# Evaluation of bone mineral density and bone turnover markers in Egyptian children with juvenile rheumatoid arthritis

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**Submitted:** 29 September 2008
**Accepted:** 1 January 2009

Arch Med Sci 2009; 5, 3: 434-442


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## Abstract

**Introduction:** To evaluate bone mineral density (BMD) and levels of bone turnover markers in Egyptian children with juvenile rheumatoid arthritis (JRA), and its relationship with disease-related variables.
**Material and methods:** A case-control study included thirty children having JRA and 25 healthy controls. They were subjected to measurement of BMD of lumbar spines (L2-L4) and femoral neck using dual-energy-X-ray absorptiometry (DXA) with laboratory evaluation of bone turnover markers including serum receptor activator of nuclear factor κB-ligand (RANKL) and osteoprotegerin (OPG).
**Results:** Patients had significantly lower femoral neck BMD than controls ($p = 0.02$), and it was significantly lower in patients with corticosteroid therapy ($p = 0.04$). Eight patients (26.7%) and only 2 (8%) controls had low BMD at lumbar spine, while 13 patients (43.3%) and 2 (8%) controls had low BMD at femoral neck. Patients showed significantly higher RANKL, OPG and deoxypyridinolin ($p = 0.0001$, $p = 0.049$, $p = 0.047$), while calcium, osteocalcin, bone-specific alkaline phosphatase and OPG/RANKL ratio were significantly lower in them ($p = 0.015$, $p = 0.031$, $p = 0.041$, $p = 0.0001$). Patients with normal BMD were significantly taller than patients with low BMD ($p = 0.035$), while the number of active painful joints and swollen restricted mobility joints were significantly higher in patients with low BMD ($p = 0.03$, $p = 0.02$), with no significant difference regarding disease duration and bone turnover markers ($p > 0.05$).
**Conclusions:** JRA patients had lower BMD, higher frequency of low BMD (more prominent in the femoral neck) and higher OPG and RANKL levels compared with healthy children; suggesting that they may be at risk of developing premature osteoporosis and fractures later in life.

**Key words:** juvenile rheumatoid arthritis, DXA, BMD, RANKL, osteoprotegrin, children, osteoporosis.

## Introduction

Juvenile rheumatoid arthritis (JRA) is a chronic systemic autoimmune inflammatory disease characterized by synovial inflammation with progressive joint damage and breakdown of cartilage and juxta-articular bone [1]. It may be associated with generalized bone loss, decreased bone mineral density (BMD) and bone mass with increased risk of osteoporosis and fractures [2, 3].

Dual energy X-ray absorptiometry (DXA) represents a major advance in osteoporosis care in the context of RA [3, 4], where osteoporosis and

growth delay have been encountered in patients with JRA as a result of disease activity [5] and corticosteroid therapy [6, 7].

Bone remodeling depends on a delicate balance between bone formation (osteoblasts) and bone resorption (osteoclasts), wherein tipping this balance in favor of osteoclasts leads to pathologic bone resorption [8].

Receptor activator of nuclear factor-κB ligand (RANKL) is produced by osteoblastic lineage cells and activated T cells in both soluble and membrane bound form and promotes osteoclast formation, differentiation, activation, and survival leading to enhanced bone resorption and bone loss [9, 10].

Osteoprotegerin (OPG) is a negative regulator of RANKL signaling [11, 12], produced by osteoblasts and acts as a soluble decoy receptor for RANKL, preventing it from binding to its receptor RANK; thereby attenuating excessive RANKL signaling and inhibits osteoclasts development thus suppressing osteoclastogenesis [13, 14].

Therefore, RANKL and OPG act as key regulators of bone metabolism and osteoclast biology [15].

OPG/RANKL ratio is an important determinant of bone mass and skeletal integrity, and predicts later joint destruction in early RA [16].

Osteoporosis is increasingly linked to bone development during childhood and adolescence [17], and recent data have indicated overlapping pathways between bone biology and biology of inflammation [18]; giving the impression that inflammation may have role in pathology of osteoporosis. Egyptian studies regarding this field in children with JRA are scarce.

The aim of this study was to evaluate BMD using DXA in parallel with serum levels of OPG and RANKL together with other markers of bone turnover in Egyptian children with JRA, and its relationship with disease-related variables, such as tender/painful/swollen joints, restricted movement of joints, disease duration, etc.

