Relation between serum IL-17 level and risk of osteoporotic fracture in premenopausal rheumatoid arthritis patients: Clinical, radiological and laboratory studies

Hamdy Khamis Korayem a, Mohamed Mostafa Rezk b, Marwa Mohamed Hassan a,*, Sarah Sayed El-Tawab a, Nesrin Ahmed Elsaid a

a Physical Medicine, Rheumatology and Rehabilitation Department, Faculty of Medicine, Alexandria University, Egypt
b Clinical and Chemical Pathology Department, Faculty of Medicine, Alexandria University, Egypt

Received 7 June 2015; accepted 18 June 2015
Available online 17 July 2015

Abstract

Introduction: Osteoporosis is a main extra-articular complication of rheumatoid arthritis (RA) which may lead to fractures. Interleukin-17 (IL-17) is one of the cytokines which plays a significant role in RA pathogenesis and promotion of osteoporosis.

Aim of the work: To study the relation between serum IL-17 levels and the risk of osteoporotic fractures in pre-menopausal RA patients.

Patients and methods: Twenty-five premenopausal RA patients and 20 matched healthy controls were included in this study. All patients were subjected to detailed history taking, thorough clinical examination, disease activity assessment using the disease activity score-28 (DAS-28) and disability was assessed using Health Assessment Questionnaire–Disability Index (HAQ-DI). Bone mineral density and serum IL-17 levels were measured in patients and the control. Fracture Risk Assessment Tool (FRAX index) was also calculated.

Results: The mean age of RA patients was 38.8 ± 7.6 years. The BMD was significantly reduced in patients compared to the control at the femur neck (p = 0.008), wrist (p = 0.046) and at the lumbar spine (p = 0.005). The Z score was below the expected range for age in 36% compared to 5% in the control (p = 0.03). Serum IL-17 concentrations were significantly higher in patients (5.99 ± 1.22 pg/ml) compared to the control (3.73 ± 2.15 pg/ml) (p < 0.001). Serum IL-17 levels showed a significant correlation with FRAX scores. Z-score interpretation showed a strong positive correlation with the risk of osteoporotic fractures.

* Corresponding author. Mobile: +20 1002773457.
E-mail address: drmarwamohamedhassan@yahoo.com (M.M. Hassan).
Peer review under responsibility of Egyptian Society of Rheumatic Diseases.

http://dx.doi.org/10.1016/j.ejr.2015.06.006
1110-1164 © 2015 The Authors. Production and hosting by Elsevier B.V. on behalf of Egyptian Society of Rheumatic Diseases.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
1. Introduction

Rheumatoid Arthritis (RA) is the most common form of inflammatory arthritis in adults and is characterized by chronic, progressive, systemic inflammation leading to substantial pain, disability, and other comorbidities [1]. It affects 0.5–1% of the adult population worldwide. Like in other autoimmune diseases females are affected more often than males [1].

Osteoporosis (OP) is recognized as the main extra-articular complication of RA and is classified into periarticular and generalized types. The risk factors for the development of OP in rheumatoid patients include the traditional OP risk factors on one side which constitute older age, female sex, menopause, lower body mass index (BMI), cigarette smoking and alcohol consumption [2,3]. On the other side the RA disease associated OP risk factors include the inflammatory process, disease activity, RA disease duration, decreased physical activity and glucocorticoid usage [1,4–6].

Osteoporotic fractures (OFs) are fractures associated with low bone mineral density (BMD) and include spine, forearm, hip and shoulder fractures. Osteoporotic fractures can cause substantial pain and severe disability, often leading to a reduced quality of life, and hip and vertebral fractures are associated with decreased life expectancy [7]. Fracture Risk Assessment Tool (FRAX index) is a computer-based algorithm developed by the WHO Collaborating Centre for metabolic bone diseases. The outputs of FRAX are the 10-year probability of major osteoporotic fractures (hip, spine, humerus or wrist fracture) and the 10-year probability of hip fracture [8].

Osteoporosis in RA is mediated by many cytokines such as interleukin-6 (IL-6), IL-1, tumor necrosis factor-α (TNF-α) and IL-17 [9]. Interleukin-17 contributes to cartilage and bone destruction by acting as a potent inducer of the expression of matrix metalloproteinases (MMPs) and receptor activator of nuclear factor (NF)-κB ligand (RANKL) in rheumatoid synoviocytes and osteoblasts, being an important promoter of osteoestroytogenesis [10].

