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Circulating Dickkopf-1 Levels in Ankylosing Spondylitis: Correlation with Disease Activity

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ABSTRACT

Background: Ankylosing spondylitis (AS) is a chronic inflammatory disorder that is characterized by inflammatory backache, enthesitis, sacroiliitis, peripheral arthritis, and anterior uveitis. Currently, a significant number of studies have been performed to seek markers to monitor disease activity in AS, and no novel markers satisfy the characteristics of use in the clinical setting. **Objective:** To assess serum Dickkopf -1 (DKK-1) level among AS patients and control individuals and evaluate their possible correlation with disease activity.

Patients and Methods: This was a case-control study which comprised 40 AS cases and 40 gender matched healthy controls. ELISA was used in measuring DKK-1 concentration in the serum of studied subjects.

Results: Mean ASDAS was 2.4±0.7, ranged from 0.8-4.1, mean BASFI was 3±1.9, ranged from 0.8-6.8, mean BASDI was 3.6±1.3, ranged from 0.9-6.8. AS patients demonstrated significantly greater DKK-1 level in comparison with controls (mean =197.7 vs. 87.1, p<0.001). There were positive significant association between DKK-1 and AS disease activity measures. There were significant positive correlations between ESR, CRP, MS, ASDAS CRP, BASFI, BASDI and DKK-1 values.

Conclusion: serum DKK-1 concentration is elevated in cases with AS, and it is significantly accompanied by disease activity and functional impairment.

Key words: AS, disease activity, Dikkoph-1, ASDS.

INTRODUCTION

AS is the prototype of immune-mediated inflammatory rheumatic disease named as spondylarthritis which is characterized by new bone formation and is significantly associated with ankylosis and functional disability [(1)]. Although the underlying pathophysiology of new bone formation among AS cases is not fully clear, there are certain reports that displayed that wingless protein (Wnt) signaling might have a role. Demonstration that the suppression of dickkopf (DKK)1, a Wnt antagonist, by specific antibodies was associated with reduced osteoclasts' activation and erosions and new bone formation in expermental studies [(2)]. Many members of wingless protein family bind to a receptor complex on the plasma membrane of mesenchymal cells leading to activation of osteoblast differentiation via engaging the intracellular protein B-catenin [(3)]. A recent report found that DKK-1 value was significantly high in AS cases [(4)]. However, other studies found that DKK-1 values were lower in AS cases compared to normal control subjects [(5)]. Another study found that DKK-1serum levels were comparable in AS cases and controls [(6)]. The results were inconsistent among studies which evaluated Wnt pathway modulators in AS [(7)].The aim of the study was to assess serum Dickkopf -1 (DKK-1) level among AS patients and control individuals and evaluate their possible correlation with disease activity.

SUBJECTS AND METHODS

Subjects:

This was a case-control study that included 40 AS cases and 40 gender matched normal controls. The AS patients' mean age was 34.2 years. They were 28 (70%) men and 12 (30%) women. In addition to 40 controls of matched age, gender and BMI. The selected cases fulfilled the modified New York criteria for AS diagnosis [(8)]. AS cases had undergone routine treatment of nonsteroidal anti-inflammatory drugs, they had received one-gram paracetamol every 8 hours if needed . All patients 19 years old or above.

All patients were not on TNF therapy, any biological therapy, and Glucocorticoids for at least 2 months. The exclusion criteria, were other sero-negative SPA, diffuse idiopathic skeletal hyperostosis, overlap syndromes, diabetes mellitus, malignancy, infection, Other spinal diseases, uncontrolled thyroid disease and primary or secondary hyperparathyroidism. Patients those whowere complicated with serious primary cardiac, hepatic, renal and pulmonary diseases. Entire cases were subjected to detailed history taking, general and systemic examination, and spinal examination.

Disease activity evaluation

The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) [(9)], the Bath Ankylosing Spondylitis Functional Index (BASFI) [(10)], and The Ankylosing Spondylitis Disease Activity Score (ASDAS) [(11)] Methods.

DKK-1 serum levels

Three cm whole blood was obtained from both AS cases and controls then centrifuged and serum was frozen at -70°C till analysis. ELISA was used in measuring DKK-1 concentration in the serum. The

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source of the kit was Wuhan Fine Biotech Co., Ltd China Cat. No: Ck-bio-430206.

Ethical approval:

An informed written consents were obtained from patients prior to participation. An approval from Research Ethics Committee in Mansoura faculty of medicine was obtained. The Declaration of Helsinki for human beings, which is the international medical association's code of ethics, was followed during the conduct of this study.

