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Elevated Serum Dickkopf-1 Levels as a Biomarker for Disease Activity and Severity in Psoriatic Arthritis Patients

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ABSTRACT

Background: Psoriatic arthritis (PsA) is distinguished by spondylitis, dactylitis, peripheral arthritis, and skin psoriasis. Dickkopf-1 (DKK-1) is thought to be the primary suppressor of the Wnt signaling pathway, resulting in decreased osteoblast proliferation.

Objective: This study determined DKK-1 serum levels and its relationship with disease severity and activity in PsA patients.

Patients and Methods: Enzyme-linked immunosorbent assay (ELISA) was used to measure serum DKK-1 levels in 45 patients with PsA and 45 healthy age and gender harmonized people as a control group. All patients were recruited from the Rheumatology, Rehabilitation, and Physical Medicine Outpatient Clinic and Inpatient Department at Benha University Hospitals. PsA Disease Activity Score (PASDAS) was utilized to assess disease activity, while Simplified Psoriatic Arthritis Radiographic Score (SPARS) and PsA Impact of Disease (PsAID) were used to assess disease severity.

Results: The mean serum DKK-1 levels in PsA patients was significantly higher than in control group [$p<0.001$]. In addition, it increased gradually in remission, low, moderate then high activity cases ($p<0.001$). As regards disease severity, unacceptable status showed significantly higher DKK-1 level when compared to acceptable status. Also, a statistically significant correlations between serum levels of DKK-1 and PASDAS, PsAID, and SPARS was discovered.

Conclusion: DKK-1 serum levels were abnormally high in PsA patients. Elevated DKK-1 levels had a significant role in the process of structural radiographic alterations, as well as disease severity and activity in PsA patients.

Keywords: Psoriatic arthritis, Dickkopf-1, Disease activity, ELISA.

INTRODUCTION

Psoriasis is a widespread inflammatory chronic skin condition defined by aberrant keratinocyte proliferation, differentiation, and death[(1)].

Psoriatic arthritis (PsA) is a comorbidity of psoriasis that affects 20–30% of psoriatic patients and is distinguished by isolated bone erosions and aberrant bone growth, which may imply an uncoupling of osteoblast-osteoclast homeostasis. The typical lag time for the onset of PsA is 10 years[(2)].

PsA typically manifests in young adults between the ages of 30 and 50. It affects both axial and peripheral joints, causing bone loss, irreversible joint damage, increased functional disability, and an impaired wellbeing's[(3)].

Psoriasis is a multifactorial disorder characterized by an aberrant immune response in genetically susceptible individuals that is brought on by additional environmental factors; the etiology and basis of these physiologic changes are not fully understood[(4)].

It has been established that the immune infiltrate within psoriatic lesions is crucial for the emergence of psoriasis and that T cells are triggered to start producing the cytokines that cause epidermal hyperplasia, acanthosis, hyperparakeratosis, and orthohyperkeratosis[(5)].

Several cytokines, including IL-17 and IL-22, generated by the Th17 subset of T lymphocytes have been shown to contribute to the pathophysiology and increase the production of Wnt proteins. Interleukin (IL)-1α, the epidermal growth factors TGF- α and vascular endothelial growth factor (VEGF), as well as other cytokines generated by the Th17 fraction of T cells, have also been linked to increase expression of Wnt proteins[(6)].

The Wnt family of signaling proteins is a set of tiny, cysteine-rich, secreted glycoproteins that regulate and govern cell proliferation, fate determination, and differentiation[(7)].

Wnt proteins interact with a membrane-bound receptor complex comprised of the Frizzled (Fz) receptor and its low-density lipoprotein receptor-related protein 5 and 6 (LRP5/6) co-receptors in order to activate multiple signaling pathways[(8)]. The production of IL-12p40 (the common subunit of IL-12 and IL-23) and IFN-γ increases as a result of this interaction. Through the maintenance of Th1 and Th17 cells and effector functions, IFN-γ and IL-23 play essential roles in the progression of psoriasis. Wnt proteins may work in concert with type I interferons, which are thought to be responsible for the development of psoriasis[(6)].

PsA is distinguished by a large influx of fibroblasts and activated CD4+ T cells into the synovial membrane, similar to the immune infiltrate of dermal cutaneous psoriatic eruptions. By interacting with receptor activators of nuclear factor κ-B (RANK), its

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ligand (RANKL), IL-7 and TNF-alpha, they induce osteoclastogenesis and bone resorption, hence causing destructive arthritis. Through a separate mechanism, IL-23 exhibits osteoclastogenesis inducing activities on RANKL, TNF, and IL-17[9].

