Inappropriate restriction of dietary gluten and associated bone acquisition and bone density in Egyptian children with coeliac disease

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Abstract

Introduction: Early onset osteoporosis is a serious complication for coeliac disease (CD). Adherence to a gluten-free diet (GFD) has been shown to restore bone density. The aim of the study was to evaluate the association between inappropriate dietary restriction of gluten and bone density in children with CD.
Material and methods: Twenty-one children with CD were diagnosed according to guidelines of the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN). All patients had persistent high level of anti-tissue transglutaminase IgA antibody (IgA-tTG). Ten males and 11 females, mean age 7.52 ±4.19 years. Thirty healthy children were included as controls. Laboratory investigation included serum osteocalcin, carboxy-terminal propeptide of type I collagen (CICP), osteoprotegerin and receptor activator of nuclear factor κB (RANKL) and urinary deoxypyridinoline (DPD). Bone mineral density and content (BMD and BMC) were measured by dual X-ray absorptiometry (DEXA) in spinal lumbar and femoral neck regions.
Results: Spinal lumbar BMD and BMC, and femoral BMD and BMC were significantly lower in patients than controls (p = 0.007, 0.003 respectively) (p = 0.002, 0.006 respectively). There were statistically significant decreases in serum calcium, osteocalcin, CICP and osteoprotegerin levels in patients compared to controls (p = 0.0002, 0.0001, 0.01 and 0.0001 respectively).
Conclusions: Egyptian children with CD not adherent to GFD had BMD and BMC values significantly lower than in controls. These results emphasize that compliance to GFD is the main challenge for the patients and physicians.

Key words: coeliac disease, children, gluten-free diet, osteoporosis.

Introduction

Coeliac disease (CD) is an autoimmune disorder that occurs in genetically predisposed individuals as the result of an immune response to gluten. It is present in approximately 1% of the population. Diarrhoea has become a less common mode of presentation (< 50% of cases). Coeliac disease, a common cause of malabsorption, is known to be associated with disorders of the skeleton. Low bone density and early onset osteoporosis are serious complications for CD in children which can lead to repeated fractures and delayed growth [1, 2].

There are conflicting data about the effect of diet on bone metabolism, after following a gluten-free diet (GFD). Intestinal absorption is restored. Bone mineral density (BMD) has been found to increase in adults; children

also experience a complete catch-up growth in height and weight, and an increase in bone mass. However, it is not definitively established whether GFD restores bone mass to normal values [3]. McFarlane *et al*. said that GFD does not always lead to improvements in BMD; they also reported that about 40% of treated patients with GFD have BMD below the normal mean [4].

Although adherence to GFD has been shown to restore calcium (Ca) absorption and bone density, it has been noted that 7 to 55% of patients with CD do not adhere to a strict GFD [5]. So, the main challenge for coeliac disease patients is dietary compliance.

Osteoporosis is characterized by low bone mass and changes in the microarchitecture of the bone. This leads to reduced bone stability and increased susceptibility to fractures. At the cellular level, bone remodelling is regulated by osteoclast and osteoblast activity. During bone loss, there is an imbalance, osteoclast activity being more pronounced [6].

Biochemical markers of bone remodelling are divided into markers of bone formation usually measured in serum (e.g. osteocalcin, carboxy-terminal propeptide of type I collagen) and markers of bone absorption determined in serum or urine (e.g. pyridinoline and deoxypyridinoline). Their assessment during growth periods in childhood and adolescence should take into consideration that their values depend on numerous variables, e.g. age, growth velocity, pubertal stage, nutritional status, and circadian and day to day variation [7]. Biochemical markers of bone turnover allow clinicians to evaluate the risk of bone loss and provide insight into response to therapy and encouraging patient compliance [8].

Osteoprotegerin (OPG), a member of the tumour necrosis factor receptor family and receptor activator of nuclear factor κB (RANKL), which is a ligand of receptor activator of tumour necrosis factor κB (TNF-κB), are involved in the process of bone turnover and have been implicated in the pathogenesis of osteoporosis and other metabolic bone diseases [9].

In this study, we evaluated the association between inappropriate dietary restriction of gluten, bone acquisition and bone density in Egyptian children with coeliac disease.

