Original Article

Study of correlation of level of expression of Wnt signaling pathway inhibitors sclerostin and dickkopf-1 with disease activity and severity in rheumatoid arthritis patients

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Summary   **This study was done with aim to assess the serum sclerostin and dickkopf-1 (DKK-1) level in patients of rheumatoid arthritis (RA) and to correlate their level with disease activity and bone mineral density. Fifty patients of RA and equal age and sex matched healthy controls were included in the study. Patients were evaluated clinically and investigated with routine blood tests along with rheumatoid factor (RF), anti-citrullinated protein antibody (anti-CCP$_2$), radiographs and bone mineral density (BMD). Serum sclerostin and DKK-1 levels of both cases and controls was assayed by using enzyme-linked immunosorbent assay (ELISA) assay [RayBio®, Georgia, USA with coefficient of variation percent (CV %), < 10%] and compared with disease activity and bone mineral density. Disease activity was measured by Disease Activity Score 28 (DAS28) along with Modified Health Assessment Questionnaire (MHAQ) score. Mean serum sclerostin and DKK-1 was significantly higher in study group as compared to control group. Serum sclerostin showed significant correlation with disease activity scores (DAS score and MHAQ score), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) level. Serum sclerostin at level of 394 pg/mL was found to have diagnostic significance with sensitivity of 100% and specificity of 90%. DKK-1 level shows significantly positive correlation with larson score which denotes radiological progression (*r* value 0.468; *p* value 0.001). More studies with larger sample size of RA patients are needed for better determination of the role of sclerostin and DKK-1 in RA. Also, the correlation of these and other bone turn over markers will help decipher their role with disease progression in RA patients.**

Keywords: Sclerostin, dikkopf 1, rheumatoid arthritis, C-reactive protein

1. Introduction

Rheumatoid arthritis (RA) is characterised by symmetrical peripheral polyarthritis of unknown aetiology. It is most common form of chronic inflammatory arthritis which results in significant joint damage and disability. Hallmark of inflammatory process in RA includes synovial inflammation and proliferation, focal bone erosion, and thinning of articular cartilage. The mechanism underlying osteolysis in RA depends on

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osteoclasts roles in shifting the normal balance between bone formation and resorption. The Wnt-β catenin signalling pathways has been found to be a critical regulator of bone and cartilage homeostasis in adult. Canonical Wnt signaling is initiated by its 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*) (*1*). Wnt signaling is modulated by soluble antagonists including dickkopf-1 (DKK1), secreted frizzled related proteins (sFRPs), and sclerostin.

Sclerostin is encoded by the *SOST* gene located on chromosome 17q12-q21 in humans. In humans, it was originally believed to be a non-classical bone

morphogenetic protein (BMP) antagonist (*2*). However, recently sclerostin has been identified as inhibitor the Wnt signaling pathway by binding to LRP5/6 receptors resulting in decreased bone formation. Sclerostin is expressed in osteocytes and some chondrocytes and inhibits bone formation by osteoblasts (*3,4*).

In RA bone loss may be limited to the peri-articular region or can be systemic. This process involves pro-inflammatory 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 DKK-1 and sclerostin, and subsequently inhibit osteoblast proliferation, maturation and progenitor differentiation (*5*).

Role of sclerostin has been found in various studies in bone remodeling in osteoarthritis and ankylosing spondylitis (*6,7*). Serum sclerostin levels depend on genetic aspects, age, sex, adiposity, renal function and presence or absence of diabetes mellitus (*8*). Sclerostin is a nonspecific product of osteocytes, however it is also produced by chondrocytes and cementocytes as well as in the liver, vascular wall and kidney (*9*). It has been reported earlier in few studies that serum levels of sclerostin and DKK-1 are increased in patients with juvenile idiopathic arthritis (JIA) and is mediated by tumor necrosis factor (TNF)-α (*10*).

Very few studies have done to find levels of sclerostin and DKK-1 in RA patients, therefore the present study was done to correlate serum sclerostin and serum DKK-1 level with disease activity, inflammatory profile and severity in rheumatoid arthritis patients.