Statistical Analysis

Data were analyzed b SPSS 22.0 for windows (SPSS Inc., Chicago, IL, US). Qualitative data were described as numbers and percent. Chi square test ($\chi 2$) and Fisher exact were utilized to compare between qualitative variables as indicated. The Independent t-test and Mann Whitney test were used for comparison between quantitative variables in 2 groups for parametric and non-parametric variables correspondingly. P-value ≤ 0.05 was set to indicate the significance of a result.

RESULTS

All AS patients were represented clinically by sacroiliac involvement, 29 patients (72.5%) of them were associated with axial involvement, while peripheral involvement was found in 12 patients. Enthesiitis was involved in 25%. Mean VAS was 5.6±2.1, ranged from 1 to 4, increase by rest decrease by exercise among all studied cases. Mean morning stiffness, was 46±19 (12-75).

Regarding spinal mobility, modified Shober test ranged from 15-18.5 cm with mean of 16.8±2.4 cm, Occiput to wall test ranged from 1-28 cm with mean of 9.1 ±4.9cm; tragus to wall test ranged from 8-35 cm with mean of 17.9±6.2cm, chest expansion test ranged from 1-4.8 cm with mean of 3.8±1.9. Regarding sacroiliac provocative tests: sacral compression test was positive in 85%, Patrick test was positive in 62.5% and Ganslen test was positive in 65%.

Regarding X-ray, 85% had sacroillitis, 47.5% had syndysmophyte, 30% had Squaring of vertebral body, 37.5% had Ossification of spinal ligaments, 22.5% had bamboo spine, while 15% had no abnormality in spines. Regarding MRI, all studied cases had sacroillitis and 50% had enhancement of spinal ligament.

Mean ASDAS was 2.4±0.7, ranged from 0.8-4.1, mean BASFI was 3±1.9, ranged from 0.8-6.8, mean BASDI was 3.6±1.3, ranged from 0.9-6.8. **Table 1.**

Table (1): Characteristics of AS patients.

				Patients (N=40)
Disease duration (years)			**mean±SD (range)**	9.1±6.4 (3-19)
Site	**Sacroiliac involvement**		**N (%)**	40(100%)
	Axial involvement		**N (%)**	29(72.5%)
	Peripheral involvement		**N (%)**	12(30%)
Morning stiffness (min)			**mean±SD (range)**	46±19 (12-75)
Spinal mobility	**Modified Shober test (cm)**		**mean±SD (range)**	16.8±2.4 (15-18.5)
	Occiput to wall (cm)		**mean±SD (range)**	9.1±4.9 (1-28)
	Tragus to wall (cm)		**mean±SD (range)**	17.9±6.2(8-35)
	Chest expansion (cm)		**mean±SD (range)**	3.8±1.9(1-4.8)
Sacroiliac provocative tests	**Sacral compression test**	**Negative**	**N (%)**	6(15%)
		Positive	**N (%)**	34(85%)
	Patrick test	**Negative**	**N (%)**	15(37.5%)
		Positive	**N (%)**	25(62.5%)
	Ganslen test	**Negative**	**N (%)**	14(35%)
		Positive	**N (%)**	26(65%)
X ray	**SIJ**	**Sacroillitis**	**N (%)**	34(85%)
	Spine	**No abnormality**	**N (%)**	6(15%)
		Syndysmophyte	**N (%)**	19(47.5%)
		Squaring of vertebral body	**N (%)**	12(30%)
		Ossification of spinal ligaments	**N (%)**	15(37.5%)
		bamboo spine	**N (%)**	19(47.5%)
M R I	**SIJ**	**Sacroillitis**	**N (%)**	40(100%)
Activity	**ASDAS**		**mean±SD (range)**	2.4±0.7 (0.8-4.1)
	BASFI		**mean±SD (range)**	3±1.9 (1.1-6.4)
	BASDI		**mean±SD (range)**	3.6±1.3 (0.9-6.8)

AS patients demonstrated significantly greater DKK-1 level in comparison with control persons, (mean±SD =197.7±87.3 versus 87.1±28.2, p<0.001) (**Figure 1).**