Material and methods

Patients

Our study is a control study and included 21 children with CD not completely adherent to GFD (group I) diagnosed according to clinical guidelines for diagnosis of coeliac disease of the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition "NASPGHAN" [10]. All patients had + ve serum anti-tissue trans-glutaminase IgA antibody IgA-tTG [11] and/or IgA-anti-endomysium antibodies (IgA-EMA) [12] which was then confirmed by jejunal biopsy. The entire study group had a persistent high level of IgA-tTG in serum although it should be normalized if they were on strict GFD. Exclusion criteria included the presence of other diseases known to affect the bone mineral density (BMD). None of the patients received hormone or dietary supplements. They were 10 males and 11 females from those attending the Tropical Pediatrics and Chronic Diarrhea Clinic at the Centre for Social and Preventive Medicine (CSPM), Cairo University Children's Hospitals. Patients were then referred to the Pediatric Clinic in the National Research Centre (NRC) in the period from March 2005 to March 2007. Thirty healthy age and sex matched children were also included in the study (group II), serving as a control group.

Informed consent was taken from the parents of the children according to guidelines of the ethical committee of NRC, Dokki, Egypt.

All patients were subjected to full history taking and general examination. Past history included drug intake, vitamin fortification, gluten-free diet, paternal or maternal smoking and social data.

Anthropometric measurements (height and weight) were measured. Weight was measured using a standard clinical balance (weight was approximated to the nearest 0.1 kg) and height was measured using a fixed stadiometer (height was approximated to the nearest millimetre). All measurements were made with the children wearing light indoor clothes without their shoes [13]. Body mass index was calculated as weight divided by height squared (kg/m^2). Pubertal status was determined and classified according to Tanner stages as prepubertal/stage I, early puberty/stages II and III, and late puberty/stages IV and V [14].

Laboratory measurements

Laboratory examination for assessment of bone turnover markers (bone formation and absorption) included:

- osteocalcin as a marker of osteoblastic activity and bone formation; osteocalcin was measured by Enzym Immuno Assay (EIA) kit, catalogue # KAP1381, BioSource Europe SA, Nivelles, Belgium [15];
- carboxy-terminal propeptide of type I collagen (CICP) as a marker of bone formation. CICP was measured in serum by METRA CICP Enzyme-Linked Immunosorbent Assay (ELISA) kit, catalogue # 8003, Quidel Corporation, San Diego, CA USA [16];
- deoxypyridinoline (DPD) as a marker for osteo-clastic activity and bone absorption; DPD level

was measured in urine by METRA CICP Enzyme-Linked Immunosorbent Assay (ELISA) kit, catalogue # 8007, Quidel Corporation, San Diego, CA USA [17];

- osteoprotegerin (OPG) was measured in serum using Osteoprotegerin Human ELISA Kit, catalogue # RD194003200, BioVendor Laboratory Medicine, Inc., Czech Republic [18]; RANKL level was estimated by using ELISA technique, catalogue # BI-20422H, Biomedica Medizinprodukte GmbH & Co KG, Wien, Austria [19].

Bone mineral measurements

Bone mineral measurements were done using dual energy X-ray absorptiometry (DEXA) (Norland –XR-46 , USA). Bone mineral content (BMC) and bone mineral density (BMD) of the lumber spine (L1-L4) and Lt femoral neck were performed at the Medical Services' Centre, NRC. Absolute values were converted to *z*-scores. Bone mineral density (BMD) was expressed in g/cm^2 and BMC was expressed in g [20].

Statistical analysis

SPSS for Windows, version 10.0 computer program was used for statistical analysis. Data were represented as frequency, percent, range and mean ± standard deviation. The *t*-test was used to compare between 2 independent means. Spearman's correlation coefficient rho was used to correlate between non-normally distributed continuous variables. Chi-square test or Fisher's exact test was used to compare between independent proportions. A *p* value of less than 0.05 was considered statistically significant.

Results

Table I shows some descriptive data of the studied coeliac children. Their mean age was 7.52 ±4.19 years (1-16 years) and the mean duration of disease was 7.02 ±4.19 years (0.5-15.5). Ten out of 21 (47.6%) had positive consanguinity. The cases were subdivided according to socioeconomic standard [5]. Three cases were of high socio-economic standard, 9 of middle and 9 of low socio-economic standard. Among 21 patients 17 (81%) children presented with chronic diarrhoea and 12 (57.1%) patients suffered from failure to thrive. All our patients were in pre-pubertal stage/stage I except 2 cases who were in early pubertal stage I/stage II.

Table II and Figure 1 show anthropometric, biochemical and DEXA data of coeliac children and controls. Height and weight *z*-score of coeliac children were – 2.69 ±2.04, –1.54 ±1.33 respectively; they were statistically significantly lower than control ($p < 0.05$, $p < 0.05$ respectively). Also BMI was statistically significantly lower compared to the control group ($p = 0.029$).

