Prevalence of IgA-antiendomysial antibody in a patient cohort with idiopathic low bone mineral density

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Abstract

AIM: To investigate the frequency of serum IgA-antiendomysial antibody positivity in patients with low bone mineral density and to assess the risk group for screening of celiac disease.

METHODS: One hundred and thirty-five patients (14 male, 121 female) with idiopathic low bone mineral density were evaluated. The median age was 57.2 years (24-81). Antiendomysial antibody was determined by the immunofluorescence method using a commercial kit (INOVA Diagnostics Inc., CA, USA), which employs a 5 μm thin cryostat section of monkey esophagus as a substrate.

RESULTS: Of the 135 patients evaluated, 13 were found to have positive IgA antiendomysial antibody test (9.6%) response. None of the patients had IgA deficiency. Endoscopic appearance and histological examination were normal in all of these patients. Seropositive patients had significantly lower age (48.9 ± 4.3 vs 59.2 ± 6.2, \( P < 0.05 \)), higher ratio of male gender (61.5% vs 49%, \( P < 0.01 \)) and pre-menopausal status (8.7% vs 13%, \( P < 0.01 \)). Lumbar spine and femoral neck z-scores, but not t-scores were significantly lower in seropositive patients. Seropositive patients had lower serum 25 (OH) vitamin D, calcium and higher serum parathormone levels than seronegative patients.

CONCLUSION: The screening of celiac disease in idiopathic osteoporosis should be restricted to patients without classical risk factors (younger, pre-menopausal, male gender) for osteoporosis. Bone mineral density measurements using z-scores should be considered for identifying risk groups for celiac disease.

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Key words: Antiendomysial antibodies; Celiac disease;
consecutive patients with a diagnosis of osteoporosis or osteopenia in the Department of Physical Therapy and Rehabilitation at Gazi University Faculty of Medicine between April 2003 and January 2006. Inclusion criteria were idiopathic low BMD (below 1 SD of the mean), normal values of serum calcium, phosphorus, alkaline phosphatase and creatinine. Exclusion criteria were diseases affecting bone metabolism (Cushing’s disease, hyperparathyroidism, hyperthyroidism, cholestatic liver diseases, osteogenesis imperfecta, acromegaly, etc.), neoplastic diseases or taking drugs known to affect bone metabolism, such as corticosteroid, antiepileptics or heparin or any other condition known to cause secondary osteoporosis were excluded from the study.

**Questionnaires**
The data on the general health status and lifestyle factors were collected by questionnaires. The questionnaire included demographic features, menarchal and menopausal age, parity, breast-feasting, consumption of dairy products, smoking, alcohol consumption, medical history including symptoms or signs of CD (diarrhea, dyspepsia, bloating, altered bowel habits resembling irritable bowel syndrome and anemia) and current diseases, physical activity and immobilization history longer than 2 mo and previous fractures.

**Bone mineral density measurements**
BMD of the proximal femur and lumbar spine was carried out using dual-energy X-ray absorptiometry (Hologic QDR 4500C, Walthom, MA, USA). BMD was expressed as standard deviation scores, which compare individual BMD determinations to those of young (T) and age/sex-matched (Z) normal populations.

**Biochemical measurements**
Biochemical parameters including serum calcium, phosphate, alkaline phosphatase and creatinine were measured by standard automated techniques. Serum intact parathyroid hormone (PTH) was measured using the chemiluminescence method (PTH Intact DPC Immulite 2000 autoanayser). 25-hydroxy-vitamin D were determined by radioimmunoassay (RIA) using a commercial kit (Biosource Inc., USA).

Hematological (hemoglobin, folic acid, vitamin B12, mean corpuscular volume, erythrocyte sedimentation rate, and immunoglobulin) tests were performed using routine methods.

**Determination of anti-endomysial antibody**
EMA was determined by the immunofluorescence method using a commercial kit (INOVA Diagnostics Inc., CA, USA), which employs a 5 μm thin, cryostat section of monkey esophagus as a substrate. Duplicate serum samples were tested at a dilution of 1:5. Total IgA levels were also determined. Sensitivity and specificity of EMA are 97.4% and 99.6%, respectively[16,17].

**Small bowel histology**
All small intestinal biopsies are obtained via videogastroscope (from distal duodenum). At least three biopsies were obtained and preserved conventionally. Pathologic assessment was done by an experienced pathologist. The histological characteristics of intestinal mucosa were assessed by conventional microscopy.

