise, body-weight at age 25, weight gain, falls during the past year, alcohol use, smoking and dietary habits, and a validated food questionnaire describing type, frequency and estimation of amount of various foods consumed per week during the previous 3 months. Fracture incidence was collected from the local diagnosis registry and from questionnaires. Subjective mental, physical and social well-being was measured by the validated Gothenburg Quality-of-Life Instrument [26], which refers to the WHO definition of health. Median sum scores of estimations from (1) "very bad", to (7) "excellent" were calculated on five topics on quality of life. Also, 11 mental and physical symptom questions were answered with "yes" or "no", on whether the woman had been troubled by any symptoms during the previous 3 months. The procedure has been described previously [25]. Informed consent was given by the participants, and

the Ethics Committee at Malmö/Lund University approved the study.

Analytical techniques

Serum samples were obtained at the same time-point as the BMD measurements were taken and had been previously analysed for S-cholesterol, S-triglycerides, S-high-density lipoprotein (HDL)-cholesterol, S-low-density lipoprotein (LDL)-cholesterol, B-glucose and S-thyroid-stimulating hormone (TSH) [1]. For this study, the serum samples were kept at -80°C until tTGAb analysis with radioligand binding assays run as described between August and November 2006 [27,28]. In brief, the tTG antigen/antibody complexes were separated with 30% protein A sepharose (PAS) conjugate 4B (Zymed Laboratories Inc., San Francisco, Calif., USA). Protein A binds all IgG subtypes with the exception of IgG3 and some IgAs and IgMs [29]. The relative amount of tTGAbs was expressed as U/ml. For this study, undetectable tTGAb levels were defined as <0.1 U/ml. Detectable tTGAb levels were seen in 117 women and the quartile distributions were 0.1–3.9 U/ml, 4–16.9 U/ml, 17–107 U/ml and >107 U/ml. A value ≥ 4 U/ml also represents the 95th percentile of normal controls [30]. In this study, comparisons by groups were made by using 4 U/ml and 17 U/ml as two different cut-off values; i.e. women with tTGAbs ≥ 4 U/ml versus women with tTGAbs of 0–3.9 U/ml and, alternatively, women with tTGAbs ≥ 17 U/ml versus women with tTGAbs of 0–16.9 U/ml were analysed.

Statistics

Calculations were performed using SPSS 12.0 (SPSS Inc., Chicago, Ill., USA). Values are given as medians (interquartile range) or frequency. Differences between groups regarding continuous variables were analysed using the Mann-Whitney U-test. The χ^2 test was used to analyse differences in categorical variables, Spearman's correlation coefficient was used for continuous variables and Kendall's tau_b for categorical variables in univariate correlation analyses. The risk of reduced BMD in relation to tTGAb levels was evaluated by multiple linear regression analysis after multivariate adjustments according to a stepwise approach. All variables with p-value less than 0.20 in univariate analysis or known risk factors for osteoporosis (i.e. age, present BMI, body-weight at 25 years of age, S-triglycerides, S-cholesterol, S-HDL-cholesterol, S-LDL-cholesterol, use of hormone replacement therapy, smoking habits, alcohol consumption, dietary habits regarding intake of fibres and vegetables and fruits) were included in the multiple linear regression model. All p-values less than 0.05 were considered statistically significant.

Results

The distribution of tTGAbs

In the 117 women with detectable values, the tTGAbs ranged between 0.1 and 219,400 U/ml. In total, 91 out of 6481 (1.4%) women had a tTGAb level ≥ 4 U/ml (median 32 U/ml) out of whom 60 women had a level ≥ 17 U/ml. The subject with a tTGAb level of 219,400 U/ml was considered as an outlier and was omitted for further statistical calculation when comparing the association of antibody levels and various exposure and effect markers. Eighteen of 6481 (0.3%) women reported they had already been diagnosed with coeliac disease at a young age, all of whom had negative tTGAb levels, which can be explained by dietary treatment normalizing the antibody response.

