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Serum Dickkopf-1 as a potential prognostic marker in patients with rheumatoid arthritis

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Abstract

Background: Rheumatoid arthritis (RA) is a disease of an autoimmune nature that involves all types of joints structures and manifested by chronic joints inflammations and thus their erosions and damage. Dickkopf-1 (DKK-1) is a molecule that has an inhibitory regulation of wingless/integrated genes (Wnt) pathway and has a major role in models of animals with arthritis or joint destruction. Increased DKK-1 levels are implicated in higher resorption of the bone in cases of rheumatoid arthritis and thus with higher probability for joint deformities, while low levels associated with formation of new bone by osteoblasts, we aimed to study the prognostic role of circulating Dickkopf-1 in rheumatoid arthritis.

Results: The present study revealed that the DKK-1 levels were significantly increased in RA patients in relation to the control group ($P=0.001$). We found a significant positive correlation between DKK-1 level and ESR ($P=0.001$), Disease Activity Score (DAS 28) ($P=0.001$), the disease duration ($P=0.001$), and the presence of bone erosions in plain X-ray of hands ($P =0.001$). Moreover, we revealed that, at cutoff value 2150, the DKK-1 in RA has 90% sensitivity and 85% specificity.

Conclusions: DKK-l serum level can be used as a potential prognostic biomarker for monitoring of joint erosions and destruction in RA patients. Furthermore, it could be a possible target molecule in the future therapy to control the process of joint destruction.

Keywords: Rheumatoid arthritis, Dickkopf-1, Bone erosions

Background

RA is chronic disease of an autoimmune nature that causes inflammation in the synovial membrane, resulting in invasion of the near cartilaginous matrix by synovial tissue, that leads to the cartilage and bone degradation inside the joints. These bone erosions are often seen in imaging as marginal erosions of the joint, and it is a predictive of a worse prognosis for the disease [1, 2].

Bony erosions which develop within the first few years after onset of the disease can cause not only pain, but also lead to gradual progressive disability that leads to heavy socio-economic burden in RA patients [3].

Therefore, identification of the key molecule responsible for bony erosion at various RA stages may be beneficial to better understand pathogenesis of the disease and improve prognosis [4].

The bone matrix destruction in RA patients is mainly caused by increase of osteoclastic activity at the junction between cartilage and inflammatory pannus [5, 6]. There is evidence that proteins which are synthesized by wingless/integrated gene cluster (Wnt) are essential mediators for genesis of osteoblasts. This Wnt signaling pathway is regulated by many groups of negative regulators, and one of these regulators is Dickkopf-1 (DKK-1) [7, 8].

Dickkopf (DKK) is a group of proteins rich in cysteine and consists of four subtypes (DKK-1, DKK-2, DKK-3, and DKK-4) or more [9, 10].

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Table 1 Comparison between control and patient groups as regards age, sex, ESR 1h., DKK–1, RF, CRP, and anti-CCP

		Control	Cases	Test	P value
Age (years)	Range	27–60	25–58	T: 0.967	0.336
	Mean ± SD	45.50 ± 9.42	42.83 ± 11.42		
Sex	Male (%)	9 (45%)	20 (25%)	X^2: 3.108	0.078
	Female (%)	11 (55%)	60 (75%)		
ESR 1h. (mm.)	Range	4–11	5–85	T: 7.556	0.001*
	Mean ± SD	8.30 ± 2.00	44.80 ± 21.50		
DKK–1 (pg/mL)	Range	421.5–2657	1650.8–10857.5	T: 6.982	0.001*
	Mean ± SD	1102.42 ± 758.29	5383.80 ± 2973.45		
RF (IU/ml)	Positive (%)	0 (0%)	64 (80%)	X^2: 44.444	0.001*
	Negative (%)	20 (100%)	16 (20%)		
CRP (mg/L)	Positive (> 6) (%)	0 (0%)	67 (83.3%)	X^2: 50.758	0.001*
	Negative (< 6) (%)	20 (100%)	13 (16.3%)		
Anti-CCP (u/ml)	Range	4–20	35–195	T: 9.632	0.001*
	Mean ± SD	12.67 ± 3.24	114.78 ± 47.18		

ESR 1h erythrocyte sedimentation rate (first hour), *DKK–1* Dickkopf-1, *RF* rheumatoid factor, *CRP* C-reactive protein, *anti-CCP* anti-cyclic citrullinated peptide antibody, *t.* test independent samples *t* test, *$P<0.05$ indicates significant difference

