Serum sclerostin level among Egyptian rheumatoid arthritis patients: relation to disease activity, bone mineral density and radiological grading

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ABSTRACT

Introduction: Bone loss in rheumatoid arthritis is caused by increased bone resorption without an increment in bone formation. The Wnt pathway is important in the control of bone formation through the regulation of osteoblast activity. Sclerostin is an important regulator of the Wnt pathway by blocking Wnt binding to its receptor and thereby inhibiting bone formation.
Aim: This study aimed to assess the serum sclerostin level in a group of Egyptian rheumatoid arthritis patients and to correlate its level with bone mineral density, disease activity and radiological grading.
Methods: Forty rheumatoid arthritis patients (mean age 48.9 ± 11.6 years, disease duration 8 ± 6.4 years) and 40 age and sex matched apparently healthy subjects were included. Serum sclerostin level was measured using enzyme linked immunosorbent assay. Plain radiographs of hands and feet and dual-energy x-ray absorptiometry test were done for all patients.
Results: No significant difference was found between rheumatoid arthritis patients and healthy controls regarding the mean value of sclerostin level. Postmenopausal healthy women had higher levels of sclerostin than premenopausal healthy women. Serum sclerostin had significantly positive correlations with the age of onset and weight of rheumatoid arthritis patients and negative correlation with erythrocyte sedimentation rate. No correlation was encountered between sclerostin level and bone mineral density, disease activity or radiographic grading.
Conclusion: For better clarification of the role of sclerostin on bone mass in rheumatoid arthritis, larger sample size is needed. More studies on serum sclerostin levels among different grades of RA activity are encouraged.

Keywords: Sclerostin; Bone loss; Rheumatoid arthritis

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INTRODUCTION

Bone is constantly undergoing bone remodeling, a complex process involving the resorption of bone on a particular surface, followed by a phase of bone formation. In normal adults, and prior to menopause in females, there is a balance between the amount of bone resorbed by osteoclasts and the amount of bone formed by osteoblasts i.e. physiologic remodeling[1].

In patients with rheumatoid arthritis (RA), this balance is altered in favor of resorption as a result of the inflammatory process, activation of osteoclasts and lack of bone repair[2]. Accordingly, several negative clinical consequences occur in patients with RA[3].Three forms of bone loss had been described in RA: focal bone erosions affecting the immediate subchondral bone and bone at the joint margins[4]; periarticular osteopenia adjacent to inflamed joints[5]; and generalized osteoporosis involving the axial and appendicular skeleton[6].

Sclerostin, an osteocyte secreted protein encoded by SOST gene, belongs to the DAN (differential screening-selected gene aberrant in neuroblastoma) family of glycoproteins[7]. The Wnt pathway is important in the control of bone formation through the regulation of osteoblast activity[8]. Sclerostin is an important regulator of the Wnt pathway by blocking Wnt binding to its receptor and thereby inhibiting bone formation[9]. Expression of Wnt antagonists such as secreted Frizzled- related protein-1 and sclerostin can be induced during inflammation and may also inhibit repair of bone erosion by suppressing bone formation[10]. Blockage of Wnt an-

tagonists such as sclerostin will trigger repair or even healing of bone erosion[11].

This study aims to assess the serum sclerostin level in a group of Egyptian RA patients and to correlate its level with disease activity, bone mineral density and radiological grading.

PATIENTS AND METHODS

This case-control study included forty patients with RA, attending the Rheumatology and Rehabilitation Department, Faculty of Medicine, Cairo University, and forty age and sex matched apparently healthy subjects. All RA patients fulfilled the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for RA; they all had definite RA as they all had a score of ≥ 6/10[12]. RA patients were excluded from the study if they had Diabetes, Hyperthyroidism, receiving thyroxin or calcium replacement therapy or steroid therapy. Written informed consents were obtained from all participants. This study was approved by the Cairo University Research Ethics Committee (REC).