2. Materials and Methods

2.1. *Study design*

A case control study was done between June 2016 to June 2018 in Division of Rheumatology/Medicine, Sir Sunderlal Hospital BHU, a tertiary centre in eastern Uttar Pradesh, India after taking approval from Institute's ethical committee. Informed consent was taken from the patients.

All RA patients of age > 15 years diagnosed by 2010 ACR/EULAR criteria (American rheumatology criteria/European League Against Rheumatism) were included in study. Exclusion criteria included patients of age less than 15 years, patients on treatment of disease modifying anti rheumatic drug, evidence of steroid use in past 1 month, calcium supplement in last 3 months and presence of diabetes mellitus, chronic kidney disease and chronic liver disease.

2.2. *Study population and sample*

A total of 50 cases of RA were taken and equal number of age-sex matched controls were taken. Each of sample

selected on basis of inclusion and exclusion criteria. No formal method of sample size calculation was made as it was a pilot study.

2.3. *Methodology and statistical analysis*

After detailed clinical history and examination all patients were evaluated with investigations like complete blood counts, liver function test, random blood sugar, RF (rheumatoid factor) titre, CRP titre, anti-cyclic citrullinated peptide (anti CCP_2).

X-ray of bilateral hands, wrist and feet were also obtained and Larsen scoring was done for each (*11*). Bone marrow density (BMD) was obtained for each patient (*12*). Disease activity was measured by Disease Activity Score (DAS28) along with Modified Health Assessment Questionnaire (MHAQ) score for all patients (*13,14*).

Serum sclerostin was done using enzyme-linked immunosorbent assay (ELISA) method [RayBio® Human SOST ELISA Kit, Georgia, USA, intra-assay coefficient of variation percent (CV%), < 10% and inter-assay CV%, < 12%)] and DKK1 measurement was done by RayBio® Human DKK-1 ELISA Kit, Georgia, USA with intra-assay coefficient of variation percent (CV%), < 10% and inter-assay CV%, < 12%.

The statistical analysis was done using statistical software SPSS for windows (Version 16.0). Chi-square test was used for categorical variables. For continuous data Student's *t* test and Mann Whitney *U* test were used. One way analysis of variance (ANOVA) test was used for comparing three groups of mean. For correlating two continuous data Spearman's correlation coefficient was used. *P*-value < 0.05 is considered as statistically significant.

3. Results

3.1. *Demographic and biochemical data*

Out of 50 cases, there were 4% patients in age group (11-20 years), 12% patients in age group (21-30 years), 32% patients in age group (31-40 years), 24% patients in age group (41-50 years), 22% patients in age group (51-60 years) and 6% patients in age group > 60 years. Among cases, males were 18% (*n* = 9) and rest 82% (*n* = 41) were females where as in control 54% (*n* = 27) were male and 46% (*n* = 23) were females. Male to female ratio in study group was 1:4.5 which shows statistically significant female predominance of disease. Comparison of various hematological and biochemical parameters of cases and control is shown in Table 1. Comparison of BMD, T score, Z score and sclerostin in case and control is shown in Table 2.

3.2. *Serum sclerostin and its correlation with other parameters*

Table 1. Comparison of hematological and biochemical parameters of cases and control