It was thought that DKK-1 has a major role in bone erosions in inflammatory articular diseases via Wnt and β-catenin pathway Wnt binds the Frizzled receptor and induces low-density lipoprotein receptor-related protein 5/6 (LRP-5/6) phosphorylation, then activates glycogen synthase kinase. This cascade releases axin from its complex with β-catenin and allows β-catenin to translocate to the nucleus. The consequence is the increased expression of many target genes encoding proteins that control osteoblastic differentiation, apoptosis inhibition, and other proteins involved in Wnt signaling. Extracellular proteins inhibiting this pathway include sclerostin (SOST) and Dickkopf-related protein 1 (DKK-1) [11]. In the present study, we aimed to study the prognostic role of circulating Dickkopf-1 in rheumatoid arthritis.

Methods
Study design and participants
This cross-sectional study was conducted on 80 RA patients and 20 healthy controls matched in age and sex.

RA patients were chosen from the inpatient unit and outpatient clinic in the Rheumatology and Rehabilitation Department at our University Hospital from June 2020 to January 2021

Inclusion criteria
All RA patients were diagnosed according to the American College of Rheumatology (ACR)/European League against Rheumatism (EULAR) 2010 criteria for the diagnosis of RA [12].

Exclusion criteria
We excluded RA patients who had any other diseases that may cause inflammatory arthritis, patients with impaired liver and kidney functions, those complaining of other autoimmune disorders, and RA patients who were in ICU.

Written informed consent was obtained from the patients and controls at the beginning of the study, and our study was approved by the ethics committee at our

Table 2 Correlation between RF and DKK-1, ESR 1h., and DAS28

RF (IU/ml)		Range			Mean	±	SD	t. test	P value
DKK–1 (pg/mL)	+ve	1650.8	–	10857.5	5116.35	±	2996.61	2.643	0.108
	-ve	1684.9	–	8957.2	6453.63	±	2706.29		
ESR 1h. (mm)	+ve	5	–	85	43.80	±	22.14	0.694	0.407
	-ve	14	–	67	48.81	±	18.79		
DAS28	+ve	3.5	–	7.5	5.45	±	1.20	0.112	0.738
	-ve	3.5	–	7.4	5.56	±	1.37		

RF rheumatoid factor, *DKK–1* Dickkopf-1, *ESR 1h.* erythrocyte sedimentation rate (first hour), *DAS28* Disease Activity Score using 28 joints count, *t. test* independent samples *t* test, *$P<0.05$ indicates significant difference

Table 3 Correlation between CRP and DKK-1, ESR 1h., and DAS28

CRP (mg/L)		Range			Mean	±	SD	*t*. test	*P* value
DKK–1 (pg/mL)	+ve	1650.8	–	10857.5	5127.03	±	2931.78	3.159	0.079
	–ve	1748.9	–	8957.2	6707.18	±	2943.83		
ESR 1h. (mm)	+ve	5	–	85	43.94	±	21.92	0.657	0.420
	–ve	18	–	67	49.23	±	19.34		
DAS28	+ve	3.5	–	7.5	5.44	±	1.19	0.239	0.626
	–ve	3.5	–	7.4	5.62	±	1.44		

CRP C-reactive protein, *DKK–1* Dickkopf-1, *ESR 1h.,* erythrocyte sedimentation rate (first hour), *DAS28* Disease Activity Score using 28 joints count, *t. test* independent samples *t* test, **P<0.05* indicates significant difference

university, approval number 33451/5/20 and it was conducted in accordance with the Declaration of Helsinki.

All groups will be subjected to detailed medical history, with complete clinical examination with assessment of disease activity using Disease Activity Score using 28 joints count (DAS28) [13], and plain X-ray on the hands for assessment of radiographic damage.

Laboratory investigations
Blood sampling
Ten milligrams of venous blood samples were collected from all participants after 12 h of overnight fasting, blood samples subdivided into 3ml on EDTA containing tubes for complete blood picture (CBC), 1.6 ml blood on 0.4 ml tri sodium citrate tube for erythrocyte sedimentation rate (ESR), and the rest of blood on plain tube which centrifuged on 3000 rpm 15 min for serum separation for measuring CRP and RF; serum was stored at −80°C until analysis.