Four members make up the Dickkopf (DKK) family, a group of cysteine-rich proteins (Dickkopf-1 – Dickkopf-4). By attaching to the LRP5/6 co-receptors and causing the internalization of the receptor complex for degradation, DKK proteins, particularly DKK-1, can inhibit Wnt signaling and reduce the number of Wnt receptors available for signaling[6].

It is revealed in several studies that cases with PsA had considerably higher DKK-1 protein serum levels than controls, suggesting that it may be valuable for prognostic and/or diagnostic purposes[9].

The study's aim is to compare DKK-1 serum levels in PsA patients to healthy controls and to look into the relationship between Dkk-1 levels and disease activity and severity in PsA patients.

PATIENTS AND METHODS

Study design:

A case-controlled study was conducted as a collaborative work between the departments of Medical Microbiology and Immunology as well as Rheumatology, Rehabilitation and Physical Medicine, Faculty of Medicine, Benha University. And Department of Clinical Pathology, Misr University for Sciences and Technology, Egypt.

Target population:

Patients group included 45 PsA patients, diagnosed in accordance with the Classification Criteria of Psoriatic Arthritis (CASPAR) [10]. The patients were selected according to the inclusion criteria from the Inpatients' and Outpatients' Clinics of the Rheumatology, Rehabilitation and Physical Medicine Department. As a control group, 45 apparently healthy, gender and age matched volunteers were recruited.

Inclusion criteria:

Patients were diagnosed according to CASPAR criteria which include: evidence of psoriasis (current, personal and family history), negative rheumatoid factor, psoriatic nail dystrophy, dactylitis (current or past history), and radiographic evidence of juxta-articular new bone formation [10].

Exclusion criteria:

Patients under the age of 18 were excluded. Patients on steroids, patients with impaired liver and kidney functions, pregnant and lactating females or those taking oral contraceptive drugs, other forms of inflammatory arthritis and the other seronegative spondyloarthropathies were excluded from the study.

Clinical and laboratory assessment:

All participants were subjected to full history taking and clinical examination. The activity of psoriatic arthritis was assessed by PASDAS [10]. Disease severity was evaluated using the EULAR PsA Impact of Disease (PsAID), which give a number between 0 and 10. More burden of the disease is indicated by a higher PsAID score. A patient-acceptable status is defined as a rating of less than 4 out of 10. A change of three points or more is regarded as a significant absolute change. All study participants were subjected to laboratory investigations such as a complete blood count (CBC), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP).

Radiographic assessment:

Both feet and hands radiographs (postero-anterior view) were taken. SPARS was utilized to assess radiological severity and structural changes in the form of erosion, joint space narrowing and bone proliferation. Total score (0-120) [11].

Assay of serum DKK-1 level by ELISA:

Venous blood samples (2 ml) were drawn from all study participants' veins under strict aseptic conditions and placed in plain tubes to coagulate for 10–20 minutes at room temperature. Following a 15-minute, 3000 rpm centrifugation to separate the serum, samples were stored at -20 °C in Eppendorf tubes until analysis. According to the manufacturer's instructions, the Human DKK-1 ELISA (Enzyme-Linked Immunosorbent Assay) Kit (Thermo Fisher Scientific, USA) was used to measure the concentration of DKK-1 in the serum. The optical density (O.D.) was measured at 450 nm in a micro-plate reader (HEALES-Multimode Plate Reader MB-580, China). The Human DKK1 solid-phase sandwich ELISA is made to gauge how much of the target is entrapped between a matched set of antibodies.

Ethical consent:

This study was ethically approved by the Institutional Review Board of the Faculty of Medicine, Benha University. Written informed consent was taken from all participants. The study was conducted according to the Declaration of Helsinki.

Statistical analysis

The collected data were analysed with the use of the Statistical Package for Social Science (IBM Corp., 2017). Version 25.0 of IBM SPSS Statistics for Windows (Armonk, New York: IBM Corporation, 2005). Student t-Test was used to assess the statistical significance of the difference in means between the two research groups. Examining the statistical significance of the difference between two nonparametric study groups using the Mann-Whitney Test (U test). The Kruskal-Wallis test was used to determine the statistical significance of a difference between more than two non-parametric study group variables. Using the Chi-Square test, the relationship between two qualitative variables

was examined. ROC Curve was also performed. Generalized linear models were used in linear regression analysis to predict risk factors.