**Diagnosis of celiac disease**
A minimal criterion for CD diagnosis was positive serology together with characteristic features of intestinal mucosal changes (villous atrophy, crypt hyperplasia, increased intraepithelial lymphocyte infiltration > 30%).

**Statistical analysis**
Statistical analyses were performed using the SPSS 15.0 statistical program. Student’s unpaired t-test was used for comparison of the differences between the EMA positive and negative patients. In EMA positive patients, Pearson’s correlation analysis was performed to assess the association between BMD and Ca, PTH, 25(OH) vitamin D levels. Data were expressed as the mean ± standard deviation (SD). Differences were considered significant, if the P values were less or equal to a level of 5% and all results are expressed at a 95% confidence level.

**RESULTS**
One hundred and thirty-five patients (14 male, 121 female) with idiopathic low BMD were evaluated. The median age was 57.2 years (24-81). Upon evaluation of the questionnaires, none of the patients was found to have signs or symptoms of CD such as malabsorption, diarrhea, weight loss or anemia.

Of the 135 patients evaluated, 13 were found to have positive IgA EMA test (9.6%). None of the patients had IgA deficiency. All of the thirteen patients with positive EMA in their sera underwent upper gastrointestinal endoscopy and duodenal biopsy. Endoscopical appearance of duodenal mucosa was normal in all of these patients. The histopathological examination revealed non-specific changes, such as mild lymphocyte infiltration in lamina propria and none of them had findings consistent with CD. We could not detect any patient with celiac disease in this population.

For the statistical analysis, the data obtained from the 13 EMA positive patients were compared with the data of 122 EMA negative patients. The demographic features and BMD values of these patients are shown in Table 1. When EMA positive patients were compared with EMA negative patients, EMA positive patients had significantly lower age (48.9 ± 4.3 vs 59.2 ± 6.2, P < 0.05), higher ratio of male gender (61.5% vs 4.9%, P < 0.01) and pre-menopausal status (8.7% vs 1.3%, P < 0.01).

Other parameters including weight, height, BMI, lumbar spine and femoral neck t-scores were similar between groups. However, lumbar spine and femoral neck z-scores were significantly lower in EMA positive patients (Table 1).

Table 2 shows laboratory findings and comparison between EMA positive and negative patients. EMA positive patients had lower serum 25(OH) vitamin D,
Asymptomatic (subclinical and silent) CD manifests with extra-intestinal features\(^\text{[3]}\). The most frequent extra-intestinal marker of subclinical CD is iron-deficiency anemia (27.77\%), alopecia and dermatitis herpetiformis (11.36\%), osteoporosis (6.81\%) and recurrent aphthous stomatitis (5.68\%). The most frequent features in silent form of CD are CD history in first-degree relatives (30\%), Basedow’s disease (25\%) and insulin-dependent diabetes (20\%).

Clinical diversity and its potential complications are the main logic behind the studies investigating asymptomatic CD. Lindh \textit{et al.}\(^\text{[15]}\) reported the first seroprevalence study of CD in idiopathic osteoporosis. They investigated 11 out of 92 seropositive patients (11\%). Duodenal biopsy was positive in three of them. Similar findings were reported in 255 osteoporotic women from Italy. The seroprevalance of CD in idiopathic osteoporosis was 9.4\% (24 patients) and celiac disease histology was positive in three of them. Similar findings were reported in another study\(^\text{[16]}\).

Our findings also indicated that serum parathormone levels were higher, however, 25(OH) vitamin D and serum Ca levels were lower in the EMA positive group than the EMA negative group (Table 2). These findings were also reported in another study\(^\text{[10]}\). O’Leary \textit{et al.}\(^\text{[21]}\) from Ireland, studied 371 female subjects attending for bone densitometry, without secondary causes of osteoporosis. Two of 115 (1.7\%) female subjects with normal bone density and five of 256 (1.9\%) female subjects with sub-normal bone density were positive for EMA.

In our study, we have found 13 EMA positive results out of 135 patients (9.6\%). None of them exhibited characteristic histological features of CD. These patients did not have any other extra-intestinal manifestations of CD.

Post-menopausal status, advanced age and female gender are the established risk factors for low BMD\(^\text{[29]}\). O’Leary \textit{et al.}\(^\text{[21]}\) from Ireland, studied 371 female subjects attending for bone densitometry, without secondary causes of osteoporosis. Two of 115 (1.7\%) female subjects with normal bone density and five of 256 (1.9\%) female subjects with sub-normal bone density were positive for EMA.