## Material and methods

This is a case-control study. Thirty children suffering from JRA (14 males and 16 females) were recruited from the Collagen Clinic of the New Childrens' Hospital, Cairo University, as well as the Pediatric Clinic of the National Research Center (NRC). Their ages ranged from 4-19 years. Diagnosis of JRA was done according to the American College of Rheumatology (ACR) criteria 1987, revised classification criteria for diagnosis of JRA [19]. Patients with any other chronic disease were excluded from the study.

Twenty five age and sex matched healthy children (16 males and 9 females) who were relatives of the medical staff of the NRC were included in the study as a control group, and their ages ranged from 5-18 years.

Parental consent to perform the study was obtained from the parents of all participant children, and the study was approved by the ethics committee for human investigation of the National Research Center.

All children included in the study were subjected to the following:

### Clinical examination

A detailed medical history, thorough clinical examination with measurement of height and weight using the same stadiometer and clinical scale, and body mass index (BMI) was calculated as kilograms per meter squared (BMI = body weight [kg]/height$^2$ [m$^2$]).

Pubertal development was established according to Tanner's stages [20]. Young children, who had not reached puberty were given the lowest value (stage 1, preadolescence). The values ranged from 1 = preadolescence to 5 = fully mature.

All children were also subjected to:

### Bone mineral density measurement

Bone status was evaluated at the time of the study by using dual energy X-ray absorptiometry (DXA) (Norland XR 46, USA), at the Medical Service Unit of the NRC. Children were examined in supine anteroposterior position using the same scan mode and all analyses were performed and read by the same investigator.

For all patients and controls, bone mineral content [BMC; g] and bone mineral density [BMD; g/cm$^2$] and bone area [cm$^2$] of total body, the lumbar spine (L2-L4) and left proximal femur (femoral neck) were measured by DXA.

Absolute values were converted into Z-scores (standard deviation from the mean of a healthy age and sex matched reference population) [21]. So, Z-score > –1 SD was considered normal and low BMD was defined as a Z-score < –1 SD.

### Laboratory investigations

**Disease-related laboratory tests*

Complete blood count (CBC), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF) and anti-nuclear antibodies (ANA) were done.

**Bone turnover-related laboratory tests*

A. Blood samples were collected from all individuals included in the study by clean venipuncture into vacutainer plane tubes under strict sterile conditions. Five ml of venous blood was obtained and put into water bath for 15 min and centrifuged at (4000 rpm). Then serum was separated and kept stored at –20°C until analysis of the following bone markers:

1) Serum calcium (Ca) level was measured using automated chemistry analyzer Olympus AU400 (Olympus Diagnostica GMBH, Japan).

2) Osteocalcin using – ELISA kit (Biosource, Europe, S.A.) which is a quantitative sandwich Enzyme Linked Immuno-Sorbent Assay [22].

3) Bone-specific alkaline phosphatase was assayed using METRA BAP immunoassay kit (Quidel corporation world wide headquarters 10165 Mc Kellar Court, San Diego, CA 92121 USA) [23].

4) Serum levels of Carboxy terminal propeptide of type I collagen (CICP) measured by METRA CICP EIA kit. It is a sandwich enzyme immunoassay in a microtiter plate format utilizing a monoclonal anti-CICP antibody coated on the plate, a rabbit anti CICP antiserum, a goat anti rabbit alkaline phosphatase conjugate and substrate to quantify the CICP in human serum [24].

5) Serum RANKL levels was estimated by using ELISA kit, (Biomedica group, Biomedica Medicine product, GmbH and Co., Cat. No. BI-20422H). It is an enzyme immunoassay for the quantitative determination of sRANKL in serum [12].