One variable is analysed at a time in a univariate regression, which looks at how one independent variable affects one dependent variable. To determine which independent variables are independently associated with the outcome, or those that maintain a significant p-value in the model despite the inclusion of other independent variables, multiple variables were analysed simultaneously for any potential association or interactions. A two tailed p value less than 0.05 was considered significant.

RESULTS

The present study encompassed 45 participants with a mean age of 42±66 years. Males were 15/45 (33.3%) and females 30/45 (66.7%). In addition, 45 healthy control subjects of similar age, gender, and BMI were employed. The disease lasted between 1.5 and 10 years. According to the (PASDAS), 11/45 (24.4%) patients were in remission, while 25 (55.6%) patients had highly active disease. The disease's severity was considered using (PsAID) and SPARS. Twenty-nine out of forty-five patients (64.4%) had acceptable status, while 16/45 (35.6%) had unacceptable status according to PsAID. Table **1** shows the features of the studied groups.

Table (1): Demographic characteristics of the studied groups.

			Psoriatic arthritis		Control		*p-value*
Age		**Mean±SD**	42.2	±6.6	41.9	±6.3	0.818
Sex	Male	**N, %**	15	33.3%	18	40.0%	0.512
	Female	**N, %**	30	66.7%	27	60.0%	
BMI		**Mean±SD**	27.0	±4.8	27.8	±4.5	0.406
Disease duration (years)		**Median, range**	5	1.5-10			
PASDAS		**Median, range**	3.5	0.5-5.2			
PASDAS	Remission	**N, %**	11	24.4%			
	Low	**N, %**	4	8.9%			
	Moderate	**N, %**	5	11.1%			
	High	**N, %**	25	55.6%			
PsAID	Acceptable status	**N, %**	29	64.4%			
	Un acceptable status	**N, %**	16	35.6%			
PsAID		**Mean±SD**	5	±1.1			
SPARS		**Mean±SD**	68	±15.31			
CRP (mg/ml)		**Mean±SD**	29.5	±6.11			
ESR (mm/h)		**Mean±SD**	46	± 11.2			
HB (g/dL)		**Mean±SD**	10.1	± 2.3			

PsAID: Psoriatic Arthritis Impact of Disease, PASDAS: PsA Disease Activity Score, SPARS: Simplified Psoriatic Arthritis Radiographic Score. Age was compared using t test; sex was compared using chi square test.

DKK-1 levels had considerably increased in patients (range: 4890-14000) compared to controls (range: 2604-4995) ($p<0.001$). Additionally, it gradually increased in cases with low, moderate, and high activity levels ($p<0.001$). As regards disease severity, unacceptable status (range: 7430-14000) showed significantly higher DKK-1 level when compared to acceptable status (range: 4890-11765) table 2, figure **1**.

Table (2): DKK-1 level among different status.

		DKK-1 level (Pg/ml)		*p-value*
		Median	**Range**	
Groups	Cases	9090	4890-14000	<0.001
	Control	4019	2604-4995	
Disease activity (PASDAS)	Remission	5950	4890-6360	<0.001
	Low	6863	6090-7020	
	Moderate	9090	7125-9450	
	High	9850	7430-14000	
Disease severity (PsAID)	Acceptable status	7020	4890-11765	<0.001
	Un acceptable status	9690	7430-14000	

Comparison between 2 groups was done using Mann Whitney test; Comparison between more than 2 groups was done using Kruskal Wallis test.