Parameter	Case (Mean ± SD)	Control(Mean ± SD)	*p*-value
Age (in years)	41.30 ± 12.971	33.58 ± 11.770	0.002
Hb (hemoglobin in gm/dL)	10.816 ± 1.6564	12.146 ± 1.7736	< 0.001
TLC (total leucocyte count/mm^3)	8,995.92 ± 3,427.541	7,803.00 ± 1,723.276	0.030
Neutrophils (/mm^3)	67.02 ± 10.103	71.28 ± 6.919	0.016
Lymphocyte (/mm^3)	22.58 ± 9.123	21.80 ± 5.026	0.598
RBS (Random blood sugar mg/dL)	105.52 ± 18.801	104.56 ± 10.958	0.756
Creatinine(mg/dL)	0.852 ± 0.2159	0.779 ± 0.2015	0.084
Urea (mg/dL)	27.58 ± 10.186	21.56 ± 5.372	< 0.001
Na (sodium mmol/L)	138.52 ± 4.205	137.28 ± 2.963	0.091
K (potassium mmol/L)	4.140 ± 0.5383	3.746 ± 0.5319	< 0.001
Total protein (gm/dL)	7.698 ± 0.6196	7.236 ± 0.4615	< 0.001
Albumin (gm/dL)	3.860 ± 0.5739	3.740 ± 0.4686	0.255
Calcium (mg/dL)	9.070 ± 0.8279	9.598 ± 0.6278	< 0.001
Phosphate (mg/dL)	3.454 ± 0.8452	3.786 ± 0.8119	0.048
Platelet (/mm^3)	2.60 (1.80 - 3.41)	2.6 (1.97-3.42)	0.828
Aspartate transaminase (AST U/L)	22 (19.00 - 33.25)	20.5 (17.75-25.00)	0.107
Alanine transaminase (ALT U/L)	21.0 (16.00 - 31.25)	22.0 (18.75-26.25)	0.926
Total bilirubin (mg/dL)	0.50 (0.30 - 0.60)	0.80 (0.50 - 0.90)	< 0.001

Table 2. Comparison of various parameter in cases and control

Variables	Case Group	Control Group	*p*-value
DAS28 score	5.25 (2.4 - 6.5)		< 0.001
MHAQ	3.0 (2.00 - 3.00)	1.0 (1.0 - 1.0)	< 0.001
MHAQ Score	> 1.8 (1.3 - ≥ 1.8)	< 1.3 (< 1.3)	< 0.001
Larsen Score	20-30 (20 - 40)	<10 (< 10)	< 0.001
RF titre	94.50 (29.98 - 175.75)	20.0 (15.00 - 21.00)	< 0.001
Anti CCP2 titre	333.50 (132.50 - 548.72)	2.0 (0.10 - 0.60)	< 0.001
CRP titer	1.84 (1.06 - 5.10)	05.0 (0.4 - 0.60)	< 0.001
ESR titer	46.0 (35.75 - 55.25)	14 .00 (10.0 - 18.25)	< 0.001
DKK-1(in pg/mL)	309.00 (196.64 - 494.45)	110.61(109.00 - 159.77)	< 0.001
BMD (gm/cm^2)	0.958 ± 0.2354	0.940 ± 0.1468	0.647
T SCORE	- 2.030 ± 1.3348	-1.248 ± 1.0737	0.002
Z SCORE	-1.416 ± 1.0305	-.926 ± 0.9722	0.016
Sclerostin (in pg/mL)	526.75 ± 54.61573	361.59 ± 24.37903	< 0.001

DAS28: Disease Activity score; RF: Rheumatoid factor; Anti CCP2: Anti-cyclic citrullinated peptide; DKK-1: Dikkopf-1; BMD: Bone mineral density; MHAQ: Modified Health Assessment Questionnaire.

Table 3. DAS 28 score and serum sclerostin level

DAS28 score	No. of patients (*n*)	Serum sclerostin level, Mean ± SD	f-value	*p*-value
2.6 - 3.2	1	488.130 ± 0.0	6.114	0.004
3.2-< 5.1	13	487.296 ± 20.5182		
> 5.1	36	542.068 ± 56.2989		
Total	50	526.748 ± 54.6157		

DAS28: Disease Activity score; RF: Rheumatoid factor; Anti CCP2: Anti-cyclic citrullinated peptide; DKK-1: Dikkopf-1; BMD: Bone mineral density; MHAQ: Modified Health Assessment Questionnaire.

Mean serum sclerostin was 526.75 ± 54.61 pg/mL was significantly higher than the control group (361.59 ± 24.37 pg/mL). Comparison of DAS28, MHAQ, Larson score and other parameter between cases and control is shown in Table 3.

In our study, we found 1 patient with low (2.6-3.2) DAS 28 score with serum sclerostin level of 488.13 pg/mL. Thirteen patients (*n* = 13) had moderate DAS 28 score (3.2-< 5.1) with mean serum sclerostin level of 487.29 ± 20.51 pg/mL and 36 patient had high DAS 28 score (542.06 ± 56.29 pg/mL) which suggested that serum sclerostin level increases with the severity of disease (Table 4).