Laboratory tests

1. Complete blood picture (CBC): RBC count, hemoglobin concentration, total and differential leucocytic count, and platelet count on ERMA PCE-210N cell counter.
2. Erythrocyte sedimentation rate (ESR in mm/h) was measured by using the Westergren method [14].
3. C-reactive protein (CRP in mg/L) using turbidmetric method on Beckman Coulter AU 480 Analyzers (lot number 2550).
4. Rheumatoid factor (RF) using nephlometric technique on Konelab 60 i Thermo Scientific (Thermo Scientific - Finland) autoanalyzer. (Catalogue Number: 981920 lot number P132).
5. The anti-CCP IgG (anti-CCP in U/ml) level was measured using electro-chemiluminescence immunoassay on automated immunoassay analyzer, Cobas e411, Roche Diagnostics GmbH, Germany, using kit Catalog no: 05031656.
6. Measurement of serum level of DKK-l using Quantikine Human DKK-1 immunoassay manufactured

and distributed by R&D kit (catalog number DKK100B) USA (Quantikine 2011) and read on the microplate reader (Stat Fax®2100, Fisher Bioblock Scientific, France).

Statistical analysis
Statistics were performed using SPSS program, version 17 (SPSS Inc., Chicago, IL, USA) and the Graph-Pad Prism software (GraphPad Prism Software Inc., San Diego, California, USA).

Statistical presentation and analysis of the present study was conducted as continuous data and was expressed as mean ± standard deviation or median. Comparison of continuous data between two groups was made by using Student's *t* test and Mann-Whitney tests, and for categorical variables, chi-square test was used. Spearman correlation between different parameters was used. Statistical significance was defined as a *P* value of ≤ 0.05. The sensitivity, specificity, and accuracy were calculated by using receiver-operating characteristic analysis (ROC curve).

Results
This study included 80 patients with RA and 20 controls. There was no significant difference between studied groups as regards age or sex (*P*= 0.336, 0.078), respectively. But there was a significant increase in serum ESR, DKK-1, RF, CRP, and also anti-CCP levels in patients in comparison with controls (*P*=0.001) (Table 1).

The RF was negative in all controls (100%). In RA patients, RF was positive in 64 out of 80 patients (80%) (*P*= 0.001).

Table 4 Correlation between serum ESR, DAS28, and DKK-1

		DKK–1 (pg/mL)	ESR 1st h.
ESR 1 h. (mm.)	*r*	0.845	
	P value	0.001*	
DAS 28	*r*	0.818	0.736
	P value	0.001*	0.001*

DKK–1 Dickkopf-1, *ESR 1h.* erythrocyte sedimentation rate (first hour), *DAS28* Disease Activity Score using 28 joints count, Pearson's correlation coefficient, **P<0.05* indicates significant difference

Fig. 1 A positive significant correlation between DKK-1 serum level and ESR (*r=0.845*) and (*P = 0.001*)
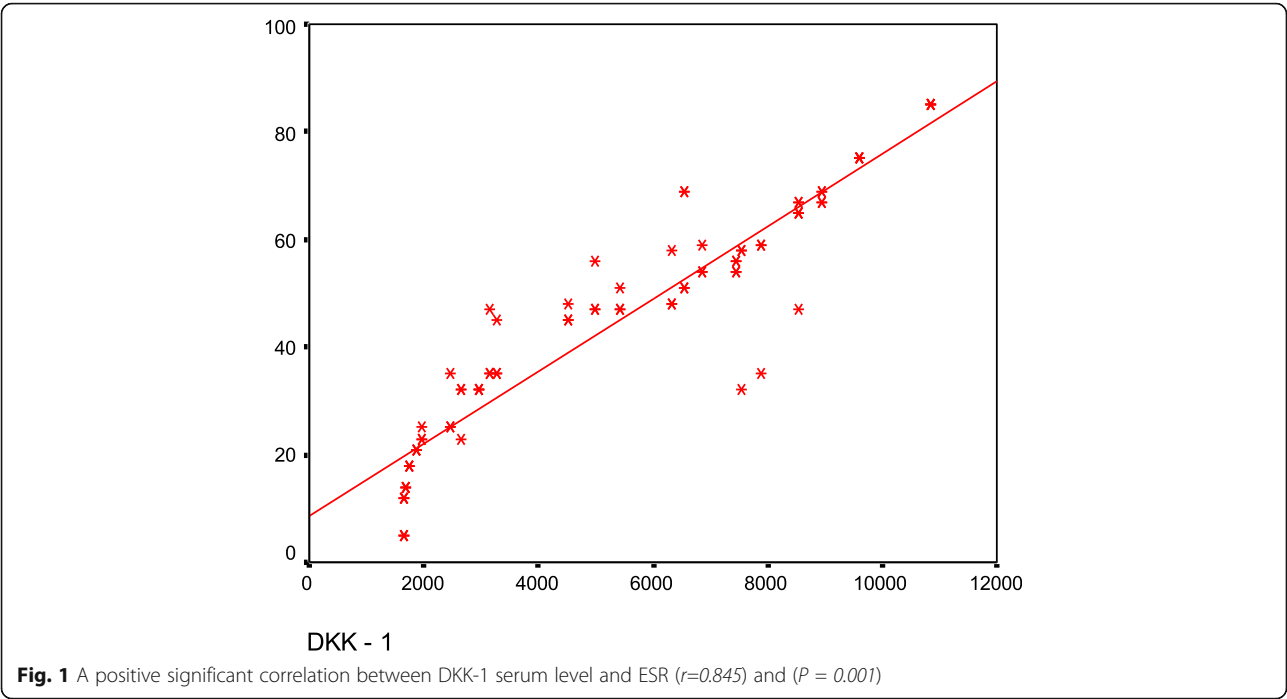
The CRP level in the control group was negative in all controls (100%). In RA patients, CRP was positive in 67 patients (83.3%) and negative in 13 patients (16.3%) (*P= 0.001*) (Table 1).

