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ORIGINAL ARTICLE

Sclerostin as an innovative insight towards understanding Rheumatoid Arthritis

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Abstract *Background:* Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by joint inflammation leading to cartilage and local bone erosion. Sclerostin is a protein that in humans has been identified as an inhibitor of the pathway and leads to decreased bone formation.

Aim of the work: This study aimed to investigate the level of serum sclerostin in RA patients, its association with inflammatory profile and its relation to disease activity and severity.

Patients and methods: Thirty-one Egyptian RA patients (28 females, 3 men) participated in this study. Their median age was 40 years. Disease activity score was assessed by the disease activity score (DAS28) and the functional status by the modified health assessment questionnaire (MHAQ). Ten matched controls were also included. Radiological severity was assessed according to the Larsen score. Serum sclerostin was measured.

Results: Median serum sclerostin in RA patients was 2000 ng/dl (800–3300 ng/dl) which was significantly higher than in controls [210 ng/dl (150–2859)] ($Z = -4.47$, $p < 0.001$). Sclerostin significantly negatively correlated with C-reactive protein and DAS28 ($p = 0.014$ and $p = 0.02$ respectively) and positively correlated with the Larsen score and total joint count ($p = 0.03$ and $p = 0.02$ respectively). At serum level 267 ng/dl sclerostin has sensitivity of 96.8% to diagnose RA and a positive predictive value of 96.6%.

Conclusion: Serum sclerostin was significantly higher in RA patients than controls and correlated with disease activity and severity which highly suggests that it may play a role in the pathogenesis of RA making it a valuable new marker of monitoring the disease progress and prognosis.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by joint inflammation leading to cartilage and local bone erosion [1]. One of the hallmarks of RA is progressive bone erosion. Research on the mechanisms by which RA

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induces osteolysis has focused on the osteoclast's roles in shifting the normal balance between bone formation and resorption. The Wnt/β-catenin signaling pathway has well-recognized roles in embryology and development, and is emerging as a critical regulator of bone and cartilage homeostasis in adult. Canonical Wnt signaling is initiated by binding to frizzled receptors and co-receptors 'LDL receptor-related proteins 5 and 6' (LRP5/6) which leads to β-catenin stabilization, nuclear translocation and activation of target genes such as Wnt-induced signaling protein-1 (WISP-1) [2]. Wnt signaling is modulated by soluble antagonists including dickkopf-1 (Dkk1), secreted frizzled-related proteins (sFRPs), and sclerostin (SOST).

Sclerostin is a protein encoded by the SOST gene. In humans, it was originally believed to be a non-classical bone morphogenetic protein (BMP) antagonist [3]. Nowadays, sclerostin has been identified as binding to LRP5/6 receptors and inhibiting the Wnt signaling pathway which leads to decreased bone formation. Sclerostin is expressed in osteocytes and some chondrocytes and inhibits bone formation by osteoblasts [4,5]. In adult cartilage in contrast, increased Wnt–β-catenin stimulates tissue breakdown rather than formation [6]. In RA bone loss may be systemic or localized to the periarticular bone due to arthritis of the affected joint. This process involves proinflammatory cytokines produced by the synovial membrane, which may increase bone resorption but also stimulate soluble antagonists of the canonical Wnt/β-catenin signaling pathway, including Dkk1 and sclerostin, and subsequently inhibit osteoblast proliferation, maturation and progenitor differentiation [7].

There is little information on changes in SOST in arthritis, with a reduction in the number of SOST-positive osteocytes noted in association with increased cortical bone density in the femoral neck of patients with hip osteoarthritis (OA) [8], and in zygapophyseal joints with OA and ankylosing spondylitis [9]. Sclerostin serum concentrations depend on genetic aspects, as well as age, sex, adiposity, kidney function and presence of diabetes mellitus [10]. Sclerostin is not a specific product of osteocytes, however, it is also produced by chondrocytes and cementocytes as well as in the liver, vascular wall and kidney [11,12]. Previously it was reported that serum levels of sclerostin and Dkk-1 increase in patients with juvenile idiopathic arthritis (JIA) and tumor necrosis factor (TNF-α) contributes to their increase [13].

The objective of this study was to investigate the level of serum sclerostin in RA patients, its association with inflammatory profile, and its relation to disease activity and severity.

2. Patients and methods

Thirty-one RA patients (28 females, 3 males) diagnosed according to the 2010 ACR/EULAR classification criteria [14] were recruited from Internal Medicine and Physical Medicine Departments of Ain Shams University Hospitals. The inclusion criteria in the study were an inadequate response to one disease-modifying anti rheumatic drug and patients were naive to anti-TNFα therapy. Ten age and sex matched apparently healthy subjects were included as a control group. The study was conducted in accordance with the World Medical Association Declaration of Helsinki for human subjects and the study was approved by the ethics committee of the Faculty of Medicine Ain Shams University, and all participants gave us their written informed consent before enrollment.

All patients underwent full medical history taking and clinical examination with special attention to tender joint count (TJC), swollen joint count (SJC) and morning stiffness. Patients who had Paget disease, multiple myeloma, breast cancer, bone metastasis and patients who were receiving biological treatment in the form of TNF-α inhibitors during the last 6 months were excluded from this study. Disease activity score (DAS28) was assessed and considered low (DAS28 $\geqslant 2.6 - < 3.2$) moderate ($\geqslant 3.2 - < 5.1$), high ($\geqslant 5.1$) and DAS28 < 2.6 as remission [15]. Modified Health assessment questionnaire (MHAQ) was calculated; eight activities were rated as 0 = without any difficulty, 1 = with some difficulty, 2 = with much difficulty, and 3 = unable to do. MHAQ scores were divided into categories of mild (MHAQ < 1.3), moderate ($1.3 <$ MHAQ < 1.8) and severe (MHAQ > 1.8) functional losses [16].

Laboratory evaluation included complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and rheumatoid factor (RF). Plain X-ray of the hands (10 MCPs) and wrists was assessed according to the Larsen method (scoring from 0 to 60) [17]. Serum level of sclerostin was measured using sandwich enzyme immunoassay (ELISA) technique (BIOMEDICA GRUPPE). The procedure was done according to the manufacturer instruction as supplied with in the kit for both patients and controls. 20 µl standard/sample/control was added into appropriate wells followed by 50 µl AB (biotinylated anti Sclerostin antibody) then the wells were covered and incubated overnight (18–24h) at room temperature (18–24°C) in the dark. Washing for 5 times was performed followed by adding 200 µl conjugate and incubated for 1 hour at room temperature in the dark. After washing was performed 200 µl substrate was added and incubated for 30 min at room temperature in the dark. 50 µl stop solution was added and the absorbance was measured at 450 nm with reference 630 nm.

2.1. Statistical methods

IBM SPSS statistics (V. 22.0, IBM Corp., USA, 2013) was used for data analysis. Data were expressed as mean ± SD for quantitative parametric measures in addition to median and percentiles for quantitative non-parametric measures. *The following tests were done*: Comparison between two independent groups for non-parametric data using Wilcoxon Rank Sum test; Comparison between more than 2 patient groups for non-parametric data using Kruskal–Wallis test; Ranked Spearman correlation test to study the possible association between each two variables among each group for non-parametric data. The probability of error at 0.05 was considered significant while at 0.01 and 0.001 are highly significant. The ROC curve (receiver operating characteristic) was used to evaluate the sensitivity and specificity of serum sclerostin in diagnosis of the patients.

3. Results

This study included 31 RA patients (28 females, 3 males). The clinical characteristics, laboratory and radiological findings,