**Table I.** Characteristics of the patients and controls

| Item | Patients | Controls |
|---|---|---|
| Number | 30 | 25 |
| Male : female (N, %) | 14/16 (46.7/53.3) | 16/9 (64/36) |
| Age [years] | 11.5 ±3.86 | 9.80 ±3.20 |
| Weight [kg] | 34.58 ±15.42 | 33.20 ±12.60 |
| Height [cm] | 132.50 ±22.21 | 133.38 ±16.12 |
| BMI [kg/m$^2$] | 18.82 ±4.12 | 18.07 ±3.47 |
| Disease duration [years] | 6.43 ±2.97 | – |
| Polyarthritis (N, %) | 21 (70) | – |
| Oligoarthritis (N, %) | 3 (10) | – |
| Systemic onset arthritis (N, %) | 6 (20) | – |
| Painful/tender joint count | 13.60 ±8.69 | – |
| Swollen joint count | 7.03 ±4.73 | – |
| ESR [mm/h] | 54.17 ±28.48 | 6.72 ±1.54 |
| RF positivity (N, %) | 5 (16.7) | – |
| ANA positivity (N, %) | 3 (10) | – |
| Fractures (N, %) | 4 (13.3) | – |
| NSAIDs (N, %) | 25 (83.3) | – |
| Corticosteroids (N, %) | 21 (70) | – |
| DMARDs (N, %) | 26 (86.7) | – |
| Vitamins (N, %) | 18 (60) | – |
| Folic acid (N, %) | 12 (40) | – |
| Calcium (N, %) | 3 (10) | – |

*Data were expressed as mean ± SD and frequency for numbers between parentheses*
*BMI – body mass index, ESR – erythrocyte sedimentation rate, RF – rheumatoid factor, ANA – antinuclear antibody, NSAIDs – non steroidal anti-inflammatory drugs, DMARDs – disease-modifying antirheumatic drugs*

6) Serum Osteoprotegerin (OPG) using ELISA technique (Biovendor Laboratory Medicine, Inc., Czech Republic), Cat. No RD 194003200. It is a biotin labeled antibody based sandwich enzyme im-munoassay [25].

B. Urine samples (preservative first morning void urine or secondary morning void urine) were taken also from all children enrolled in the study and stored at –20°C for determination of deoxy-pyridinoline (DPD). Urinary deoxypyridinoline (DPD) concentration was measured by METRA DPD EIA kit, Cat no. 8007. METRA DPD assay is a competitive enzyme immunoassay, in microtiter strip-well format utilizing a monoclonal anti-DPD coated on the strip to capture DPD in the sample and compete with conjugate DPD alkaline phosphatase for antibody and the reaction is detected with substrate. Metra DPD results are corrected for urinary concentration by creatinine in urine [26].

### Statistical analysis

Statistical package for social sciences (SPSS) program version 11 was used for analysis of data. Data were described in terms of mean ± SD and percentage. Analysis of two quantitative in-dependent variables was done by using non parametric test (Mann Whitney U test) and $\chi^2$ test was used for qualitative variables. Spearman correlation was done. *P*-value is considered significant if < 0.05.

## Results

Characteristics of JRA patients and controls are summarized in Table I. Duration of disease ranged from 1 to 12 years and the patients were classified into polyarticular JRA; 21 patients (70%), oligo-articular JRA; 3 patients (10%) and systemic onset JRA; 6 patients (20%). Three patients (10%) were ANA positive, and 5 patients (16.7%) were RF positive. Four patients (13.3%) had previous fractures and they were all males. None of the fractures were vertebral.

Regarding therapy, non steroidal anti-inflam-matory drugs (NSAIDS) were used by 25 patients (83.3%) and 21 patients (70%) were on corti-costeroids. Disease-modifying antirheumatic drugs (DMARDs) were used by 26 patients (86.7%) including hydroxychloroquine 21 (70%), methotrexate 20 (66.7%), leflunomide 6 (20%) and sulfasalazine 1 (3.3%). Also, 18 patients (60%) were receiving multi-vitamin supplementation, 12 (40%) were receiving folic acid and 3 patients (10%) were receiving calcium.

At the time of the study 20 (66.7%) patients had active disease (joint swelling with pain and reduced mobility in several joints). Active JRA was defined by the presence of at least three of the following criteria ≥ 6 tender or painful joints, ≥ 3 swollen joints, ESR ≥ 28 mm/h and morning stiffness ≥ 45 min in duration [27].

Table II illustrates DXA measurements and demonstrates that BMD of the femoral neck was significantly lower in JRA patients than in the control group ($p$ = 0.02). Although other bone measurements were lower in patients compared to controls; yet the difference was not significant ($p$ > 0.05).

Total fat percentage was higher in patients than in healthy children and approached significance ($p$ = 0.05).