Physical status

When comparing the 90 women (outlier omitted) with a tTG level ≥ 4 U/ml with the 6390 women with a tTG level 0–3.9 U/ml, there was no difference in age, body-weight, height, BMI or weight gain over time (Table I). However, women with tTGAb levels ≥ 4 U/ml were heavier at 25 years of age (58.5 (8.8) kg versus 56.0 (8.0) kg, $p=0.012$) and had later menarche (14.0 (5.0) years versus 13.0 (2.0) years, $p=0.022$), while no difference was seen in menopausal age ($p=0.697$) or hormone replacement therapy (32.4% versus 37.6%, $p=0.497$). The group of women with tTGAb levels ≥ 4 U/ml had lower S-TSH (1.10 mU/l versus 1.50 mU/l, $p=0.004$) as well as lower S-cholesterol (5.60 mmol/l versus 5.90 mmol/l, $p=0.013$) and S-HDL cholesterol (1.52 mmol/l versus 1.71 mmol/l, $p<0.001$) compared with the remaining women with tTGAb levels of 0–3.9 U/ml, Furthermore, there was an inverse relationship ($r=-0.250$) between tTGAb levels ($n=116$) and S-HDL cholesterol ($p=0.011$) and this association remained in the multivariate adjusted linear regression analysis (regression coefficient -706.4; 95% CI $=-1405.7$–7.2) ($p=0.048$).

Bone density

There were no differences in bone density or prevalence of osteopenia or osteoporosis between the 90 women with tTGAb levels of ≥ 4 U/ml and the 6390 women with tTGAb levels of 0–3.9 U/ml (Table II). However, the 59 women with tTGAb levels ≥ 17 U/ml had lower BMD (0.41 g/cm^2 versus

Table I. Subject characteristics according to tissue transglutaminase autoantibody (tTGAb), with two alternative threshold levels of positivity: 4 U/ml and 17 U/ml, respectively.

	tTGAb level (U/ml)					
Variable	0–3.9 U/ml ($n=6390$)	4–16224 U/ml ($n=90$)	p-value	0–16.9 U/ml ($n=6421$)	17–16224 U/ml ($n=59$)	p-value
Age (years)	56.1 (4.8)	56.3 (4.6)	0.951	56.1 (4.8)	55.9 (4.5)	0.446
Body-weight (kg)	67.3 (14.0)	67.4 (18.0)	0.918	67.3 (14.0)	65.4 (16.4)	0.305
Height (m)	1.65 (0.08)	1.66 (0.08)	0.052	1.65 (0.08)	1.67 (0.09)	**0.036**
Waist:hip ratio (cm/cm)	0.78 (0.08)	0.77 (0.09)	0.726	0.78 (0.08)	0.76 (0.08)	0.089
BMI (kg/m^2)	24.7 (5.0)	24.5 (4.2)	0.600	24.7 (5.0)	24.1 (3.7)	0.078
Weight gain $\geq$5 kg/last 5 years (%)	42.1	32.9	0.088	42.2	23.6	**0.006**
Body-weight at age 25 years (kg)	56.0 (8.0)	58.5 (8.8)	**0.012**	56.0 (8.0)	59.0 (8.0)	**0.026**
Weight gain/last 25–30 years (kg)	4.0 (4.1)	3.7 (3.9)	0.191	4.0 (4.1)	3.1 (3.7)	**0.008**
BMI at age 25 years	20.6 (2.8)	21.1 (2.6)	0.086	20.6 (2.8)	21.1 (2.4)	0.113
BMI increase $\geq$25% last 25–30 years (%)	36.1	30.5	0.294	36.1	23.6	0.055
Menarche (year)	13.0 (2.0)	14.0 (5.0)	**0.022**	13.0 (2.0)	14.0 (1.8)	0.113
Menopause (year)	50.0 (4.0)	50.0 (4.0)	0.697	50.0 (4.0)	50.0 (2.8)	0.362
S-triglycerides (mmol/l)	1.47 (0.98)	1.31 (0.89)	0.072	1.47 (0.98)	1.28 (0.88)	0.083
S-cholesterol (mmol/l)	5.90 (1.43)	5.60 (1.32)	**0.013**	5.90 (1.43)	5.63 (1.32)	0.076
S-HDL cholesterol (mmol/l)	1.71 (0.60)	1.52 (0.55)	**<0.001**	1.71 (0.60)	1.46 (0.58)	**<0.001**
S-LDL cholesterol (mmol/l)	3.42 (1.28)	3.30 (1.06)	0.811	3.41 (1.28)	3.52 (1.10)	0.347
B-glucose (mmol/l)	5.87 (1.34)	5.83 (1.80)	0.808	5.87 (1.34)	5.70 (1.56)	0.463
S-TSH (mU/l)	1.50 (1.10)	1.10 (1.02)	**0.004**	1.50 (1.10)	1.10 (0.70)	0.129

Abbreviations: BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein; TSH = thyroid-stimulating hormone.

Data are presented as medians (interquartile range) or percentages. The tTGAb levels 4 U/ml and 17 U/ml correspond to the lower limit in quartiles 2 and 3, respectively, among the total group of women ($n=117$) with detectable tTGAb ($\geq$0.1 U/ml); see Methods.