All patients included in this study were subjected to full clinical and family history taking, clinical examination, Modified Health Assessment Questionnaire (MHAQ) for measuring the functional ability of patients with RA and assessment of disability index[13]. Disease activity in the patients was assessed using the disease activity score 28 (DAS 28)[14].

Rheumatoid factor (RF) was assayed with a quantitative immunonephelometry test (Behring, Marburg, Germany). RF was considered positive when the concentration was higher than the cut-off value of the kit (15 IU/ml). Anti-cyclic citrullinated peptide (Anti--CCP) was measured using the microparticle enzyme immunoassay (MEIA) method with the Abbott AxSym (Chicago, IL, USA). Normal reference level is up to 5 U/ml.

Serum sclerostin was assayed using enzyme-linked immunosorbent assay (ELISA) assay (TECO medical®, USA, intra-assay coefficient of variation percent (CV %), 3.5 – 5.5% and inter-assay CV %, 5–7%).

Plain radiographs for hands, wrists and feet were taken for each patient. Radiological scoring was performed according the final van der Heijde modification of the Sharp erosion score[15].It includes both erosions and joint space narrowing. For erosions, 16 areas from each hand and wrist and 12 areas from the feet were evaluated. Erosions were scored from 0 to 5, as follows: (0 = normal, 1 = discrete erosions, 2 to 3 = larger erosions according to surface area involved, 4 = erosions extending over middle of the bone, and 5 = complete collapse). Joint space narrowing score includes 15 areas from the hands and wrists and six areas from the feet. Joint space narrowing was scored from 0 to 4, as follows: (0 = normal, 1 = focal narrowing, 2 = reduction of less than 50% of joint space, 3 = reduction of greater than 50% of joint space, and 4 = ankylosis). The total van der Heijde radiographic score ranges from 0 to 448.

For the assessment of the bone mineral density (BMD), the dual-energy x-ray absorptiometry (DXA) test was done for all 40 RA patients using the DXA scanner from Norland Medical Systems, Inc. T-score was evaluated according to the definition of World health organization (WHO) for osteopenia and osteoporosis as follows: normal when T scores > -l, Osteopenia when T scores -1 to - 2.5, osteoporosis when T scores ≤ -2.5, severe osteoporosis when < -2.5 with fragility fracture[16].

Data was analyzed using IBM Statistical Package for the Social Science (SPSS) Advanced Statistics version 20.0 (SPSS Inc., Chicago, IL, USA). Numerical data was expressed as mean and standard deviation (SD) or median and range, as appropriate. Qualitative data was expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relationship between qualitative variables. For quantitative data, comparison between two groups was done using independent sample t-test or Mann-Whitney test and comparison between 3 groups was done using Kruskal-Wallis test. Pearson product-moment was used to estimate correlation between numerical variables. Spearman-rho method was used to test correlation between ordinal variables. P value < 0.05 was considered significant.

RESULTS

This study included two groups; RA patients and healthy control subjects. The RA patients group included 40 patients, 26(65%) females and 14(35%) males. Their mean (±SD) age was 48.9 ± 11.6 years. The mean (± SD) age of RA onset was 41.2±10.8 years. The median (range) of the disease duration was 8(3-25) years. Sixteen (61.5%) female patients were postmenopausal and 10 (38.5%) were premenopausal.

TABLE I. THE DEMOGRAPHIC CHARACTERISTICS OF RA PATIENTS

Patients' characteristics	Value
Age (years) †	48.9 ± 11.6
Age of RA onset (years) †	41.2 ± 10.8
Sex‡	
Female	28 (70)
Male	12 (30)
Disease duration (years) †	8 ± 6.4

‡Data is presented as number (percent), †Data is presented as Mean± SD

TABLE II. THE GENERAL CHARACTERISTICS AND ASSOCIATED COMORBIDITIES IN THE RA GROUP

Patients' characteristics	Value
Cigarette smoking ‡	10 (25)
Hypertension‡	5 (12.5)
Family history of rheumatic disease‡	3 (7.5)
Family history of osteoporosis‡	5 (12.5)
Weight (kg) †	69.5 ± 12.2
Height (Cm) †	158.6 ± 8.6
BMI (kg/m^2) †	27.8 ± 5.7