DAS score, MHAQ score, Larsen score, rheumatoid factor (RF) level, anti CCP level, CRP titer and ESR titer was found significantly higher in cases as compared to control group suggesting that as disease severity increases the levels of these parameters increase. Serum sclerostin level showed significant correlation with disease activity scores (DAS score and

Table 4. Correlation of serum sclerostin and DKK1 with various parameters (significant values *p* < 0.05 are in bold)

Items		Sclerostin	DKK1	DAS28 Score	MHAQ	MHAQ Score	LARSEN Score	RA	Anti CCP	CRP	ESR	BMD	T-SCORE	Z-SCORE
Sclerostin	r	1.000												
	p	.												
DKK1	r	0.409	1.000											
	p	**0.003**	.											
DAS28 Score	r	0.635	0.151	1.000										
	p	**0.000**	0.297	.										
MHAQ	r	0.498	0.235	0.559	1.000									
	p	**0.000**	0.100	**0.000**	.									
MHAQ Score	r	0.591	0.063	0.882	0.632	1.000								
	p	**0.000**	0.662	**0.000**	**0.000**	.								
Larsen Score	r	0.649	0.468	0.230	0.312	0.144	1.000							
	p	**0.000**	**0.001**	0.109	**0.000**	0.317	.							
RA	r	0.117	0.208	0.006	0.129	0.075	0.125	1.000						
	p	0.418	0.147	0.964	0.372	0.604	0.386	.						
Anti-CCP	r	-0.055	0.243	0.035	-0.020	-0.052	0.219	0.362	1.000					
	p	0.703	0.089	0.811	0.889	0.717	0.127	**0.010**	.					
CRP	r	0.404	0.136	0.318	0.320	0.376	0.208	0.189	-0.048	1.000				
	p	**0.004**	0.346	**0.024**	**0.023**	**0.007**	0.147	0.189	0.739	.				
ESR	r	0.491	0.193	0.475	0.235	0.432	0.244	0.216	0.177	0.437	1.000			
	p	**0.000**	0.180	**0.000**	0.100	**0.002**	0.087	0.131	0.220	**0.001**	.			
BMD	r	0.011	-0.343	0.176	0.193	0.238	-0.056	0.077	-0.072	0.221	0.024	1.000		
	p	0.941	**0.015**	0.221	0.179	0.096	0.697	0.595	0.617	0.123	0.870	.		
T-SCORE	r	-0.245	-0.661	-0.063	-0.110	0.058	-0.419	-0.124	-0.254	-0.016	-0.172	0.519	1.000	
	p	0.087	**0.000**	0.662	0.446	0.689	**0.002**	0.392	0.075	0.909	0.234	**0.000**	.	
Z-SCORE	r	-0.207	-0.661	-0.070	-0.131	0.047	-0.386	-0.153	-0.335	-0.018	-0.163	0.498	0.961	1.000
	p	0.148	**0.000**	0.628	0.366	0.748	**0.006**	0.289	**0.017**	0.902	0.258	**0.000**	**0.000**	.

MHAQ score), ESR and CRP level. Serum sclerostin at level of 394 pg/mL showed a diagnostic positive predictive value (PPV) and negetive predictive value (NPP) of 90.9% and 100% respectively and had diagnsotic sensitivity of 100% and specificity of 90%.

3.3. *Serum DKK-1 and its correlation with other parameters*

DKK-1 level was found significantly higher in cases as compared to control group. Among cases it was found to be 309.00 pg/mL (196.64-494.45) in cases and in controls it was 110.61 pg/mL (109.00-159.77).

Eighteen ($n = 18$) patient with RA with T score < –2.5 (more negative) had higher mean DKK-1 level of 512.59 pg/mL as compared to 269.44 pg/mL found in rest 32 RA patients with T score > –2.5 (more positive). However, it was not significant.

DKK1 level shows significantly positive correlation with larson score which denotes radiological progression (r value 0.468; p value 0.001). A significant negative correlation was also found with with BMD (r value –0.343) and T score(r value 0.661).