0.44 (0.08) g/cm^2, $p=0.001$) and lower T-scores (-1.40 ± 1.28 versus -0.90 ± 1.40, $p=0.003$) as well as a higher prevalence of osteoporosis (13.4% versus 6.5%, $p=0.008$) than that of the remaining 6421 women with tTGAb levels of 0–16.9 U/ml (Table II). Levels of tTGAb ($n=116$) further correlated negatively ($r=-0.27$) with both BMD ($p=0.003$) and T-score ($r=-0.26$, $p=0.004$). Levels of tTGAbs were still associated with lower BMD (T-score) in a multiple linear regression analysis (regression coefficient -382.1; 95% CI = -673.6–90.7, $p=0.011$) after multivariate adjustments for age, BMI, body-weight at 25 years of age, S-triglycerides, S-cholesterol, S-HDL-cholesterol, S-LDL-cholesterol, hormone replacement therapy, smoking habits, alcohol consumption, dietary habits regarding intake of fibres, vegetables and fruits.

In the 18 women with already diagnosed coeliac disease, BMD was 0.398 (0.11) g/cm^2 T-score -1.60 (1.33), which did not differ from the 6390 tTGAb-negative women ($p=0.108$ and $p=0.128$), the 59 ($p>0.95$ and $p>0.95$) nor the 90 ($p=0.452$ and $p=0.432$) women with positive tTGAb at levels $\geq$4 U/ml and $\geq$17 U/ml, respectively. However, 4/18 (22.2%) women with coeliac disease had osteoporosis, which was increased compared with the 6.5% of 6390 tTGAb-negative women ($p=0.024$), but not when compared with tTGAb levels >17 U/ml and >4 U/ml, where osteoporosis was found in 8/59 (13.4%) ($p=0.526$) and 9/90 (10.0%) ($p=0.400$) of the women, respectively.

Fractures

In all, 37 different fractures were identified and the frequency of each type and localization of fractures were minor. The total prevalence of fractures during the study period was 24.4% (22/90) in women with tTGAb levels $\geq$4 U/ml compared with 18.8% (1201/6390) in women with tTGAb levels of 0–3.9 U/ml ($p=0.174$) (Table II). Fracture frequency was more pronounced in women with tTGAb levels $\geq$17 U/ml, out of whom 32.2% (19/59) had fractures during the study period compared with 18.8% (1204/6421) among women with tTGAb levels of 0–16.9 U/ml ($p=0.009$). The most common fracture was of the distal radius, which occurred among 8.9% (8/90) of women with tTGAb levels $\geq$4 U/ml compared with 4.8% (308/6390) among women with tTGAb levels of 0–3.9 U/ml ($p=0.081$). There was a weak correlation ($r=0.18$) between levels of tTGAbs and the number of fractures ($p=0.023$).

Associated symptoms, psychosocial habits and quality of life

There were no differences in numbers of falls during the previous year, or in leisure physical activity, regular medical surveillance or frequency of hospital

Table II. Bone mass density and frequency and numbers of fractures according to tissue transglutaminase autoantibody (tTGAb) levels with two alternative threshold levels of positivity: 4 U/ml and 17 U/ml, respectively.

	tTGAb level (U/ml)					
Variable	0–3.9 U/ml ($n=6390$)	4–16224 U/ml ($n=90$)	p-value	0–16.9 U/ml ($n=6421$)	17–16224 U/ml ($n=59$)	p-value
Bone mass density						
gram/cm^2	0.437 (0.08)	0.429 (0.07)	0.077	0.437 (0.08)	0.408 (0.08)	**0.001**
T-score (SD)	−0.90 (1.40)	−1.15 (1.30)	0.104	−0.90 (1.40)	−1.40 (1.28)	**0.003**
Normal (%)	51.0	45.6	0.319	51.0	33.3	**0.008**
Osteopenia (%)	42.5	44.4		42.5	53.3	
Osteoporosis (%)	6.5	10.0		6.5	13.4	
Fracture frequency (%)						
Self-reported[a]	6.3	5.5		6.3	6.8	
Diagnosed between 1995 and 2006[b]	12.5	18.9		12.5	25.4	
Total	18.8	24.4	0.174	18.8	32.2	**0.009**
Number of women with fractures[b]						
1 fracture	665	16		667	14	
2 fractures	115	1		115	1	
3 fractures	17	0		17	0	
4 fractures	3	0		3	0	
Total (1–4 fractures)	800	17	0.079	802	15	**0.004**

Data are presented as median values (interquartile range) or percentages or numbers. The tTGAb levels 4 U/ml and 17 U/ml correspond to the lower limit in quartiles 2 and 3, respectively, among the total group of women ($n=117$) with detectable tTGAb (≥ 0.1 U/ml); see Methods.