‡Data is presented as number (percent), †Data is presented as Mean± SD

TABLE III. THE CLINICAL PARAMETERS OF RA PATIENTS

Patients' characteristics	Value
Morning stiffness (minutes)‡	13 (0-300)
Deforming arthritis†	7 (17.5)
Erosive arthritis†	30 (75)
Subcutaneous nodules†	10 (25)
Dry eyes†	9 (22.5)
Dry mouth†	7 (17.5)
Disease activity†	
High-disease activity	22 (55)
Moderate disease activity	16 (40)
Low disease activity	1 (2.5)
Remission	1 (2.5)

‡Data are presented as Median (range), †Data are presented as number (percent)

TABLE IV. THE DISEASE ACTIVITY, FUNCTIONAL ABILITY AND RADIOLOGICAL SCORING OF STUDIED RA PATIENTS (N=40)

Patients' clinical characteristics	Value
DAS 28	5.56 (2.5-8.58)
MHAQ	0.68 (0-2.6)
van der Heijde modification of the Sharp score	44.5 (6-158)

DAS: Disease Activity Score, MHAQ: Modified Health Assessment Questionnaire. Data is presented as Median (range)

Forty healthy controls were included, 28 (70%) females and 12 (30%) males. Their mean (±SD) age was 44.4 (±12.1) years. Seventeen (60.7%) females were post-menopausal and 11 (39.3%) were premenopausal. Five patients were hypertensive and receiving antihypertensive treatment. The demographic characteristics of RA patients are presented in Table I. The general characteristics and comorbidities in RA patients are summarized in Table II. The clinical parameters of RA patients are shown in Table III. The disease activity, functional ability and radiological scoring of the RA patients are summarized in Table IV.

All patients were using non-steroidal anti inflammatory drugs (NSAIDs), 28 (70%) patients were using methotrexate (MTX), the median (range) of the cumulative dose of methotrexate was 4.6 (0.75 - 14.4) grams. Ten (25%) patients were receiving antimalarial drugs, 14 (35%) patients were receiving leflunomide, 16 (40%) patients were receiving MTX only, 8 (20%) patients were receiving leflunomide only, 7 (17.5 %) patients were using combination of MTX and antimalarial drugs, 3 (7.5%) patients were on combination of MTX and leflunomide, 2 (5%) patients were on combination of MTX and leflunomide and antimalarial drugs and 1 (2.5%) patient was on combination of leflunomide and antimalarial drugs. There were three (7.5%) newly-diagnosed RA patients not receiving any disease-modifying anti-rheumatic drugs (DMARDs). No patients were on biologics. There were no patients receiving antiosteoporotic or anabolic treatment (bisphoshphonate, hormonal replacement therapy, teriparatide or denosumab). Five patients were receiving antihypertensive treatment (captopril, furosemide, amlodipine).

According to DXA test, eleven (27.5%) patients had normal bone density, 15 (37.5%) patients had osteopenia, and 14 (35%) patients had osteoporosis. Nine osteoporotic patients were found to have a fracture risk. Fourteen (35%) patients had normal lumbar spine density, 15 (37.5%) patients had normal femoral neck den-

TABLE V. DXA FINDINGS IN RA PATIENTS (N=40)

Variable	Result
BMD (g/cm²)†	
Lumber spine	0.992 ± 0.14
Neck of femur	0.845 ± 0.132
Wrist	0.75 ± 0.155
T score ‡	
Lumber spine	–1.6 (-5-1.8)
Neck of femur	-1.5 (-4.1-1.4)
Wrist	-1.5 (-4.7-1.0)
Z score ‡	
Lumber spine	-1.4 (-3.2-1.0)
Neck of femur	-1.2 (-3.1-1.3)
Wrist	-1.2 (-4.6-1.1)