The disease duration in the patients' group ranged from 2 to 12 years with mean+SD, 6.9937+2.78633.

In this study, we did not find significant correlation between both RF and CRP and other markers DDK-1, ESR, and DAS28 (*P= 0.108, 0.407, 0.738*) (*P= 0.079, 0.420, 0.626*) (Tables 2 and 3, respectively).

A positive significant correlation was found between DKK-1 levels and ESR (*P=0.001*), DKK-1, and DAS28

Fig. 2 A positive significant correlation between serum DDK-1 level and DAS28 (*r = 0.818*) and (*P= 0.001*)
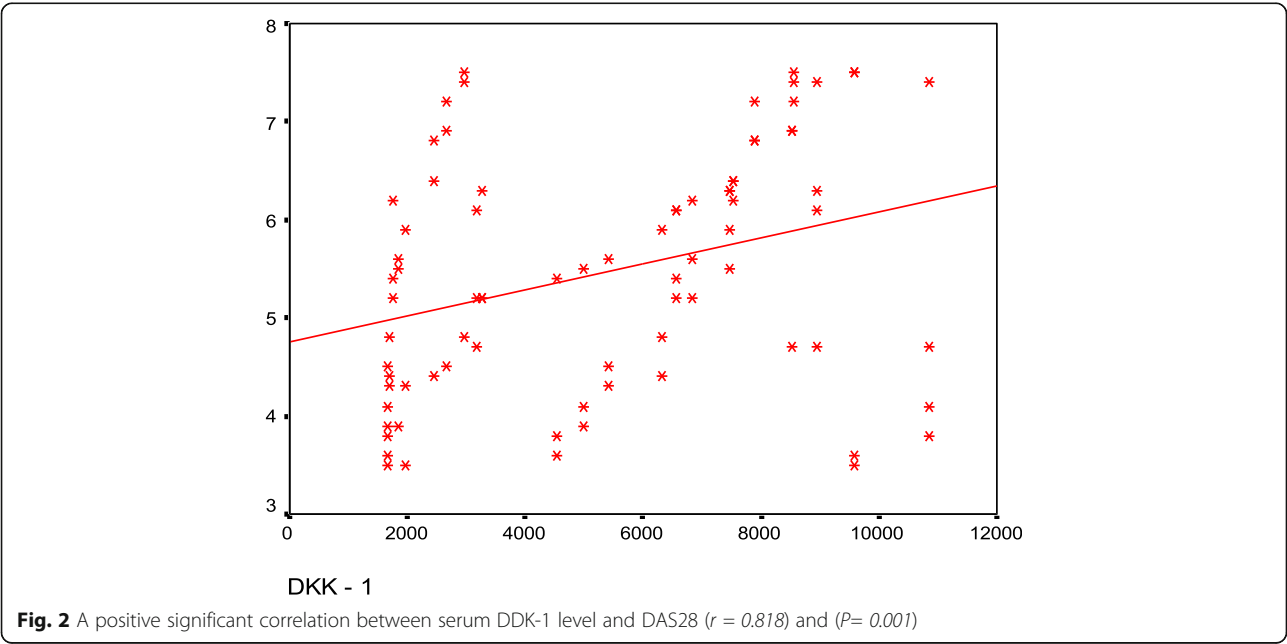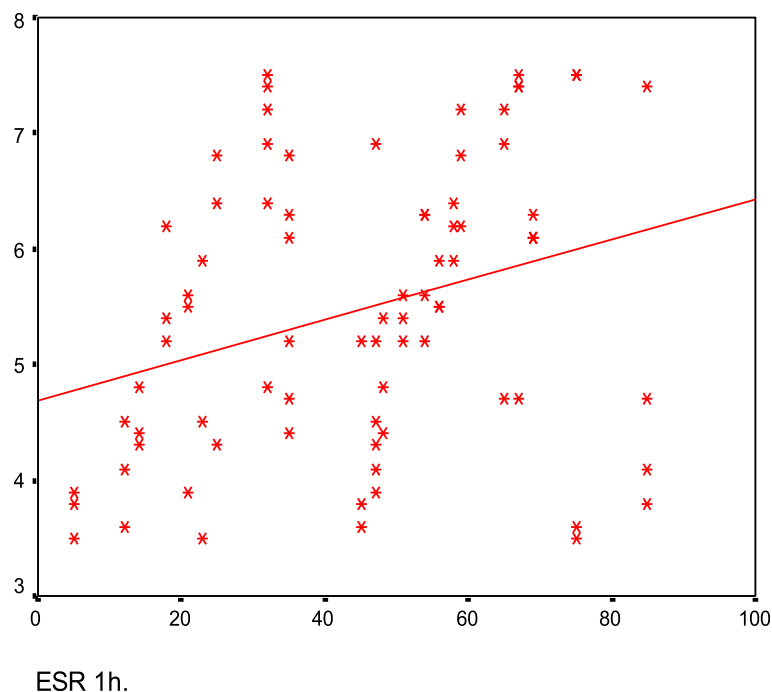
Fig. 3 A positive significant correlation between ESR and DAS 28 (*r =0.736*) and (*P= 0.001*)