IL-17 is a pro-inflammatory cytokine that is secreted as a dimer by T helper 17 (TH17) cells. It is already demonstrated that in human RA, IL-17 plays a key role in the synergistic or additive effects expressed together with TNF-α and IL-1 [11]. The direct proinflammatory effects of IL-17 may be small when compared to those of IL-1 and TNF-α. However, IL-17 enhances many of the effects of IL-1 and TNF-α and stimulates their production from human macrophages. IL-17 also enhances IL-1-mediated IL-6 production by RA synovial tissue fibroblasts, as well as TNF-α induced synthesis of IL-1, IL-6 and IL-8. In short, a major role of IL-17 may be amplifying the effects of macrophage derived proinflammatory cytokines and hence be the missing link between T cells in the RA joint and the effector phase of RA [12].

The aim of the current study was to study the relation between serum IL-17 levels and risk of osteoporotic fractures (hip, vertebral and wrist) in pre-menopausal RA patients.

2. Patients and methods

The present case control study included 25 premenopausal females with RA that constitute patients group. They fulfilled the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for RA [13]. They were collected from the outpatient clinic of the Physical Medicine, Rheumatology and Rehabilitation Department, Faculty of Medicine, University of Alexandria. We excluded patients with a history or current use of any medications for management of OP (except for calcium and/or vitamin D) including bisphosphonates, hormone replacement therapy or raloxifene. Patients with other systemic or local bone disease that lead to OP were excluded. Twenty healthy premenopausal women matching the age of the patients were selected to constitute the control group. Both groups gave their informed consent to participate in the study. The study was approved by the institutional ethics committee.

All the enrolled patients were subjected to a detailed history taking, thorough clinical examination, disease activity assessment using the disease activity score 28 (DAS-28) [14] and the disability was assessed by using Health Assessment Questionnaire–Disability Index (HAQ-DI) [15].

The Bone Mineral Density (BMD) measurements were done by dual-energy X-ray absorptiometry (DEXA) for all subjects by a Lunar Prodigy Advanced DEXA scanner system. As all the studied females in this study were premenopausal and according to The International Society for Clinical Densitometry (ISCD) recommendation, instead of T-scores, ethnic or race adjusted Z-scores were used. Z-score was calculated as a relationship between 2 norms by comparing the patient’s BMD to the expected BMD for the patient’s age and sex. The difference between the patient’s score and the norm is expressed in SD above or below the mean.

Fracture assessment was done using the FRAX index for patients and controls. The outputs of FRAX index are the 10-year probability of a major osteoporotic fracture (hip, spine or wrist fracture) and the 10-year probability of hip fracture [16,17]. Probability is calculated from age, sex, BMI and dichotomized risk factors comprising prior fragility fracture, parental history of hip fracture, current tobacco smoking, ever
use of long-term oral glucocorticoid use, rheumatoid arthritis, other causes of secondary osteoporosis and high alcohol consumption.

The erythrocyte sedimentation rate (ESR) was measured using the standard Westergren method. The C-reactive protein (CRP) was assessed by the nephelometer assay, and Anti–citrullinated protein antibody (ACPA) was measured for all patients. Serum IL-17 levels were assessed using enzyme-linked immunosorbent assay (ELISA) technique for both patients and control. The C-reactive protein consumption.

Other causes of secondary osteoporosis and high alcohol use of long-term oral glucocorticoid use, rheumatoid arthritis, other causes of secondary osteoporosis and high alcohol consumption.

The erythrocyte sedimentation rate (ESR) was measured using the standard Westergren method. The C-reactive protein (CRP) was assessed by the nephelometer assay, and Anti–citrullinated protein antibody (ACPA) was measured for all patients. Serum IL-17 levels were assessed using enzyme-linked immunosorbent assay (ELISA) technique for both patients and control [Human IL-17 Platinum ELISA, eBioscience, USA].