†Data is presented as Mean ±SD, ‡ Data is presented as Median (range)

sity and 19 (47.5%) patients had normal forearm density. Sixteen (40%) patients had osteopenia of lumbar spine, 19 (47.5%) had osteopenia of neck of femur, and 9 (22.5%) patients had osteopenia of wrist bones. Ten (25%) patients had osteoporosis of lumbar spine, 6 (15%) patients had osteoporosis of neck of femur and 12 (30%) patients had osteoporosis of wrist bones. The DXA findings in RA patients are shown in Table V. The DXA findings of post-menopausal RA patients were as follows, the means (SD) BMD of the spine, hip, forearm were 0.9(0.1), 0.7(0.1) and 0.64(0.12) g/cm², respectively. The T score means (SD) of the spine, hip and forearm were -2.3(0.9), -1.7(0.9) and -2.7(1.3), respectively.

Serum sclerostin measured in patients and controls showed no statistically significant difference, as seen in Table VI and there was no significant difference in sclerostin level among different age groups (Table VII).The mean (SD) sclerostin level among female RA patients (N=26) and male RA patients (N=14) was 0.39±0.25 and 0.41±0.24 respectively, with no statistically significant difference (p=0.8). The mean (SD) sclerostin level among female controls (N=28) and male RA patients (N=12) was 0.43±0.33 and 0.48±0.25 respectively, with no statistically significant difference (p=0.2). Sclerostin level was significantly higher in postmenopausal healthy females than the pre-menopausal healthy females. In addition, sclerostin level was higher in postmenopausal RA patients but with no statistically significant difference (Table VI).

The median (range) RF titer was 152(16-768) IU/ml in RA patients. There were 27 RF positive RA pa-

TABLE VI. SCLEROSTIN LEVELS IN RA PATIENTS AND CONTROLS AND ITS LEVELS IN THE PRE-MENOPAUSAL AND POST-MENOPAUSAL RA PATIENTS AND CONTROLS

Variable	RA (N=40)	Controls patients (N=40)	p value
Serum sclerostin (ng/ml)	0.4±0.2	0.5±0.4	0.14
	Pre-menopausal RA patients (N=10)	**Post-menopausal RA patients (N=16)**	
Serum sclerostin (ng/ml)	0.29±0.18	0.46±0.26	0.1
	Pre-menopausal controls (N=11)	**Post-menopausal controls (N=17)**	
Serum sclerostin (ng/ml)	0.32±0.14	0.5±0.39	0.02

Data is presented as Mean±SD

TABLE VII. SCLEROSTIN LEVELS IN RA PATIENTS AND CONTROLS IN THE DIFFERENT AGE GROUPS

Patients	20-39 years (N=6)	40-60 years (N=27)	>60 years (N=7)	p value
Serum sclerostin (ng/ml)	0.24±0.18	0.44±0.25	0.41±0.23	0.1
Controls	**20-39 years (N=5)**	**40-60 years (N=26)**	**>60 years (N=9)**	**p value**
Serum sclerostin (ng/ml)	0.44±0.24	0.5±0.42	0.62±0.2	0.1

Data is presented as Mean±SD

tients and 13 RF negative RA patients; the serum sclerostin level was 0.39±0.22 and 0.42± 0.29 ng/ml respectively, with no statistically significant difference between the RF positive and RF negative RA patients (p = 1.0). There were 29 anti-CCP positive RA patients and 11 anti-CCP negative RA patients, the serum sclerostin level was 0.36±0.28 and 0.38± 0.26 ng/ml respectively, with no statistically significant difference between the anti-CCP positive and anti-CCP negative RA patients (p value, 0.8). The median (range) of the erythrocyte sedimentation rate (ESR) was 32.6 (5-130) mm/hr. There was significant negative correlation between sclerostin and ESR (r= -0.34, p= 0.03).