(*P= 0.001*) and between ESR and DAS28 (*P= 0.001*) (Table 4, Figs. 1, 2, and 3, respectively).

Our results found a significant positive correlation between duration of the disease and serum levels of DKK-1 (*P= 0.001*). The duration of disease in RA patients ranged from 2.00 to 12.00 years with mean $\pm$ SD (6.9937 $\pm$ 2.78633) (Table 5, Fig. 4). We recorded a significant positive correlation between serum DKK-1 and anti-CCP levels (*P=0.001*) (Table 6, Fig. 5).

We found that 30 out of 80 patients (37.5%) which had bony erosions in plain X-ray of the hands, and they had significant higher levels of serum DKK-1 than the other 50 patients (62.5 %) who had no erosions detected in their X-rays (*P=0.001*) (Table 7).

At cutoff value 2150, the DKK-1 in RA has 82% accuracy, 75% negative predictive value, 95% positive predictive values, 90% sensitivity, and 85% specificity (Table 8).

Table 5 Correlation between serum DKK-1 levels and duration of the disease

		DKK–1
Duration of the disease	*r*	0.824
	P value	0.001*

DKK–1 Dickkopf-1, Pearson's correlation coefficient, **P<0.05* indicates significant difference

Discussion

RA is a disease of autoimmune nature that affects mostly the articular structures and synovial membranes causing chronic inflammation involving multiple joints and body systems. The small joints of the hands and feet are often firstly affected, followed by larger joints. RA patients have periodic flare-up that can lead to irreversible joint damage, systemic affection, and organ damage as the heart and lungs [15].

Until now, although new modalities of treatment, severe joint damage and disability are common. Thus, early and effective treatment can change the prognosis of the disease, decrease mortality, and improve joint functions [16]. To meet the needs to improve the diagnostic and prognostic tests, multiple serum biomarkers are studied in RA, including many autoantibodies [17]. Wnt proteins are among these important biomarkers that related to disease prognosis.

The Wnt proteins were recognized as essential elements in normal homeostasis of the bone, specially formation of the new bone by osteoblasts [18] and also responsible for many cellular processes as maturation, differentiation, and programmed death.