[a]Self-reported fractures that occurred outside the county after 40 years of age and that were not documented in the local diagnosis registry; [b]fractures that were diagnosed and documented in the diagnosis registry from 1 December 1995 to 15 September 2006.

visits between the 90 women with tTGAb levels ≥ 4 U/ml and the remaining 6390 women with tTGAb levels of 0–3.9 U/ml, and no differences were seen in smoking- alcohol- or dietary habits ($p=0.072$–0.656) (data not shown). A self-estimated quality-of-life scale did not show any differences in general health, appetite, mood or energy, or in abdominal pain, nausea, diarrhoea, constipation or loss of weight (Table III). However, dizziness ($p=0.003$) and general fatigue ($p=0.034$) were less common among women with tTGAb levels ≥ 4 U/ml.

Discussion

To our knowledge, the material from the WHILA study utilized in the present study belongs to one of the largest cohorts of women prospectively screened for osteoporosis and associated fractures [25]. In this study we extended our previous observations related to the development of osteoporosis and fracture risk to also include analysis of tTGAbs in all participants from serum samples collected at the same time-point as measurement of BMD. By this procedure we were able to hypothesize that tTGAb as a marker for coeliac disease, a common chronic malabsorptive disorder in the Caucasian population, was associated with osteoporosis and fractures. We measured radius BMD, which is likely to reflect the risk of forearm fractures, although it may not be a good index of osteoporosis in other parts of the skeleton. However, we validated the wrist DXT technique through a supplementary dual-energy X-ray absorptiometry of hip BMD on nearly every fifth woman with wrist osteoporosis, and found a correlation, albeit weak, between BMD of the radius and the hip in 81 women of the WHILA cohort (data not shown).

In our material, the prevalence of osteoporosis and osteopenia was 6.5% and 42.5%, respectively, and the fracture incidence was 18.8%, which are representative figures for middle-age women and in accordance with previous reports on females aged 50 years or older [31]. More than 90% of the women were postmenopausal, among whom 40% were receiving hormone replacement therapy, but no differences were seen with regard to the presence of tTGAbs. Thus, the risk of bias owing to the influence of hormone therapy on bone mass must be considered a minor one.

We found tTGAbs to be present in 1.4% or 1 in 71 of the individuals, while only 0.3% or 1 in 463 women had self-reported coeliac disease. Interestingly, there were no differences in gastrointestinal symptoms of intestinal failure between individuals with or without tTGAbs, indicating that coeliac disease generally remains asymptomatic or silent in a majority of adult people. Despite the lack of clinical signs of coeliac disease, an association between tTGAb and osteoporosis as well as

Table III. Self-estimated well-being according to tissue transglutaminase autoantibody (tTGAb) levels with two alternative threshold levels of positivity: 4 U/ml and 17 U/ml, respectively.

	tTGAb level (U/ml)					
Variable	0–3.9 U/ml ($n=6390$)	4–16224 U/ml ($n=90$)	p-value	0–16.9 U/ml ($n=6421$)	17–16224 U/ml ($n=59$)	p-value
Quality of life 0–7: 0 lowest, 7 highest						
General health	6.0 (1.0)	6.0 (2.0)	**0.032**	6.0 (1.0)	6.0 (1.0)	0.142
Bodily perception	5.0 (2.0)	5.0 (2.0)	0.322	5.0 (2.0)	5.0 (1.8)	0.123
Appetite	7.0 (1.0)	6.5 (2.0)	0.408	7.0 (1.0)	7.0 (2.0)	0.825
Mood	6.0 (1.0)	6.0 (1.0)	0.367	6.0 (1.0)	6.0 (1.0)	0.650
Energy	5.0 (2.0)	6.0 (1.0)	0.054	5.0 (2.0)	6.0 (1.0)	0.165
Subjective symptoms (% yes)						
Dizziness	25.6	12.1	**0.003**	25.6	10.0	**0.006**
General fatigue	59.3	48.4	**0.034**	59.2	51.7	0.235
Abdominal pain	25.3	24.2	0.800	25.3	26.7	0.810
Nausea	12.3	9.9	0.489	12.3	8.3	0.352
Diarrhoea	13.1	12.1	0.770	13.1	10.0	0.473
Constipation	14.2	9.9	0.237	14.3	6.7	0.094
Loss of appetite	3.6	3.3	0.884	3.6	3.3	0.918
Loss of weight	1.9	2.2	0.851	1.9	3.3	0.427
Joint pain	44.7	36.3	0.107	44.7	36.7	0.215
Backache	48.5	40.7	0.136	48.5	40.0	0.190
Pain in the legs	43.5	37.4	0.244	43.5	30.0	**0.036**