The RA patients with low BMD had higher levels of sclerostin (0.43±0.25 ng/ml) than patients with normal BMD group (0.3± 0.21 ng/ml) but with no statistically significant difference (p=0.1).

There was no significant correlation between sclerostin level and age of patients (r=0.16, p=0.1) and controls (r=0.13, p=0.4). Also, there was no significant correlation between sclerostin level and the disease duration in RA patients (r=0.02, p=0.8), age of menopause (r=0.06, p=0.6) or duration of menopause in the postmenopausal RA patients(r=-0.05, p=0.7). However, there was a significant positive correlation between the sclerostin level with the age of onset (r=0.32, p=0.03) and weight (r=0.32, p=0.04) of RA patients, and no significant correlation with BMI (r=0.25, p=0.1) was found.

Results demonstrated no significant correlation between sclerostin level and disease activity measured by DAS 28 (r=0.01,p=0.9), MHAQ (r=0.06, p=0.6), van der Heijde modification of the Sharp score (r=0.07, p=0.6), BMD or T scores of lumbar spine (r=0.06, p=0.6), (r=0.05, p=0.7), hip (r=-0.03, p=0.9), (r=-0.02, p=0.8) and forearm (r=0.1, p=0.5), (r=0.05, p=0.7), respectively. Also, there was no significant correlation between serum sclerostin and cumulative dose of MTX (r= -0.03, p= 0.87).

DISCUSSION

Sclerostin as a Wnt antagonist has role in both local and systemic bone loss in RA. Limited repair of bone erosions in RA seems to involve the induction of signals that block new bone formation. Expression of the Wnt antagonist – sclerostin can be induced during inflammation and may also inhibit repair of bone erosion by suppressing bone formation[10]. Blockage of Wnt antagonists such as sclerostin will trigger repair or even healing of bone erosion, however, this needs more research[11]. To our knowledge there are few studies focusing on the role of sclerostin on bone mass in RA patients. This is the second study to identify serum sclerostin level in RA patients. The first study measuring serum sclerostin level in RA was done by Vis *et al.*[17], but the present study is the first case-control study analyzing the relation between serum sclerostin levels and the BMD, disease activity and radiographic joints damage, in a sample of Egyptian RA patients.

In the current study, it has not been found that there is significant difference in serum sclerostin between RA patients and healthy controls. On the contrary, Vis *et al.*[17] found that sclerostin levels were significantly higher in female RA patients than healthy control Dutch females. This difference may be explained by the differences in the make-up of the study population and the difference in ethnicity.

This study showed higher levels of sclerostin in postmenopausal healthy and RA females than premenopausal healthy and RA females, respectively. However, it was statistically significant on comparing healthy postmenopausal to healthy premenopausal females only. This observation is consistent with previously reported studies[17-19]. The higher sclerostin levels in postmenopausal women were supposed to be associated with the increased bone turnover occurring in the postmenopausal state as a cause or effect, or combination of both[18].

In the present study, males had higher sclerostin level than females in the patients and controls, but the differences were statistically non-significant. This finding was confirmed by Gennari *et al.*[20]. A similar finding was observed by Amrein *et al.*[21], however, after adjustment for age, bone mineral content, physical activity, BMI, and renal function, sclerostin levels did not differ. They believed that as circulating sclerostin levels might reflect total-body skeletal mass, accordingly the larger skeleton in men might produce and release more sclerostin into the circulation[21].

In the current study, there was no significant correlation of serum sclerostin with age of RA patients and controls. Similar finding was observed by Polyzos *et al.*[22].However; this was in contrast to Vis *et al.*[17] and Szulc *et al.*[23]. In the current study, there was a positive correlation with the age of RA onset, which might indicate that the older the patients develop RA, the higher sclerostin level they have and this may later on predict a lower bone mass.