The frequency of low BMD (Z score < –1 SD) at the level of femoral neck was significantly higher (40%) in children with JRA than healthy children (8%) ($p$ = 0.013), and although the frequency was higher at the lumbar spine of the patients (26.7%) vs. controls (8%) it was not significant ($p$ = 0.074), with no sex related difference (Figure 1). Only one patient (3.3%) was found to be osteoporotic in the femoral neck.

Serum levels of Ca, osteocalcin and bone-specific alkaline phosphatase were significantly lower in JRA patients compared to healthy controls ($p$ = 0.015, $p$ = 0.031 and $p$ = 0.041 respectively). On the other hand, OPG, RANKL and DPD were significantly elevated in JRA patients compared to healthy children ($p$ = 0.049, $p$ = 0.0001, $p$ = 0.047 respectively). The ratio of OPG/RANKL levels was significantly lower in JRA patients than controls ($p$ = 0.0001). No difference was found regarding CICP ($p$ > 0.05) (Table III).

Regarding corticosteroid therapy; femoral BMD was significantly lower in patients with corticosteroid therapy than patients without (0.58 ±0.16 vs. 0.71 ±0.16 g/cm$^2$, respectively) ($p$ = 0.04). But, there was no significant difference regarding bone turnover markers.

There was no significant difference in bone turnover markers regarding stages of pubertal development ($p$ > 0.05).

On classifying JRA patients into 2 groups according to the state of BMD; we found that height and lean mass were significantly higher in patients with normal BMD than patients with low BMD (139.06 ±20.93 vs. 123.92 ±21.61 cm respectively for height) ($p$ = 0.035), and (25214.29 ±8992.86 vs. 17904.69 ±10182.12 g respectively for lean mass) ($p$ = 0.013). The number of active painful joints and swollen joints with restricted mobility were significantly lower in patients with normal BMD (10.71 ±7.98 vs. 17.39 ±8.37 for active painful joints) ($p$ = 0.03) and (5.12 ±3.22 vs. 9.54 ±5.32 for swollen joints with restricted mobility) ($p$ = 0.02). On the other hand, there was no significant difference between JRA patients with normal BMD and patients with low BMD regarding disease duration and markers of bone turnover ($p$ > 0.05).

Age correlated positively with lumbar spine BMD ($r$ = 0.680, $p$ = 0.0001), lumbar spine BMC ($r$ = 0.782, $p$ = 0.0001), femoral BMD ($r$ = 0.534, $p$ = 0.002) and femoral BMC ($r$ = 0.657, $p$ = 0.0001).

Femoral BMD correlated positively with age, weight, height and BMI ($r$ = 0.534, $p$ = 0.002; $r$ = 0.764, $p$ = 0.0001; $r$ = 0.797, $p$ = 0.0001 and $r$ = 0.434, $p$ = 0.016 respectively) and inversely with corticosteroids ($r$ = –0.382, $p$ = 0.037).

DPD correlated inversely with lumbar spine BMC ($r$ = –0.382, $p$ = 0.037).

## Discussion

In spite of the increasing interest in bone metabolism in patients with JRA, few data exist on

**Table II.** DXA measurements in juvenile rheumatoid arthritis (JRA) patients and control group

| Items | JRA patients ($N$ = 30) | Controls ($N$ = 25) | $P$-value |
|---|---|---|---|
| Lumbar spine (L2-L4) BMC [g] | 17.88 ±9.57 | 18.20 ±9.05 | 0.67 |
| Lumbar spine (L2-L4) BMD [g/cm$^2$] | 0.57 ±0.17 | 0.60 ±0.14 | 0.23 |
| Femoral neck BMC [g] | 2.47 ±1.06 | 2.75 ±0.95 | 0.48 |
| Femoral neck BMD [g/cm$^2$] | 0.62 ±0.17 | 0.73 ±0.13 | 0.02 |
| Total lean mass [g] | 22 046.80 ±10 054.43 | 22 310.04 ±8091.27 | 0.73 |
| Total fat mass [g] | 11 491.86 ±7094.93 | 9341.28 ±6878.60 | 0.09 |
| Total fat % | 31.86 ±10.22 | 26.00 ±12.08 | 0.05 |