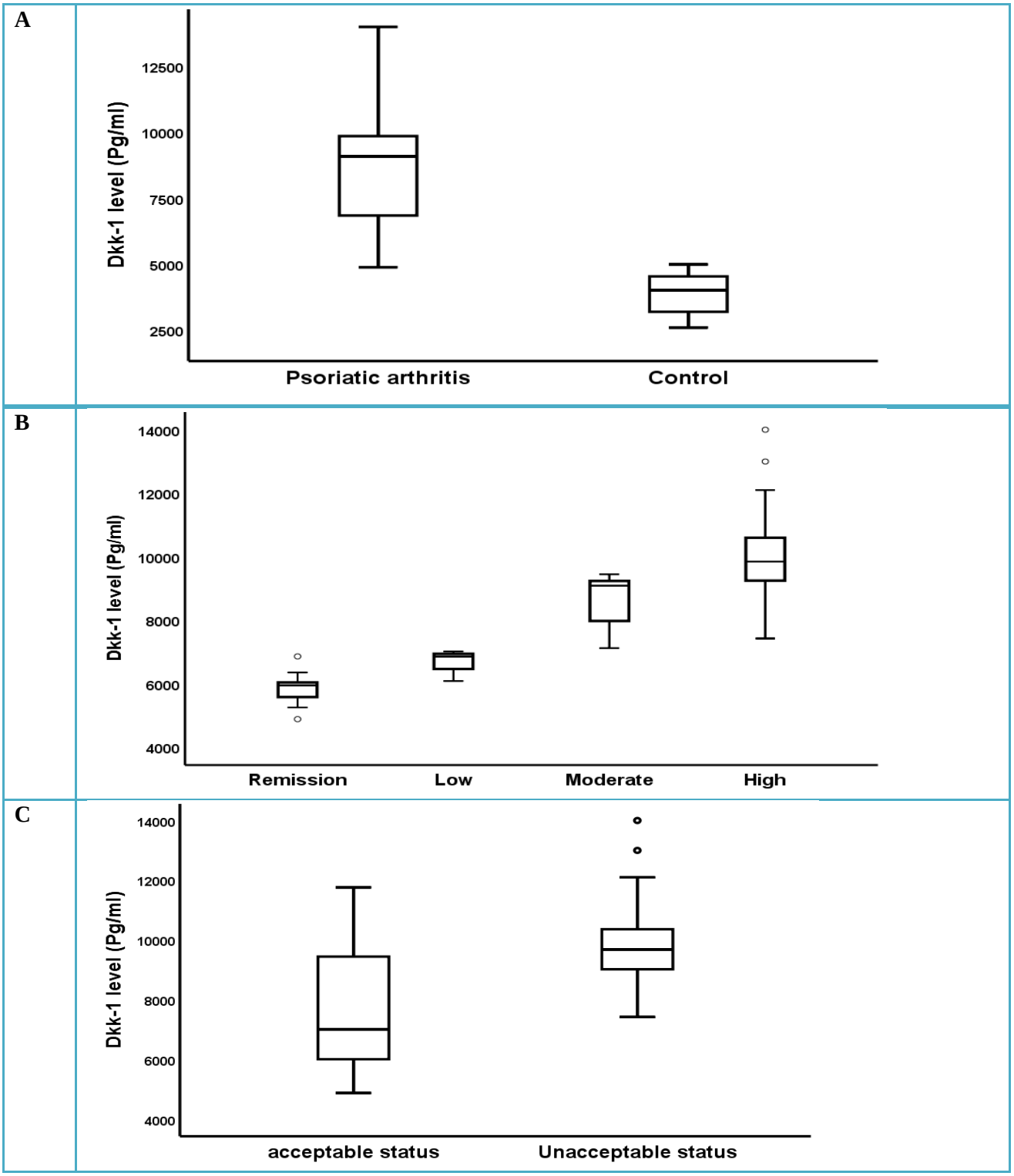


Figure (1): Box plot showing DKK-1 level among (A) cases and controls, (B) remission, low, moderate, high activity and (c) Acceptable and unacceptable status.

DKK-1 ROC analysis was performed to differentiate between different groups (figure **2**). It demonstrated high accuracy AUCs for both PsA diagnostic ability and disease activity prediction (AU=0.999, 0.992, respectively). While it had a moderate AUC for predicting disease severity (AUC =0.773). All cases were classified as low (≤SPARS median) or high (>SPARS median) based on their SPARS level. When the DKK-1 ROC curve was examined for discrimination between low and high SPARS subgroups, a moderate AUC (AUC=0.877) was discovered. The table (**3**) shows the cut off values, sensitivity, and specificity.