Statistical analysis: was done using the IBM SPSS software package version 20.0. Qualitative data were described using number and percent. Quantitative data were described using mean and standard deviation. Comparison between categorical variables was tested using the Chi-square test. The distributions of quantitative variables were tested for normality using Kolmogorov–Smirnov test, Shapiro–Wilk test and D’Agostino test, also Histogram and QQ plot were used for vision test. For normally distributed data, comparison between two independent populations was done using independent t-test, correlations between two quantitative variables were assessed using the Pearson coefficient. Abnormally distributed data were assessed using Mann–Whitney test, and correlations between two quantitative variables were assessed using Spearman coefficient. Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison between the RA patients and control according to FRAX index (major osteoporotic fractures and hip fracture).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage median (min.–max)</td>
<td>RA patients (n = 25)</td>
</tr>
<tr>
<td>10 year probability of:</td>
<td></td>
</tr>
<tr>
<td>Major osteoporotic fractures</td>
<td>4 (1.4–10)</td>
</tr>
<tr>
<td>Hip fracture</td>
<td>0.1 (0–2.1)</td>
</tr>
<tr>
<td>RA: Rheumatoid arthritis, FRAX: Fracture Risk Assessment Tool. Bold values are statistically significant at p ≤ 0.05.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Bone mineral density measurements in RA patients and control.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD (g/cm²) Mean ± SD</td>
<td>RA patients (n = 25)</td>
</tr>
<tr>
<td>Femur neck</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Wrist</td>
<td>0.64 ± 0.1</td>
</tr>
<tr>
<td>L1–L4</td>
<td>1.07 ± 0.2</td>
</tr>
<tr>
<td>RA: Rheumatoid arthritis, BMD: bone mineral density, L1–L4: lumbar vertebrae 1–4. Bold values are statistically significant at p ≤ 0.05.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Comparison between the RA patients and control according to Z-score interpretation.</th>
</tr>
</thead>
<tbody>
<tr>
<td>z score interpretation</td>
<td>RA patients (n = 25)</td>
</tr>
<tr>
<td>Normal for age</td>
<td>16 (64)</td>
</tr>
<tr>
<td>Below the expected range for age</td>
<td>9 (36)</td>
</tr>
<tr>
<td>RA: Rheumatoid arthritis. Bold values are statistically significant at p ≤ 0.05.</td>
<td></td>
</tr>
</tbody>
</table>

3. Results

The mean age of RA patients in the study was 38.8 ± 7.6 years and of the control 38.7 ± 7.2 years (p = 0.95). The mean duration of the disease was (7.04 ± 5.47) years ranging from (1 to 20) years.

According to the DAS-28, 15 (60%) patients showed severe activity, 9 (36%) showed moderate activity, but only one (4%) showed mild disease activity score. Regarding the HAQ-DI in RA patients, 15 (60%) patients experienced a mild to moderate disability, 5 (20%) showed moderate to severe disability and 5 (20%) showed severe to very severe disability in their activity of daily living.

Fracture Risk Assessment Tool (FRAX) showed a 10 year probability of major osteoporotic fractures in the patients ranging from 1.4 to 10%, with a median of 4%, while in the control group it ranged between 0.9 and 2.7%, with a median of 1.85% (p < 0.001) (Table 1). The range of 10 year probability of hip fracture in the patients ranged between 0 and 2%, with a median of 0.1%, while in the control group it ranged between 0 and 0.3%, with a median of 0.035% (p < 0.001) (Table 1).

The means of BMD in RA patients in femur neck, wrist and lumbar vertebrae (L1-L4) were 1.0 ± 0.1, 0.64 ± 0.08, 1.07 ± 0.18 (g/cm²) respectively while the means of the control were 1.11 ± 0.14, 0.68 ± 0.07, 1.21 ± 0.12 (g/cm²) in the three measured sites. The BMD was significantly lower in patients compared to control p = 0.008, p = 0.046 and p = 0.005 respectively (Table 2). A Z score of −2.0 or lower was considered below the expected range for age. Z-score in 16 (64%) patients was normal for age compared to 19 (95%) for control. While 9 (36%) patients compared to control (1 to 20) years.

The range of 1st hour ESR in the study group ranged between 11.0 and 120 mm/h, with a mean level of 47.5 6 ± 29.51 mm/h. The CRP in the patient group ranged between 1.8 and 52 mg/l, with a mean level of 16.37 ± 14.36 mg/l. The range of ACPA in the patients group ranged between 11.4 and 525 U/ml, with a mean level of 105.34 ± 120.92. 80% of the studied patients were ACPA positive.
The mean level of serum IL-17 concentrations in the patients was 5.99 ± 1.22 pg/ml, while in the control was 3.73 ± 2.15 pg/ml and the difference was statistically significant \( (p < 0.001) \) (Fig. 1).

Serum IL-17 levels showed a significant correlation with the FRAX index (10 year probability of major osteoporotic fractures and hip fracture) in RA patients \( (p = 0.005 \text{ and } p = 0.013 \text{ respectively}) \) (Figs. 2). The correlation was insignificant in the control for both major osteoporotic and hip fractures \( (p = 0.24 \text{ and } p = 0.18 \text{ respectively}) \).

Z-score interpretation showed a significant correlation with FRAX index (10 year probability of major osteoporotic fractures and hip fracture) in RA patients \( (p = 0.002 \text{ and } 0.03 \text{ respectively}) \) (Figs. 3). Regarding the control group, the correlations were insignificant.