*P-value is significant if < 0.05*
*DXA – dual energy X-ray absorptiometry, BMC – bone mineral content, BMD – bone mineral density*

**Table III.** Biochemical markers of bone turnover in juvenile rheumatoid arthritis (JRA) patients and healthy children

| Items | JRA patients ($N$ = 30) | Controls ($N$ = 25) | $P$-value |
|---|---|---|---|
| Calcium [mg/dl] | 8.44 ±1.93 | 9.61 ±1.07 | 0.015 |
| Osteocalcin [ng/ml] | 46.22 ±24.25 | 62.16 ±23.78 | 0.031 |
| BAP [U/l] | 50.47 ±29.35 | 64.84 ±21.36 | 0.041 |
| CICP [ng/ml] | 405.77 ±248.59 | 407.58 ±179.74 | 0.93 |
| OPG [pmol/l] | 3.79 ±1.70 | 2.93 ±1.36 | 0.049 |
| RANKL [pmol/l] | 4.53 ±2.40 | 0.20 ±0.06 | 0.0001 |
| OPG/RANKL ratio | 1.84 ±3.76 | 16.01 ±8.94 | 0.0001 |
| DPD [mmol/mmole of creatinine] | 61.42 ±32.73 | 43.42 ±28.53 | 0.047 |

*P-value is significant if < 0.05*
*BAP – bone-specific alkaline phosphatase, CICP – carboxy terminal propeptide of type I collagen, OPG – osteoprotegerin, RANKL – receptor activator of NF-κB ligand, DPD – deoxypyridinoline*

bone mineral status in Egyptian children. Lifetime fracture probability is considerably greater in JRA patients than that of older individuals due to their younger ages, their greater life expectancy and more years of declining bone mass with risk of osteoporosis [28].

This study evaluated for the first time BMD using DXA in parallel with serum levels of OPG and RANKL together with other markers of bone turnover in Egyptian children with JRA.

We found that JRA patients had significantly lower BMD of the femoral neck as compared to the control group which is in agreement with several studies [29–32]. The frequency of low BMD observed in a previous study was 25% at lumbar spine (L2-L4), and 31% at femoral neck [30]; which was lower than the observed frequency in the femoral neck of our patients. Lien *et al.* [30] also found that 5% of patients were osteoporotic in the lumbar spine, and 8% were osteoporotic in the femoral neck, which is higher than our results where only 3.3% of our patients were osteoporotic in the femoral neck with a history of femoral fracture and no one was osteoporotic at the lumbar spine; indicating less severe reduction of bone mass in our patients. This might partly be explained by the younger mean age of our patients. Still, they demonstrated a relatively high frequency of low BMD during their early stages of life, which may be associated with a potentially higher risk of osteoporosis later in life.

In the current study body composition was altered in JRA patients with a higher body fat percentage approaching significance found in them compared to the healthy children. Similar results have previously been reported [31, 33]. The altered body composition is probably related to chronic inflammation and reduced physical activity, which means that the muscle force applied to bone, is diminished and may result in low bone mass [34].

Regarding corticosteroid therapy: we observed that femoral BMD of patients with corticosteroid therapy was significantly lower than patients without corticosteroids. Previous studies have found that patients with current corticosteroids had lower bone mass [30, 31]. It was reported that short-term administration of glucocorticoids might participate in the development of glucocorticoid-induced osteoporosis via an enhancement of bone resorption and suppression of bone formation [35]. Furthermore, glucocorticoids up-regulate RANKL expression, and down-regulate OPG [36], and also suppresse serum osteocalcin [35]. But, in our study there were no significant differences of RANKL, OPG or osteocalcin in relation to corticosteroid therapy. Unfortunately, we haven't got the cumulative steroid dose for our patients.