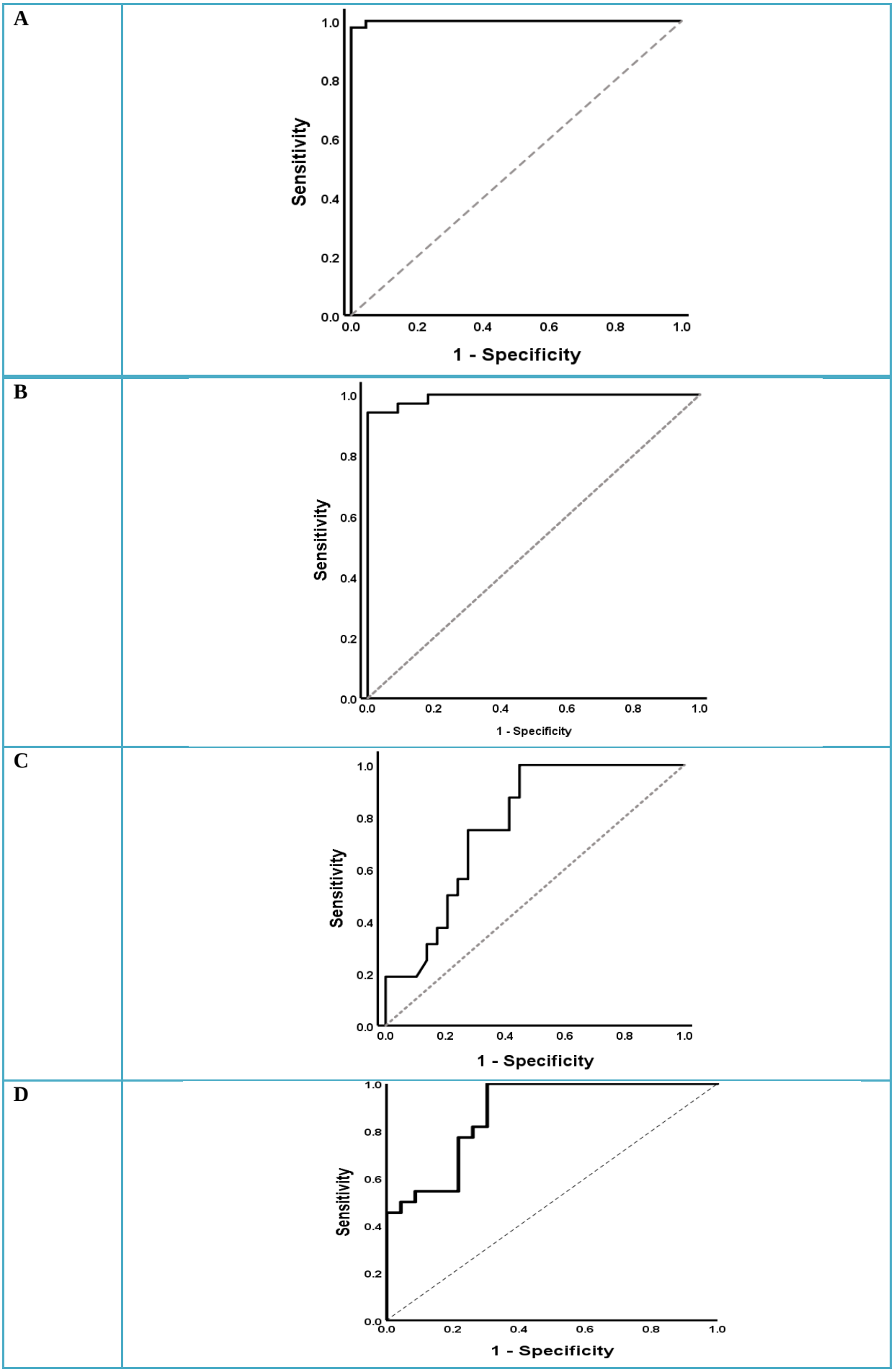


Figure (2): ROC curve of DKK-1 level for prediction of (A) PsA diagnosis, (B) disease activity and (C) disease severity (D) low and high SPARS.

Table (3): Validity of DKK-1 level for prediction of PsA diagnosis, disease activity, clinical and radiographic severity.