Consistent to the work of Polyzos *et al.*[22], results of the present study showed no significant correlation between serum sclerostin and age or duration of menopause, in both RA patients and healthy controls. Also, there was a statistically significant positive correlation between serum sclerostin and weight of RA patients. This finding was also observed by Polyzos *et al.*[23] and Sheng *et al.*[24].

Concerning the relation of serum sclerostin level with the disease activity in RA using DAS 28, Vis *et al.*[17] have found that sclerostin level is inversely correlated to DAS 28.The Vis *et al*. finding was in contrast to the present study where there is no significant correlation between serum sclerostin and DAS 28. However, our results partially coincide with Vis *et al.*[17] in negative correlation with disease activity, however, not with the whole DAS 28 score but with one of its variables which is (ESR).

Correlating serum sclerostin to radiological joints damage, using van der Heijde modification of the Sharp score, Vis *et al.*[17] found no correlation between van der Heijde score and sclerostin levels which is consistent with the results of this study.

In the present study there is no significant correlation between serum sclerostin and BMD or T scores of lumbar spine, hip and forearm in RA patients. On the contrary of 2 studies by Ardawi *et al.*[19,25], significant negative correlations between serum sclerostin and BMD were encountered. However, after adjusting for age and BMI, sclerostin levels were not significantly correlated with BMD. Ardawi *et al.*[25] supposed that mineral loss (or bone mass loss) in postmenopausal women is associated with more local mechanical stress stimulating sclerostin production by osteocytes. Several studies found positive correlation of circulating sclerostin levels with BMD[22,24,26,27]. Ardawi *et al*.[25] attributed such contradictory results to variations in the selection criteria of women studied, inclusion of men, health status, and the mean age of the group examined, or the environmental or lifestyle and/or genetic effects on bone mass, hence the serum sclerostin level.

Dickkopf 1 (DKK-1), like sclerostin, is a natural inhibitor of Wnt signaling[28]. It was studied earlier in RA than sclerostin, and plays a key role in the remodeling of bone and impairs local bone formation, which is particularly deleterious in RA[29].Wang *et al*.[30] showed that DKK-1 levels in patients with RA were significantly higher than levels in healthy controls, it was correlated with the Sharp score of radiologic change (r = 0.449, p = 0.001) in RA.

Given that sclerostin inhibits osteoblastic activity, a significant higher level in the osteoporotic group would be expected. Comparing serum sclerostin level between population with normal BMD and others with low BMD, Polyzos *et al.*[22] found that serum sclerostin was significantly higher in the post-menopausal females with normal BMD group compared with osteoporotic post-menopausal females. They were not able to provide explanation for this seemingly paradox at that time. Sheng *et al.*[24] compared women with normal BMD and women with low BMD. They found that osteoporotic women had a significantly lower level of serum sclerostin. On the contrary, in the present study, serum sclerostin level was higher in RA patients with decreased BMD than RA patients with normal BMD but this difference was statistically insignificant. This data indicates that the decreased bone formation in RA may not be caused by increased sclerostin levels and may be against the involvement of sclerostin in the pathogenesis of low bone mass in RA patients.

There are case reports of fragility fractures in adults on long-term, low-dose MTX therapy for conditions such as RA and psoriatic arthritis[31,32]. In the present study, there is a negative correlation (-0.03) between serum sclerostin and the cumulative dose of MTX, however with no statistically significant difference (P=0.87) and this is in agreement with Abdelhadi et al.[33] and Minaur et al.[34] who suggested that methotrexate appears to have no significant negative effect on bone.

We acknowledge the limitations of the present work such as the sample number of patients, methodological issues such as using isolated serum samples, and the low sensitivity of ELISA. Also due to limited funding, the calcium and vitamin D metabolism, as well as PTH, calcium intake, which are markers of bone remodeling status, were not conducted in this study.

More studies on sclerostin, in larger RA population are needed for better discrimination of the role of sclerostin in RA patients. Also, more studies on serum sclerostin levels among different grades of RA activity and serum sclerostin level in early onset and late onset RA are encouraged.

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