Data are presented as median values (interquartile range) or percentages. The tTGAb levels 4 U/ml and 17 U/ml correspond to the lower limit in quartiles 2 and 3, respectively, among the total group of women ($n=117$) with detectable tTGAb (≥ 0.1 U/ml/l); see Methods.

frequency of fractures was found in individuals with antibody levels in the upper two quartiles, i.e. a tTGAb level ≥ 17 U/ml. This finding remained after multivariate adjustments for known variables associated with osteoporosis. It is well known that levels of tTGAb correlate with severity of intestinal damage in coeliac disease [32], suggesting that women in this study with high tTGAb levels may have had more widespread coeliac disease. We could also demonstrate a negative correlation between increased levels of tTGAb and S-HDL-cholesterol as a sign of intestinal failure. This is in agreement with similar screening showing a linear inverse relationship between levels of tTGAb, BMD and cholesterol [19], further supporting our findings.

A limitation of this study, however, is the lack of confirming biopsies in tTGAb-positive women. There is thus a possibility that false-positive tTGAb women have been misclassified as having asymptomatic coeliac disease. In our experience, these individuals are mainly detected with low or borderline levels of tTGAb [33]. Therefore, two alternative cut-off levels for a tTGAb response were set for this study. As previously discussed, there was only clear association between tTGAb and osteoporosis using the higher discriminating level, suggesting that measurement of bone density could be restricted to subjects with distinctly elevated tTGAb levels.

The strength of this study, on the other hand, is the measurement tTGAb levels in all included women. In contrast, previous studies have made such assessments only in subjects with positive EMA. The detection of IgA-tTG shows high agreement with EMA [34–39], and some assays even display a higher diagnostic sensitivity than EMA [40]. We used an immunoprecipitation assay where IgG-specific autoantibodies bind to a low amount of radioactive tTG in a solution and antibodies directed against both linear and conformational epitopes are precipitated by conjugated protein A beads. The diagnostic performance of radioligand binding tTGAb assays based on protein A displays a sensitivity and specificity of 99% and 96%, respectively, in adults [41] and a 99% concordance with IgA-tTG [42]. Furthermore, coeliac disease in individuals with IgA deficiency is detected [33], a disorder found in 1 in 500 of the general population and at a 10-fold risk for coeliac disease [43]. Previous studies may therefore have missed some individuals with coeliac disease by only using EMA, IgA-tTG or both antibodies.

The clear association between the presence of tTGAb and osteoporosis or fractures demonstrated in the present study and by West et al. [44] is more likely to be explained by the inclusion of individuals from a large unselected population with previously undetected coeliac disease. In contrast, other studies show no [45] or only a slightly increased risk for fractures [44,46]. However, these latter surveys were carried out on patients in whom coeliac disease had

already been diagnosed and who were probably receiving treatment. Other investigators have shown that fractures are mainly detected in patients with coeliac disease before diagnosis, but who had distinct symptoms of the disease, or in non-compliant patients consuming a gluten-containing diet [47]. It has been demonstrated that a gluten-free diet results in healing of the intestinal mucosa and consequently leads to improvement of BMD [48]. It is therefore likely that the frequency of osteoporosis or fractures in patients with unrecognized or non-compliant coeliac disease could be higher. In contradiction to this argument was the finding that the tTGAb-negative women with already known coeliac disease in this study showed a similarly reduced BMD to that of the women with tTGAb levels of 17 U/ml or higher, suggesting that dietary treatment is of no benefit to these women with respect to risk for osteoporosis and related fractures. However, the lack of information about these women concerning duration of coeliac disease and their compliance in adhering to a gluten-free diet limits further interpretations.

In conclusion, this study suggests that the specific marker for coeliac disease, tTGAb, is frequently found in 1 out of 71 Swedish women between 50 and 64 years of age. Individuals with distinct levels of tTGAb are more likely to have a lower BMD and an increased risk for associated fractures. As asymptomatic coeliac disease may nevertheless lead to malabsorptive disorders and consequently will only be detected by screening or a case-finding methodology, undetected coeliac disease should therefore be considered as one of the possible causes in middle-aged or older women with newly diagnosed osteoporosis or associated fractures. Furthermore, measurement of BMD should be considered in women detected with high levels of tTGAb.

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