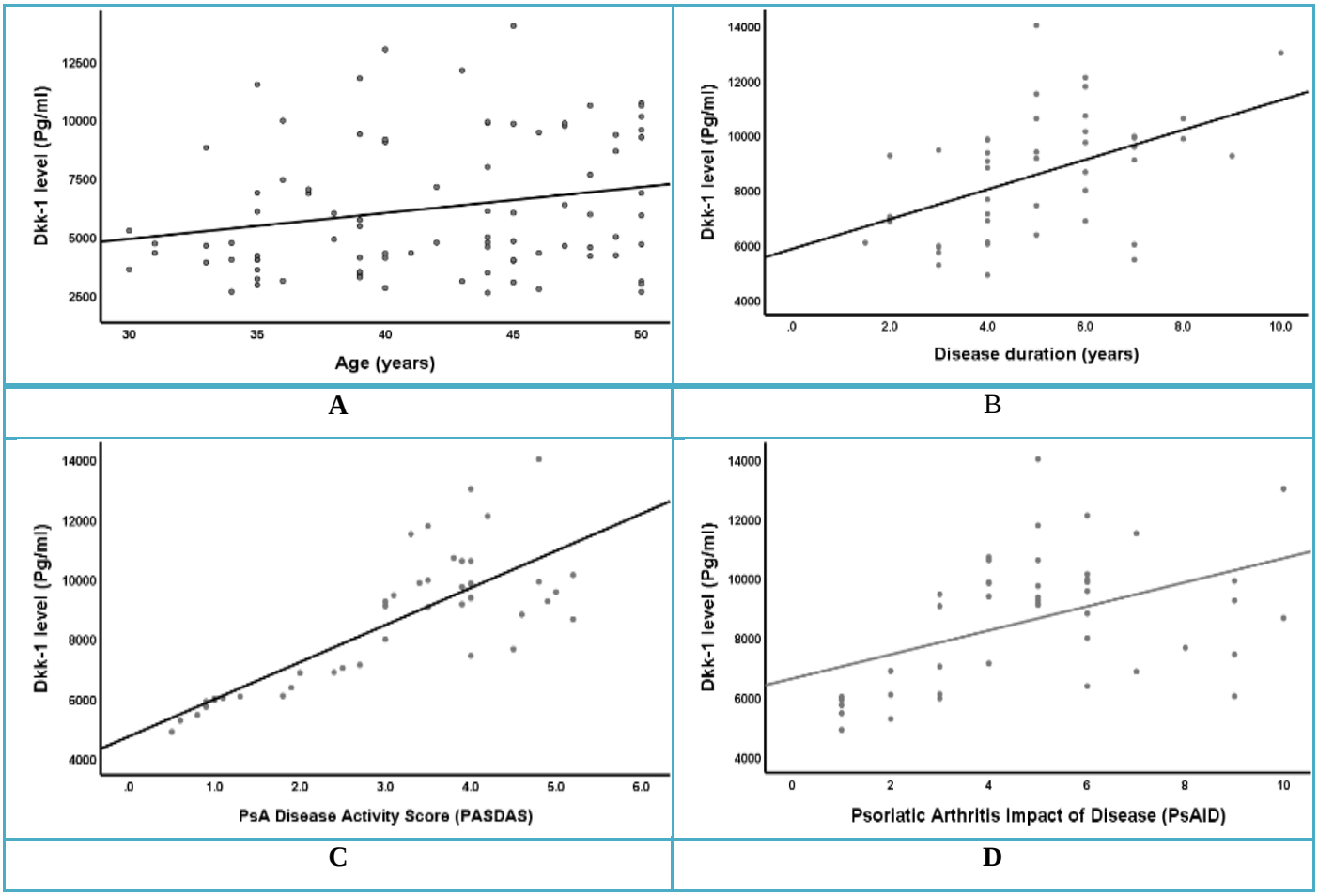
	Area	95% CI	Cut off	Sensitivity (%)	Specificity (%)
Psoriatic arthritis	0.999	0.996-1	5126.50	97.8	100
Disease activity (PASDAS)	0.992	0.974-1	6870.00	94.1	100
Clinical severity (PsAID)	0.773	0.639-0.907	9245.00	75	72.4
Radiographic severity (SPARS)	0.877	0.779 -0.976	9090	77.3	78.3

DKK-1 serum concentrations correlated significantly with age, disease duration, PASDAS, PsAID, PsAID, CRP, and ESR (table 4, figure 3). There was no correlation found between BMI, HB, and DKK-1 serum concentration.

Table (4): Correlation of DKK-1 with other parameters.

	DKK-1 level (Pg/ml)	
	Coefficient	**p-value**
Age	0.220	0.037
BMI	-0.140	0.189
Disease duration	0.489	0.001
PASDAS	0.745	<0.001
PsAID	0.487	0.001
SPARS	0.722	<0.001
CRP	0.781	<0.001
ESR	0.825	<0.001
HB	-0.198	0.193

PsAID: Psoriatic Arthritis Impact of Disease, PASDAS: PsA Disease Activity Score, SPARS: Simplified Psoriatic Arthritis Radiographic Score. Spearman's correlation test was used.