Table I. Descriptive data of the studied children with coeliac disease

Parameters		Mean ± SD
Age [years]		7.52 ±4.19
Weight-for-age z-score „WAZ"		–1.54 ±1.33
Height-for-age z-score „HAZ"		–2.69 ±2.04
Body mass index „BMI" [kg/m^2]		16.62 ±3.35
		Number (%)
Gender „*n* (%)"	Male	10 (47.6%)
	Female	11 (52.4)
Socioeconomic status „*n* (%)"	Low	9 (43%)
	Moderate	9 (43%)
	High	3 (14%)
Clinical presentation „*n* (%)"	Chronic diarrhoea	17 (81%)
	Failure to thrive	12 (57.1%)

Table II. Anthropometric, biochemical and dual X-ray absorptiometry data of coeliac children and controls

	Patients (mean ± SD)	Controls (mean ± SD)	Value *p*
Weight-for-age z-score „WAZ"	–1.54 ±1.33	0.511 ±1.67	< 0.05*
Height-for-age z-score „HAZ"	–2.69 ±2.04	–0.44 ±1.55	< 0.05*
Body mass index „BMI" [kg/m^2]	16.62 ±3.35	19.85 ±6.27	0.029*
Serum calcium [mg/dl]	8.41 ±1.21	9.74 ±0.92	0.0002*
Serum osteocalcin [mg/dl]	9.27 ±9.68	45 ±12.09	0.0001*
Serum CICP [ng/ml]	302.46 ±187.24	470.62 ±203.62	0.01*
Serum osteoprotegerin [ng/ml]	3.63 ±3.05	13.05 ±4.33	0.0001*
Plasma RANK-L [ng/ml]	33.86 ±65.52	0.171 ±0.8	0.006*
Urinary DPD [mmol/mmol creatinine]	29.79 ±28.71	68.68 ±29.91	0.006*
Femoral bone mineral content [g]	1.6804 ±0.8719	2.5 ±1.10	0.006*
Femoral bone mineral density [g/cm^2]	0.5771 ±0.1515	0.71 ±0.14	0.002*

CICP – carboxy-terminal propeptide of type I collagen, RANKL – receptor activator of nuclear factor κB, DPD – urinary deoxypyridinoline

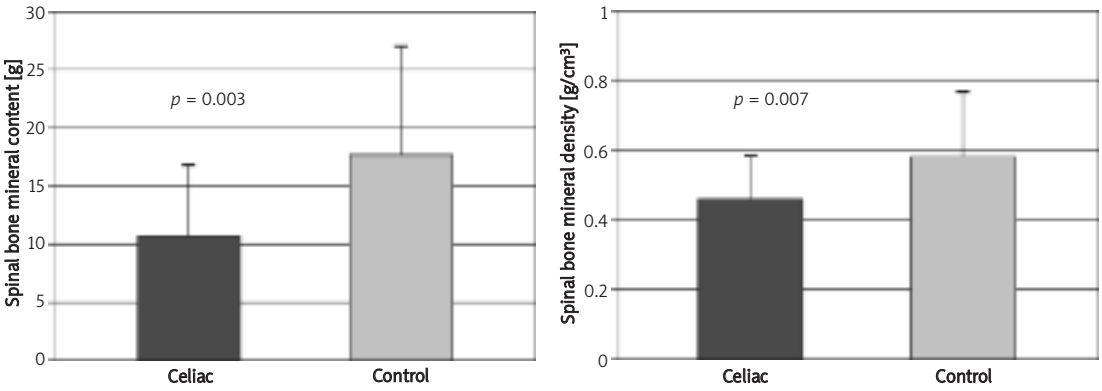

Figure 1. Spinal BMC and spinal BMD in celiac patients and control

Osteopenia, z-score lower than – 1.0 in femoral or spinal BMD, was found in 6 cases (30%) of coeliac disease and one case (3.3%) in controls with a statistically significant difference (p = 0.012). Spinal BMD and BMC were significantly lower in coeliac patients than in the control group (p = 0.007, p = 0.003 respectively); also femoral BMD and BMC in coeliac disease patients were significantly lower than in the control group (p = 0.002, p = 0.006 respectively).

As regards biochemical parameters of bone turnover in coeliac disease patients, there were highly statistically significant decreases in serum Ca, osteocalcin, CICP and osteoprotegerin levels in coeliac patients compared to controls (p = 0.0002, 0.0001, 0.01 and 0.0001 respectively). On the other hand, there was a statistically significant increase in serum level of RANKL in cases compared to controls (p = 0.006). A positive correlation was found between osteocalcin and osteoprotegerin in cases (r = 0.8382, p = 0.009), and between BMI and osteocalcin (r = 0.7529, p = 0.003) (Figure 2).