4. Discussion

The choice of premenopausal state was selected to exclude effect of menopause on the occurrence of osteoporosis. In a study on Egyptian RA patients, impaired bone formation and uncoupling of bone turnover were evident in postmenopausal cases with a higher risk of fracture hip [18]. DAS-28 in the current study represented all grades of disease activity among the studied RA patients and also reflected how much our patients had severely active RA. Regarding the HAQ-DI, it covered all grades of functional disability among the studied RA patients. The current results showed that RA patients had significantly higher FRAX index scores than healthy subjects. Gonzalez-Lopez et al. [19], and Lee et al. [20] had reported similar results but on Mexican and Korean population respectively.

The current results showed that RA patients had significantly higher IL-17 serum level than healthy subjects. Rosu et al. [11] reported that serum IL-17 was high in RA patients and this supports the hypothesis that IL-17 family cytokines are involved in the pathogenesis of RA as seen in previous reports [9,12,21,22]. The current results showed that RA patients had significantly lower Z-scores mean values in all measured sites (femoral neck, wrist and lumbar vertebrae L1–L4) compared to healthy subjects. This finding runs in accordance with previous published studies [6,23,24].

The current study showed no correlation between serum levels of IL-17 and the disease activity. Failure to demonstrate an association with DAS-28 is not to downplay the importance of IL-17 in RA disease activity but it appears that not all our patients were in the same activity state at time of sample withdrawal. Moreover, the studied patients were established RA, previously treated with a wide variety of DMARDs regimens, which could influence both IL-17 behavior and the correlation with the activity markers and the disease duration. Similarly, in another study on Egyptian RA patients, IL-17 was significantly increased but did not correlate with any of the clinical findings, laboratory and radiographic scores [25]. On the contrary, serum IL-17 was significantly increased in RA patients and significantly correlated with DAS-28 and bone erosions.

Figure 1  Comparison between the RA patients and the control according to serum IL-17 levels (pg/ml).

Figure 2  Correlation between serum IL-17 levels with FRAX index (10 year probability of major osteoporotic fracture) (Left) and with FRAX index (10 year probability of hip fracture) in RA patients (Right).
suggesting an important role in the pathogenesis of the inflammatory and destructive pattern characteristic of the disease [26]. This discrepancy could be also attributed to the small sample sizes as well as the different regions in Egypt from which the patients were enrolled for each study.

As regards the relation between serum levels of IL-17 and HAQ-DI, no significant relationship between both parameters could be detected. This result agreed with Yue et al. [27] who found no correlation between serum levels of IL-17 and HAQ. These results could be explained by that the HAQ-DI is a subjective questionnaire which differs from one patient to another according to assistance received from their relatives, socioeconomic class of the patients, different levels of disease activity or it may be attributed to the small sample size. In contradiction to the previous results, Genovese et al. [28] reported a strong relationship between serum IL-17 levels and HAQ-DI.

The current study showed a significant correlation between serum IL-17 levels and the FRAX index (both major osteoporotic and hip fractures). Also, IL-17 significantly correlated with the Z-score, where the serum IL-17 level was higher in patients with Z-score below the expected range for age. This result could be explained by the findings of Chabaud et al. [9] who reported that high serum IL-17 levels were linked to progressive destruction of cartilage and bone in RA patients. Also, Lubberts et al. [29] clarified that IL-17 is a crucial cytokine in osteoclastogenesis and bone resorption. The Z score interpretation as expressed by ISCD was found to be strongly correlated with FRAX index. This might explain that our premenopausal RA patients were found to have high z-score interpretation (below the expected range for age) and high serum levels of IL-17 and also a high FRAX index which in turn explains the role of IL-17 in the occurrence of OP and increases the risk of fractures even in premenopausal females.

Finding these results despite our selection of premenopausal patients might be referred to their bad dietary habits which are deficient in calcium, corticosteroid intake, bad sleeping habits which hinder their exposure to useful sunlight as being the natural source of vitamin D, in addition to the chronic inflammatory state. In another study on Egyptian RA patients, vitamin D levels were significantly lower than those in the control [30].

In rheumatoid arthritis (RA) patients, the risk of both vertebral and non-vertebral fractures is roughly doubled, which is for an important part caused by inflammation-mediated amplification of bone loss and by immobilization [31]. Down regulation of IL-17 and IL-17-triggering cytokine production was found to alleviate RA which highly supports its role in the disease pathogenesis [32]. It is recommended that future studies are conducted on larger sample sizes longitudinally to verify our results.

In conclusion, the premenopausal rheumatoid arthritis patients showed a high fracture probability. Interleukin-17 serum levels are associated with higher liability to fractures among rheumatoid patients.

Conflict of interest

None.

References