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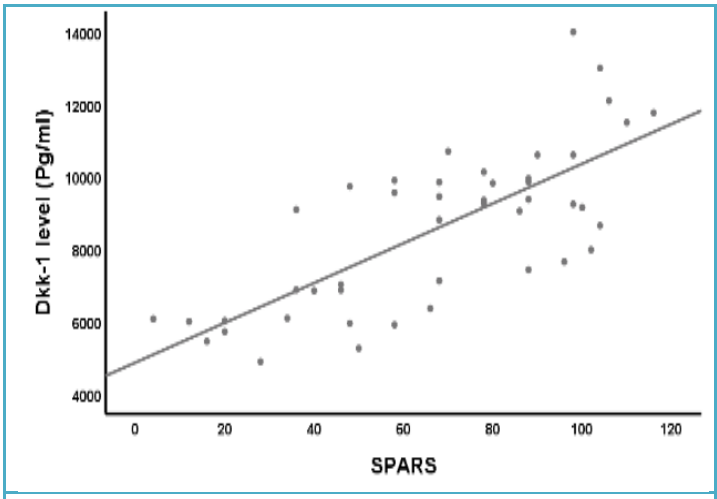

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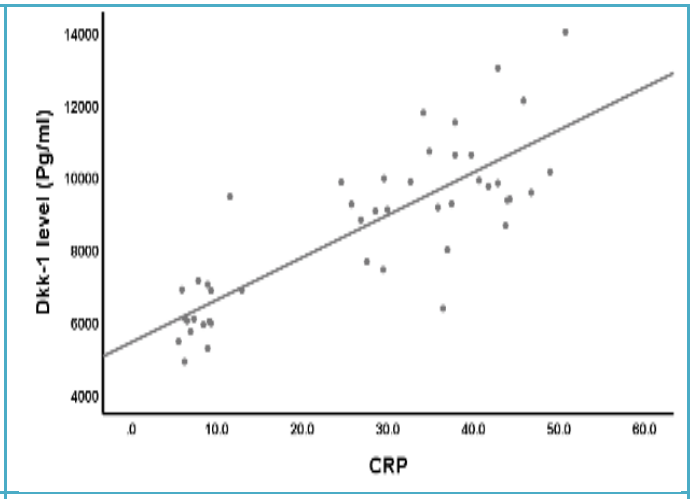

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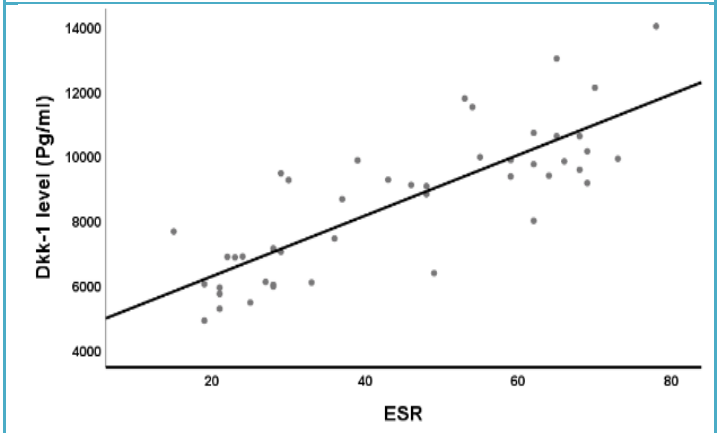

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Figure (3): Scatter plots showing correlation of DKK-1 with (A) age, (B) duration, (C) PASDAS, (D) PsAID, (E) SPARS, (F) CRP and (G) ESR.

Linear regression analysis was conducted for prediction of disease activity (PASDAS), clinical severity (PsAID) and radiological severity (SPARS), using age, gender, BMI, duration, CRP, ESR and DKK-1 as confounders. Uni and multivariable analyses were conducted. Higher CRP and DKK-1 was considered predictors of disease activity (PASDAS). Moreover, higher CRP, ESR and DKK-1 were considered predictors of clinical severity (PsAID) and radiographic severity (SPARS). P values < 0.05 were deemed statistically significant (*) while p values > 0.05 were deemed insignificant. P value < 0.01 was deemed highly significant (**) (Table 5).

Table (5): Regression analysis for prediction of disease activity (PASDAS), clinical severity (PsAID) and radiographic severity (SPARS).