These functions occur via attachment of Wnt proteins to a co-receptor complex that consists of low-density lipoproteins that called (receptor related proteins LRP5 or LRP6). Multiple pathways were found, but the Wnt b-Catenin pathway is the major one involving bone stasis.
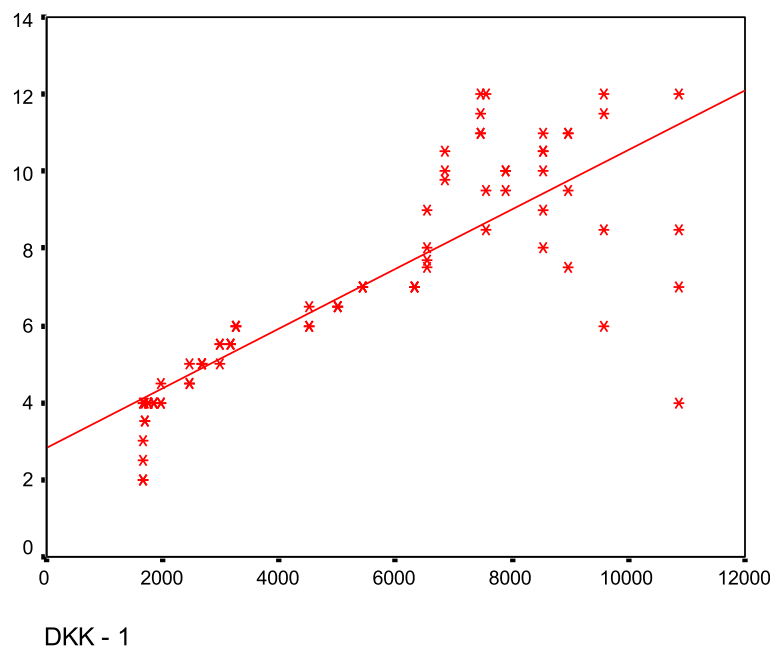
Fig. 4 Correlation between serum DKK-1 levels and duration of the disease (*r*=0.824) and (*P*= 0.001)

In this pathway, the attachment of Wnt proteins with the previously mentioned receptors results in inhibition of b-catenin degradation and so, an increase in intracellular b-catenin [19].

DKK-1 was proved to inhibit regulation of the Wnt pathway. In experimental trials on animals that had arthritis, it was found to have a role as a marker for joint destruction. Elevated DKK-1 levels were positively correlated to bone resorption, whereas its low levels were related to new bone formation. Moreover, *Diarra et al.* showed a negative regulation between DKK-1 and OPG. So that, DKK-1 was proven to have an indirect effect on genesis of osteoclasts and subsequent bony resorption and erosions. So, this dual action demonstrated that DKK-1 is a key regulator of pathogenic remodeling of joint, and this remodeling is based on the interplay of anabolic with catabolic pathways [18]. In other studies, DKK-1 blockage was found to make fusion of the sacroiliac joints in animals that had arthritis [20].

Table 6 Correlation between serum DKK-1 levels and anti-CCP titer

	DKK–1	
Anti CCP	*r*	*P* value
	0.870	0.001*

DKK–1 Dickkopf-1, *anti-CCP* anti-cyclic citrullinated peptide antibody, Pearson's correlation coefficient, **P≤0.05* indicates significant difference

The aim of this work was to study the prognostic role of circulating DKK-1 in the pathogenesis of RA and its correlation to other laboratory markers of disease activity.

The results of this work showed no significant difference between the studied groups according to age and sex (Table 1).

As regards ESR, we found that the values of ESR were significantly high in RA patients in comparison to healthy subjects (Table 1). These results were in accordance with the results of *Daoussis et al.* He noticed a significant increase in ESR level in patients of RA compared to controls [21]. Also, we found a significant difference between the studied groups regarding RF (Table 1). These results were in agreement with that of *Vittecoq et al.* [22] who found that high titer of RF was important in predicting continuation of radiographic damage in RA.

Our results demonstrated that there was no significant difference as regards ESR in RF negative and RF-positive patients (Table 2), and this was in agreement with *Vajpayee et al.* who demonstrated that RF showed a low correlation with ESR [23].