Discussion

Coeliac disease, a common cause of malabsorption in childhood, is frequently associated with skeletal disorders such as osteoporosis, rickets and osteomalacia. Several studies have demonstrated the presence of low bone mineral density in up to 75% of adults and children with untreated coeliac disease [21]. In our study, all cases were non-adherent to GFD or partially adherent, proved by persistence of IgA-tTG antibodies in their serum, and the mean duration of the CD was 7.02 ±4.19 years. Dietary non-adherence is the most common cause of unresponsive coeliac disease. The highest rates of adherence are reported among patients with the diagnosis in childhood and those with severe symptoms at presentation. Studies from France and Belgium show that less than half of adults with coeliac disease adhered strictly to the diet for more than a year after diagnosis [22]. Meanwhile in a study from the United Kingdom, the rate of adherence was low for both teenagers and adults [23]. In a study from Italy, adolescents

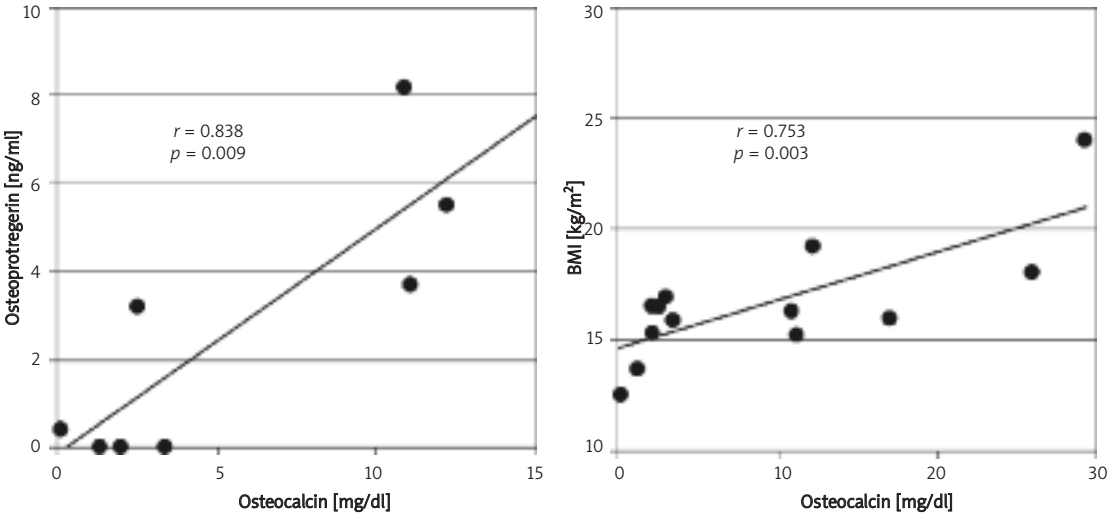

Figure 2. Scatter diagram and regression line of serum osteocalcin vs. serum osteoprotregerin and BMI in celiac children

and adults in whom the diagnosis was established on the basis of mass serological screening had poor adherence [24]. In another study, many people in whom the disease was diagnosed in childhood became non-adherent to a strict gluten-free diet as adults [25]. Goddard and Gillett stated that compliance with the diet can be expected to be poor, especially in patients with minimal symptoms, and the precise diet that should be recommended is still controversial [26]. Factors associated with poor compliance include older age of diagnosis, poorer baseline parent education and low family social class. The later significantly predicts poor dietary compliance [5]. Our results showed that 43% had low socioeconomic status, 43% middle and only 14% high socioeconomic status, so the highest percentages of poor compliance were in low and middle classes. However, some cases belonged to the high levels. An interesting point is the presence of dietary non-adherent cases in the high socioeconomic level group, which may be due to unavailability of GFD in regular stores and poor information about the hidden gluten in our diet.

Height, weight and BMI of our cases were significantly lower than controls; height was affected more than weight and this may point to the chronicity of the disease process due to inappropriate restriction of dietary gluten and the unbalanced diet of our cases.

As the nutritional adequacy of the GFD can vary considerably among individuals with CD, the use of standard multivitamins with minerals is recommended for all newly diagnosed patients [27]. Their use should also be considered for previously diagnosed patients who may have increased needs or simply have difficulty getting adequate amounts of nutrient dense foods to meet normal needs [28].