	PASDAS				**PsAID**				**SPARS**			
	Univariable		**Multivariable**		**Univariable**		**Multivariable**		**Univariable**		**Multivariable**	
	B	***P***	***B***	***p***	***B***	***P***	***B***	***p***	***B***	***p***	***B***	***p***
Age	0.085	0.022	0.006	0.753	0.080	0.241			1.253	0.118		
Gender	0.037	0.935			1.333	0.195			2.133	0.824		
BMI	0.036	0.425			0.043	0.597			1.153	0.224		
Disease duration	0.234	0.032	0.095	0.132	0.563	0.003	0.322	0.071	5.550	0.016	0.308	0.853
CRP	0.078	<0.001	0.080	<0.001	0.092	<0.001	0.170	<0.001	1.443	<0.001	1.484	0.001
ESR	0.053	<0.001	0.024	0.064	0.046	0.020	0.111	0.003	0.927	<0.001	0.852	0.015
Dkk-1	0.001	<0.001	<0.001	0.004	0.001	0.021	0.013	0.036	0.010	<0.001	0.008	0.002

B, regression coefficient. Linear regression test was used.

DISCUSSION

The Wnt signaling pathway is thought to be essential for maintaining bone homeostasis. Dickkopf-1 (DKK-1) is an important Wnt pathway regulator and inhibitor of osteoblast activity. Its serum concentrations have been linked to the evolution of bone degradation in rheumatoid arthritis [12].

This study included 45 PsA adult patients ranging in age from 20 to 50 years, as diagnosed by CASPAR criteria[10], and 45 healthy control subjects of matched age, gender, and BMI.

In the present work, serum DKK-1 levels had considerably increased in PsA patients as opposed to controls, which agrees with **Chung *et al.*** [3], who unearthed that serum DKK-1 levels had increased considerably in PsA patients in comparison to RA patients and controls. This supported the hypothesis that DKK-1 may contribute to the genesis of PsA. Then again, a research from New Zealand indicated that cases with PsA had significantly higher blood DKK-1 concentrations than controls [13].

Contrary to our findings, **Fassio *et al.*** [14] found that PsA cases had considerably decreased DKK-1 levels compared to controls. This could be explained by the fact that levels of DKK-1 vary between phenotypes.

In accordance with the current study, DKK-1 concentrations were higher in active PsA patients, also these levels gradually rose as the disease activity increased, with a significant P value. Increased DKK-1 levels may indicate higher disease activity, according to research by **Chung *et al.*** [3], who found that cases with higher DKK-1 levels, had more cases of swollen joints than normal DKK-1 levels.

In terms of structural radiological changes, our study demonstrated a highly statistically significant relation between serum DKK-1 values and the SPARS as an indicator of bone damage. This goes in parallel with **Chung *et al.*** [3], who noticed that PsA patients with raised DKK-1 experienced significantly more bone erosions than those with normal DKK-1.

Aydemir *et al.* [15] observed that the serum levels of sclerostin and DKK-1 were considerably higher in the RA group than in the control group. **Grandaunet *et al.*** [16] reached the same result after discovering that baseline DKK-1 plasma levels had a noticeable increase in patients with periarticular bone loss after one year compared to persons who did not develop periarticular bone loss. Low levels of DKK-1 were pertaining to high bone mineral density and rapid remodeling, indicating that DKK-1 and sclerostin antagonists might be utilized to treat osteoporosis in rheumatoid arthritis patients. In addition to rheumatoid arthritis, increased levels of sclerostin and DKK-1 in the blood strongly indicate joint inflammation in psoriatic arthritis, mixed connective tissue disease, and systemic lupus erythematosus. This is consistent with the release of DKK-1 and sclerostin by inflammatory cells, the high serum concentrations of DKK-1 and sclerostin in individuals with severe systemic inflammation, and the fact that inflammatory cells produce sclerostin and DKK-1.

A link between PsA and disease-related patient-reported outcomes (PROs) has not, to the best of the authors' knowledge, been previously shown. Using the PsAID as a surrogate for PROs, we found a statistically significant link between its values and DKK-1 concentrations. PsA patients with an unacceptable clinical status had significantly higher DKK-1 levels than those with an acceptable disease condition.

However, several restrictions apply to the current study. One of which is the comparatively small sample size. Additionally, the medications' relationship with DKK-1 could not be assessed due to the small sample size. Another limitation is the single opportunity participation of cases. Fourth is, complete longitudinal data such as patients' classification as having PsA with or without the radiographic axial disease was not applied.

CONCLUSION

DKK-l serum levels have the potential to be used as a prognostic biomarker for psoriatic arthritis disease activity, patient reported outcomes, and structural radiological joint erosions and damage.

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