Also in our study, a significant difference was found between the studied groups regarding CRP (Table 1). These results were in agreement with that of *Raghav et al.* who demonstrated that CRP as an acute phase protein was shown to differ by changes in clinical disease activity more than ESR, usually elevated in untreated RA
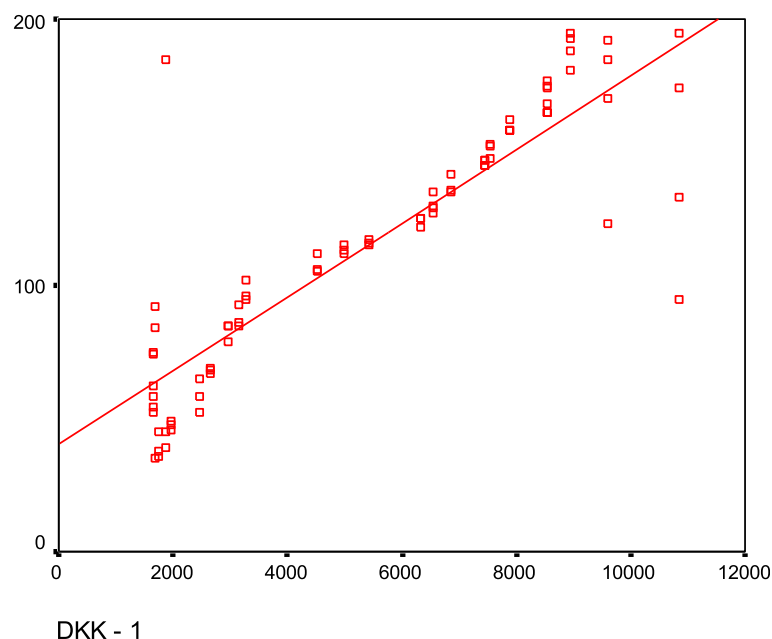
Fig. 5 Correlation between serum DKK-1 levels and anti-CCP titer (*P= 0.001*)

and degree of elevation correlated roughly with the severity of inflammation [24].

But in contrary with *Orr CK et al.* which conducted his study on 223 RA patients with knee arthralgia, synovial examination was done under microscopy using hematoxylin and eosin (H&E) staining and immunohistochemistry, after synovial samples were taken from the symptomatic knee through arthroscopy. Synovial examination was correlated to DAS28-CRP, ESR, and CRP in the blood samples taken immediately before arthroscopy. They found that, inspite of a significant positive correlation between CRP and inflammation intensity in the biopsy, there was synovial inflammation in patients with normal CRP. They concluded that CRP had a moderate correlation with RA activity [25].

This study showed that there was a positive significant correlation between ESR and DAS 28 (Fig. 3). These results are in disagreement with that of *Keenan et al.* who demonstrated that poor correlation of ESR with RA activity as ESR is affected by age, sex, menstrual cycles, smoking, drugs, RBC characteristics, and dietary lipids [26].

The results illustrated that DKK-1 levels were significantly high in patients with RA when compared to control group (Table 1). Also, positive correlation was found between DKK-1 and DAS 28 (Table 4, Fig. 2). This matched the results of *Diarra et al.* who assessed that DKK-1 was closely related to clinical disease activity [18]. We found the same issue with correlation between DKK-1 and Anti-CCP, as we reported a positive significant correlation between them which the matter that support relation of DKK-1 with disease activity (Table 6, Fig. 5). These results were in disagreement with *Aydemir ZS et al.* who found that, no correlation between markers of RA activity and levels of DKK-1 in patients group [27].

In our results, we reported that DKK-1 showed significant higher levels with longer duration of disease (Table 5, Fig. 4). So, these results were in agreement with *Liu et al., 2010* who studied the potential role of DKK-1 in all RA stages. He correlated serum DKK-1 levels in early RA patients in relation with its levels in patients with long duration disease [28] and reported significantly

Table 7 Comparison between DKK-1 levels in patients with versus without bony erosions in hand X-rays

DKK–1 (pg/mL)		Range			Mean	±	SD	*t* test	*p* value
Erosions in Hand X-ray	Present	1748.9	–	10857.5	6260.06	±	2804.28	3.660	0.001*
	Absent	1650.8	–	9587.6	3923.38	±	2695.85		

DKK–1 Dickkopf-1, *t. test* independent samples *t* test, **P<0.05* indicates significant difference

Table 8 ROC curve between the studied groups as regards DKK-1

	Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy
DKK–1 (pg/mL)	2150	0.923	90	85	95	75	82

DKK–1 Dickkopf-1, cut-off value

higher serum DKK-1 levels in patients with long disease duration, so that bony erosions that usually occur with long disease duration in RA were proved to be associated with increasing serum DKK-1 (Fig. 6). Also, *Diarra et al.* stated that a profound decrease in levels of DKK-1 was found in RA patients taking anti TNF therapy [18].