Thirty percent of our cases showed osteopenia with z-score BMD lower than – 1.0 in the femoral neck and lumber spine. This is in agreement with Kalayci *et al*., who found that osteopenia (*z*-score lower than –1.0) was found in 50% of patients with CD (62.5% in newly diagnosed cases and 37.5% in those who follow GFD strictly) [29]. This was also in agreement with the other studies performed on patients with CD in childhood [30]. In addition, Tau *et al*. noticed that axial BMD below –1 SD was found in 58% of children with coeliac disease before treatment. Following GFD, BMD increased more than 1 SD in most children under 4 years of age; a similar increase was only observed in 50% of children more than 4 years of age, some of whom did not follow GFD strictly [31]. Comparing BMD of our cases and controls, there was a statistically significant decrease in lumber spine and femoral neck BMD, which was the same result reported by Fiore *et al*. [9].

Determinants of BMD are age, sex, genetic-ethnic factors, hormonal status, calcium intake, physical activity, height, and weight, and the major ones of BMD are age, sex, and pubertal stage [32]. Genetic factors are among the predictors of peak bone density. The measures of growth and stage of pubertal development are primarily genetically determined. The dependence between BMD and growth parameters has been observed in several studies of normal healthy children [32], but the relevance of this relationship when assessing BMD in disorders in which growth may be affected has not been usually appreciated. It may be important to say that all our patients were in pre-pubertal stage except 2 cases who were in early pubertal stage.

The primary mechanism of low bone mineral in youths with coeliac disease remains unknown. Some evidence suggests a possible role of interleukins and auto-antibodies in the genesis of osteopenia. These elements might be more relevant in the developing skeleton than are nutritional factors. Maro *et al*. reported that the difference between patients with positive antibodies (t TGA antibodies) and those with negative antibodies was statistically significant in BMD responding to GFD [33]. This finding could indicate that bone metabolism may be affected by immunological alterations, which could impair normal function of bone cells and ultimately affect bone mineralization. Sugai *et al*. had explained that these antibodies recognize bone tissue transglutaminase as the autoantigen, and based on the localization of the immunoreactivity, they speculated that these might have an active role in the pathophysiology of coeliac disease-associated bone complications [34].

Calcium levels in our cases were statistically significantly lower than in controls, which was the same as reported by Tau *et al*. [31]. The slight hypocalcaemia could be related to chronic Ca malabsorption.

In our study, there was a positive correlation between osteocalcin and serum OPG level, which was the same result in a study of Icelandic men and women between serum OPG and the bone formation marker osteocalcin [35].

Regarding results of OPG and RANKL in our patients, there was a lower serum level of OPG and higher level of RANKL than in controls. Fiore *et al*. found that serum OPG and RANKL levels were significantly higher in CD patients than in controls [9]; the role of elevated OPG in this study is unclear, but it might point to a transient dynamic compensatory mechanism against other factors that promote bone damage. Osteoprotegerin functions as a soluble decoy receptor to RANK-L and competes with RANK for RANK-L binding. Consequently, OPG is an effective inhibitor of osteoclast maturation and activation. Inhibition of RANK-L function via OPG might therefore prevent

bone destruction and cartilage damage, so OPG ameliorates the osteopenic condition and prevents excess bone destruction [36]. This may explain the low level of plasma OPG in our cases. However, the clinical utility of serum OPG and soluble RANKL (sRANKL) measurements as markers of disease activity requires further investigation. It should be remembered that it is the ratio of OPG/RANKL that determines the net effect on osteoclast activity and that measuring each molecule in isolation has its limitations. In the case of serum OPG measurement, attention should be paid to the development of assays that specifically detect the active form of OPG (the homodimer). The usefulness of circulating sRANKL measurements remains uncertain because the major proportion of RANKL in bone is membrane bound. In both cases, rigorous testing of assays should be carried out and the sources of pre-analytical variability identified [37].

In conclusion, Egyptian children with CD not on strict GFD had BMD and BMC values significantly reduced in lumbar spine and femoral neck bones compared to controls. These results emphasize that compliance to GFD is the main challenge for the patients and the physicians. Gluten-free diet is more expensive, unavailable in regular stores, and has poor palatability; patients are exposed to pressures from the surrounding community, peers and friends; also there is poor medical follow-up and poor information available about the hidden gluten in our food. The use of GF cookbooks and GF special foods, the inclusion of nutritious alternative grains in their food, including uncontaminated oats if appropriate, periodic follow-up with a registered dietician, and participation in local and national support groups can improve dietary compliance and quality of life for individuals with CD. These problems should be solved in addition to health education and culture changes, which are required to optimize the growth and quality of life of these children.

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