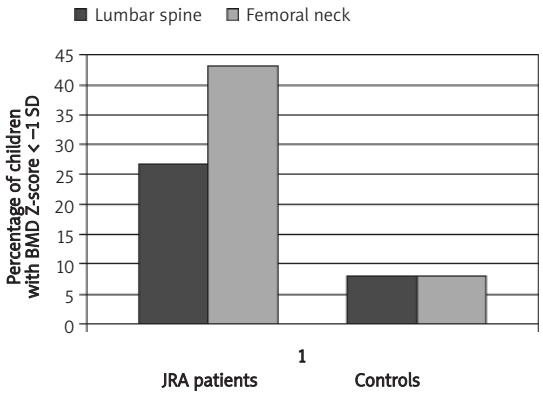


**Figure 1.** Frequency of low BMD at lumbar spine L2-L4 and femoral neck in children with JRA and controls

We found that patients with low BMD were significantly shorter and had lower lean mass than did patients with normal BMD, and they had higher number of active painful and swollen restricted mobility joints, which is similar to other studies [30, 37]. It was reported that patients with low BMD had higher disease activity values than did patients with normal BMD [30, 31]. An association between disease activity and low bone mass has also been found in several other studies of childhood arthritis [5, 28, 33, 38, 39].

Our study revealed that serum OPG concentrations were significantly higher in JRA patients than healthy children. Higher serum OPG levels have been described in children with JRA and also in adults with RA compared to healthy controls [40-45]. This observation may represent a compensatory response to the enhanced osteoclastic bone resorption, or might be the result of the inflammation. Also, DMARDs inhibit osteoclast formation by increasing the secretion of OPG through modulating the RANKL-RANK-OPG interaction [46], and 86.7% of patients of the current study were on DMARDs; which may be a contributing factor of increased OPG levels in our study. In a previous study the authors speculated that patients with more severe disease could have higher levels of serum OPG due to a compensatory self-defense response for keeping under control immune mechanisms responsible of bone and cartilage destruction. They also found that serum OPG was higher in patients with radiographic erosions and attributed this to the probable abundant cytokines in the inflamed synovial tissues in those patients which are important modulators of bone and cartilage erosion and could also have a role in stimulating OPG production [41]. Further explanation was that higher OPG levels could be secondary to a decreased clearance [47].

The present study also, showed that serum level of RANKL was significantly higher in JRA patients

compared to healthy controls, which was consistent with other studies [42, 44, 48]. The increased RANKL level may be attributed to the increased frequency of disease activity in our patients since 66.7% of them had active disease at the time of the study. It was reported that RANK/RANKL pathway was altered as part of the inflammatory process which leads to destructive synovitis in those children [48]. Activated T cells in inflammatory or autoimmune disease states produce RANKL and pro-inflammatory cytokines which in turn can induce RANKL expression in osteoblasts and bone marrow stromal cells [10]. Kotake *et al.* [49] suggested that excess production of RANKL by activated T cells increases the level of soluble RANKL and may contribute to osteoclastic bone resorption in RA patients. It was observed that inhibition of RANKL function via OPG might prevent bone destruction in RA but has no effect on inflammation [50]. So, elevated RANKL levels in JRA patients; implies that treatment with recombinant OPG could suppress the ongoing bone destruction, especially in patients treated with corticosteroids. On the contrary, other investigators observed that the amount of RANKL was lower in patients in comparison with controls [41]. They attributed this to the fact that the serum RANKL test kit they have used may be an enzyme immunoassay designed to determine soluble, uncomplexed RANKL; and it is possible that part of the molecule could bind to its receptor and part to the OPG, so that free RANKL is less available in the serum. Furthermore, it was reported that RANKL concentrations in children with JRA and controls did not differ significantly [40].

These contradictory findings can be explained by different technologies used, different patient populations and differences in age, disease duration, disease activity and medications used; where they may modify the interpretation of the results.

There was no significant difference between male and female subjects in terms of OPG and RANKL levels among JRA patients or age-matched healthy individuals which was consistent with others [40, 42], and may be attributed to the lack of sex hormone effects on bone resorption at this age group [51].

We found that OPG/RANKL ratio in our study was considerably lower in JRA patients than healthy children due to marked increased levels of RANKL in the patients with shift of the equilibrium; indicating osteoclastic activity in those patients. This is in accordance with Geusens *et al.* [16] who stated that OPG/RANKL has a central role in RA-associated joint destruction and further added that, the balance between protective signals (OPG) and activating signals (RANKL) is so critical that a single value obtained at baseline is indicative of 5-year progression of joint damage; suggesting that osteoclast activation by RANKL is a specific constitutive feature in a proportion of patients. On the other hand, Masi *et al.* [41] reported that OPG/RANKL ratio was higher in patients than in controls, which agrees with their result of a higher compensatory production of OPG. However, other investigators found the ratio was similar in both patients and controls [42].