4. Discussion

RA is associated with both local and systemic bone loss. Bone remodelling is effected by sclerostin and DKK-1 which act by inhibiting the Wnt/β-catenin signalling pathway (*15*). The study aimed to find a correlation between these inhibitors and disease activity in RA patients.

In the study, we found mean haemoglobin level in RA group to be significantly lower than the control group and anaemia was found in overall 82% of cases. Previous studies have shown that anaemia develops in 30-70% of patients with RA (*16*). Similar results were seen in a study by Smyrnova Ganna *et al*. where 64% of cases were found to have anaemia compared to healthy population (*17*). Above observation reinforced the fact that anaemia is common in patient with rheumatoid arthritis as compared to healthy population and usually anaemia of chronic disease was seen.

Mean calcium level was also found to be significantly lower in RA group (9.070 ± 0.8279 mg/dL) as compared to healthy control (9.598 ± 0.6278 mg/dl). In a study conducted by Scott *et al*. mean calcium level was found to significantly lower compared to healthy group (*18*). It suggested that RA patient are associated with low calcium level as compared to healthy population and importance of adding calcium supplement with other treatment options in RA.

Serum sclerostin levels were found to be significantly higher in cases as compared to controls ($p < 0.001$). Further, significant correlation between serum sclerostin with DAS28 score suggested that as DAS28 score increases serum sclerostin increases. In our study serum sclerostin at level of 394 pg/mL has positive predictive value (PPV) and negetive predictive value (NPP) of 90.9% and 100% respectively and had diagnsotic sensitivity of 100% and specificity of 90%. In a study by El-Bakry S *et al*., serum sclerostin at a level of 267 ng/mL showed a diagnostic sensitivity of 96.8% and specificity of 66.7% with PPV and NPV of 96.6% and 90% respectively (*19*). Results from above studies suggests that it can be used for new diagnostic tool for rheumatoid arthritis.

In our study, we found that mean serum sclerostin in RA patient with osteoporosis was 536.95 pg/mL which was similar to serum sclerostin in RA without osteoporosis (521.01 pg/mL). Similar finding were found in study by Mehaney DA *et al*., where no significant correlation was found between sclerostin level, BMD or T scores were found (*20*). From above finding it could be suggested that although serum sclerostin has diagnostic significance and correlate well with disease activity, it cannot be used as marker of bone destruction.

The mean DKK-1 level was also found to be significant higher in RA patients as compared to controls and correlated significantly with larsen score, BMD and T score which suggest bone destruction. Another study by Wang *et al*. showed that DKK-1 levels in RA patients were significantly higher than levels in healthy controls, and correlated with the Sharp score of radiological changes ($r = 0.449$, $p = 0.001$) in RA (*5*). Study by Raphaèle Seror *et al*. also showed that the mean baseline DKK-1 level was higher among RA patients with radiological changes than without radiological progression (29.6 ± 13.3 *vs*. 26.63 ± 12.4 pmol/L) ($p = 0.0084$) (*21*). In study by Jun Tian *et al*. mean DKK-1 in control with osteoporosis was 55.25 ± 10.13 ng/mL and in control without osteoporosis was 40.19 ± 10.69 ng/mL (p value < 0.001) (*22*). Above results suggests that as rheumatoid arthrtis is chronic disease having bone destructive effect, therefore DKK-1 in RA patient was found higher than the control healthy group. Higher DKK-1 in healthy population with osteoporosis than non-osteoporosis suggest that DKK-1 can be used as a marker of osteoporosis.

Results comparision between our study and previous studies suggest that as disease progress and bone distruction increase, serum level of DKK1 will increase. Hence serum DKK1 can used as marker for bone destruction and osteoporosis.

Limitations of our study includes small sample size, use of low sensitivity ELISA method and limited investigation with respect to bone turnover and calcium and vitamin D metabolism. More studies on larger population is needed to delineate the role of sclerostin in RA patients as diagnostic marker. Also, more studies on serum sclerostin, DKK-1 and other bone turn over markers will delineate their role in bone remodelling mechanism in rheumatoid arthritis.

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