Also *Garnero et al.* underwent assessment of DKK-1 levels in a group of randomly selected early RA patients who were receiving disease-modifying anti-rheumatoid drugs (DMARDs) for only 1 year, and they found higher radiologic progression in patients with higher serum levels of DKK-1. Each 1 (SD) standard deviation increase in serum DKK-1 was correlated with a relative risk of progression of 1.65. So, they suggested that DKK-1 can be as a marker to predict patients who have high risk of progression [29]. These results were in accordance with our study results, as we found that 37.5% from RA patients who had bony erosions in their plain X-ray of the hands, had higher levels of serum DKK-1 than other patients who had normal X-rays (Table 7).
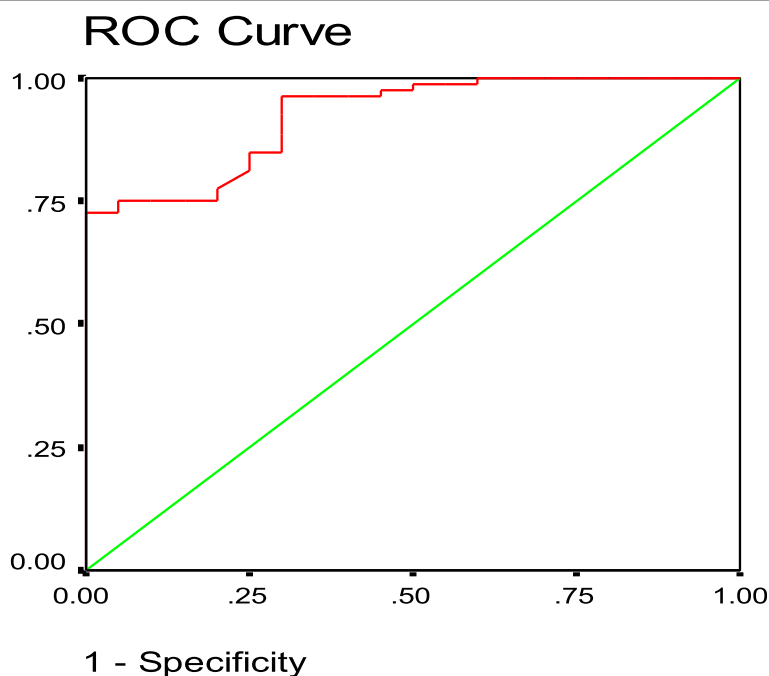
In contrast to our results, *Dalbeth et al.* did not detect any significance between serum DKK-1 and bone pathology, and serum levels of DKK-1 had no correlation with the presence of bony erosion or new bone formation [30].

Conclusions

DKK-l serum level can be used as a potential prognostic biomarker for monitoring of joint erosions and destruction in RA patients. Furthermore, it could be a possible target molecule in the future therapy to control the process of joint destruction.

Limitations

1. The study had limited number of rheumatoid arthritis patients, and larger number can provide more accurate statistical results.
2. We had no exclusion criteria about drug history that can affects bone metabolism as Parathyroid hormone (PTH), vitamin D, glucocorticoids, and estrogens.
3. We evaluated just the presence or absence of bony erosions at plain X-ray without using Larsen's scoring system.

Fig. 6 ROC curve between the studied groups as regard DKK-1

Authors' contributions
DAA: Study designing and retrieving the literature. **DAA** and **RME:** performing the routine and molecular investigations and analyzing data. **DME:** Patients' selection, collecting the data from the patients and control, and performing the clinical examination. **HAM:** the corresponding author and was involved in writing the manuscript. **RLY** participated in assessing the patients and writing the manuscript **All authors:** writing the original draft, reviewing, and final manuscript approval. The authors have read and approved the manuscript.

Availability of data and materials
All data generated or analyzed during this study are available.

Declarations

Ethics approval and consent to participate
This study was approved by the ethics committee of Faculty of Medicine, Tanta University, approval number 33451/5/20, and written informed consent was obtained from all individual participants included in the study.

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

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