Serum levels of osteocalcin and bone-specific alkaline phosphatase were significantly lower in our patients than in the control group which was similar to several studies [31, 33, 52] suggesting decreased bone formation. Also, serum calcium concentration was significantly lower in children with JRA, which coincide with other investigators who attributed this to inappropriate parathyroid hormone and 1,25-dihydroxyvitamin D levels in JRA [52]. On the other hand, urinary DPD was significantly elevated in JRA patients versus the control group and as a marker of bone resorption it indicates increased bone resorption in those patients.

In brief the results of bone turnover markers of the current study revealed uncoupling of bone turnover with elevated bone resorption, suggesting osteoclastic activation as a possible mechanism of bone loss in JRA patients.

We also found no significant differences of serum levels of OPG or RANKL, between patients with normal BMD and those with low BMD, which is in agreement with other studies [53]. Also, no significant difference was found between both groups regarding other bone turnover markers.

Age correlated positively with lumbar spine BMD and BMC and with femoral BMD and BMC which was consistent with another study [29]. In contrast; Oelzner *et al.* [53] found that age related negatively with lumbar spine BMD and femoral BMD of their patients since their study was performed in old patients including those above 60 years old, and age related osteoporosis only occurs in the elderly [54].

Femoral BMD was associated with age, weight, height and BMI which is in accordance with other investigators who suggested that bone mass could attain maximal development before the end of the second decade of life [55], and that the relationship between height, weight and bone mass is highly correlated [56, 57].

Our study like other studies [41] showed lack of correlations between bone mass measurements and disease duration, disease activity variables and markers of bone turnover; except for DPD which correlated inversely with spinal BMC. This may be explained by the fact that DPD is a marker of bone resorption and is elevated in patients with JRA indicating increased bone resorption.

The lack of correlation between bone measurements and OPG and RANKL may limit the use of these markers in the diagnosis of low BMD,

since none of them has proven useful as a single diagnostic index. They reflect alterations in the metabolism of the entire skeletal envelope. However, they may be useful in evaluating the physiology and pathophysiology of bone metabolism, and in elucidating the pathogenesis of bone disease [58]. They perhaps may be used to indicate bone activity but not absolute bone mass, and may be useful in directing, monitoring and following therapy.

Neither OPG nor RANKL were found to be related to disease activity variables in the current study which is consistent with several studies [40-43, 53]. Wu *et al*. [50] reported that inhibition of RANKL function via OPG has no effect on inflammation.

Only 10% of patients with JRA of the current study were receiving oral Ca supplementation and 60% were receiving multivitamins but not regularly. It was reported that Ca intake was a positive determinant of BMD in children with JRA [39]. Lovell *et al.* [59] found that Ca supplementation resulted in a statistically significant, increase in BMD in children with JRA. Besides it may be useful in prevention of bone loss. Also, Bowden *et al.* [60] recommended the priority of vitamin D supplementation in the management of pediatric patients with osteopenia or osteoporosis. Since peak bone mass is achieved before the end of the second decade of life; efforts to increase bone mineralization in children with JRA must be started at an early age, so, Ca and vitamin D supplementation and enriched foods may be beneficial to patients with JRA by increasing bone mineralization.

In conclusion, this study showed that JRA patients had lower BMD and a higher frequency of low BMD (more prominent in the femoral neck), together with higher serum levels of RANKL and OPG compared with the healthy children; indicating that there is a process leading to diminished bone mass in those patients; with the femoral neck being more affected. Those patients may be at risk of developing premature osteoporosis and associated fractures later in life. So, we recommend follow up of BMD in those patients.

Absence of correlations between serum levels of OPG, RANKL and bone measurements may limit the use of these markers in the diagnosis of low BMD in such patients. Still they may be used to indicate bone activity but not bone mass. They may be helpful in directing and following therapy, as well as the application of new therapeutic strategies that could suppress the ongoing bone destruction, especially in patients treated with corticosteroids.

It appears to be encouraging to suggest oral Ca and vitamin D supplementation and enriched food to increase bone mass in such patients with controlled use of corticosteroids.

Further large and longitudinal studies are needed using lager sample size since Watała [61] reported that the larger the sample size, the greater the power [61].

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