In our study, EMA positive patients had significantly lower age (48.9 ± 4.3 vs 59.2 ± 6.2, \(P < 0.05\)), higher ratio of male gender (61.5\% vs 49.9\%, \(P < 0.01\)) and pre-menopausal status (8.7\% vs 1.3\%, \(P < 0.01\)). These patients have lower risk factors for low BMD. In this sub-group (pre-menopausal, younger patients or males), serological screening led to higher frequency of EMA positive patients.

Our findings also indicated that serum parathormone levels were higher, however, 25(OH) vitamin D and serum Ca levels were lower in the EMA positive group than the EMA negative group (Table 2). These findings were also reported in another study\(^\text{[10]}\). Armagan \textit{et al.}\(^\text{[6]}\) studied 89 premenopausal women with idiopathic osteoporosis. Of the 89 patients evaluated, 17 were found to have positive IgA AGA tests (19\%) and 9 were found to be positive for EMA (10.11\%). They also reported lower levels of 25(OH) vitamin D and serum Ca in EMA positive patients. However, the main limitation of this study is the lack of endoscopic and histologic evaluation of EMA positive patients.
Chronic gastrointestinal diseases can affect bone remodeling by altering both systemic and local regulatory factors, which means that bone loss can be induced by Ca and phosphate alterations, hormones, and local factors such as growth factors and cytokines. Although decreased bone mass is clearly documented in celiac patients, the underlying pathological mechanisms are still controversial. It seems that at least two main mechanisms should be considered. The first is due to intestinal malabsorption, which can lead to not only Ca and vitamin D deficiency, but also to general malnutrition and a reduced BMI; the second is related to the presence of inflammation. Absence of gastrointestinal and other symptoms of CD is not a prerequisite for the development of bone related complications. Malabsorption and inflammation may not be the sole factors for decreased BMD in CD. Sugai et al. reported increased prevalence of anti-bone antibodies (51.5%) in CD and these antibodies significantly decreased after gluten-free diet. Thus, biochemical abnormalities, which were observed in our study in EMA positive patients, might be a consequence of pathogenetic mechanisms other than malabsorption in CD.

Controversial results obtained from previous studies might be the result of the study cohort. Most of the studies on post-menopausal women failed to find an increased prevalence of CD in osteoporotic patients. However, premenopausal women with low BMD have an increased seroprevalence of EMA. The results should also be evaluated according to the baseline prevalence of CD in the population studied. Such as in Ireland, where the prevalence of CD is high, the prevalence of CD among osteoporotic women and controls might be similar.

Predominance of older, postmenopausal patients in our study might affect our EMA seroprevalence. However, postmenopausal patients might also have silent CD and this might augment the degree of osteoporosis. For this reason, we have not selected a premenopausal patient cohort in this study.

Prevalence of CD in Turkey is reported in a recent study on school-age children. Although adult prevalence is lacking, prevalence of biopsy-proven CD in Turkey is 1/115 in children. This prevalence approximates the prevalence in many European and North American countries. For this reason, the increased EMA seroprevalence in our study (9.6%) is not augmented by the background general prevalence of the disease.

In EMA positive patients, z-scores but not t-scores were lower than EMA negative patients (Table 1). These two scores are expressed as the number of standard deviations by which BMD deviates from the mean value. The t-score is expressed in relation to the mean value in subjects aged 30 years and the z-score to the mean age-matched value. At the age of 30, the z-scores are close to the t-scores. The t-score is an absolute measure, and as BMD decreases with age, an increasing proportion of subjects will fall below a t-score of -2.5. For this reason, z-score might be more sensitive for detecting changes related to secondary (not postmenopausal bone loss related to age) causes of bone loss, such as CD.

In conclusion, we have detected higher EMA seroprevalence in patients with idiopathic low BMD than the general population. EMA positive patients are younger and of premenopausal status or male gender predominates. These patients have low serum values of Ca, vitamin D, BMD (z-scores) and higher serum PTH. The screening of CD in idiopathic osteoporosis should be restricted to patients without classical risk factors for osteoporosis (advanced age, post-menopausal). A novel finding in our study is that z-scores are more accurate in determining risky groups for CD. However, larger studies are needed for clarifying this issue.

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