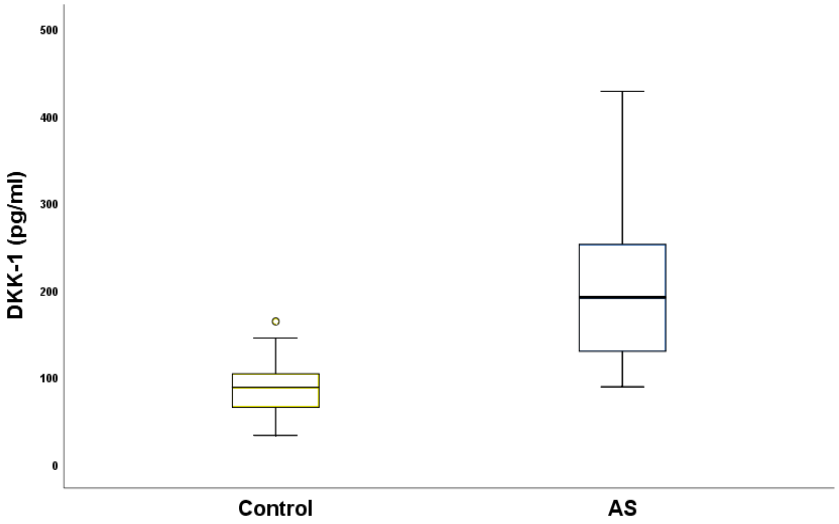
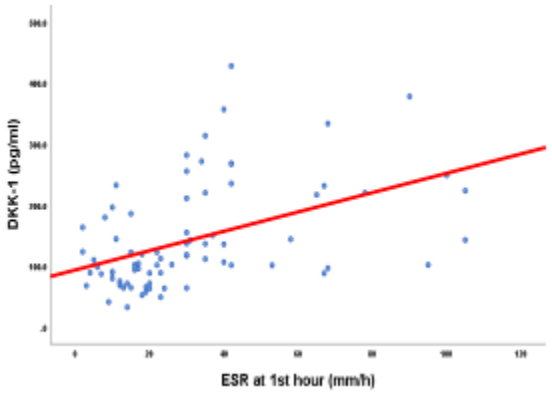
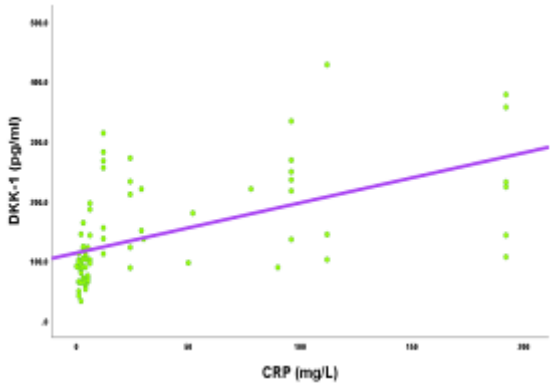


Figure (1): DKK-1 level among studied groups.

There were significant positive correlations between DKK-1 level with ESR, CRP, MS, BASMI, ASDAS CRP, BASFI, BASDI **(Figure 2).**



A



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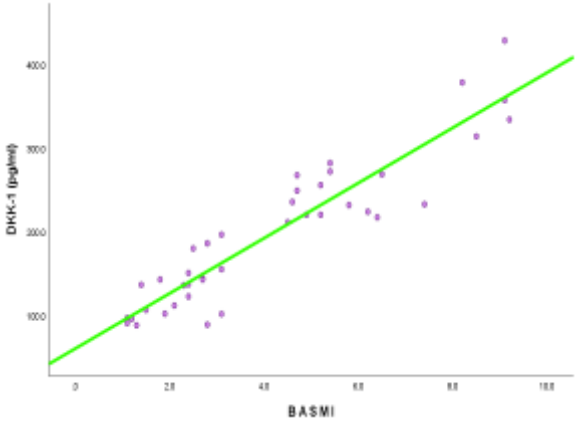

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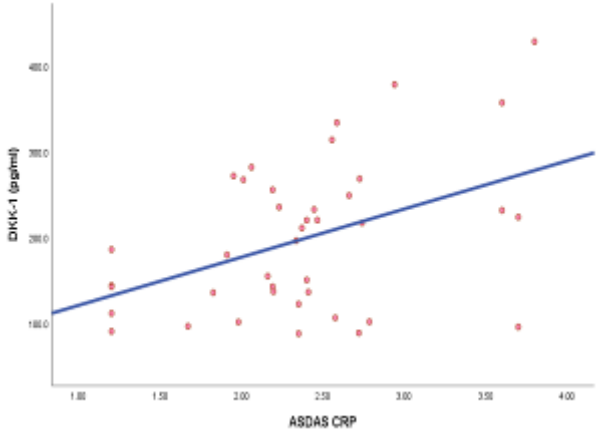

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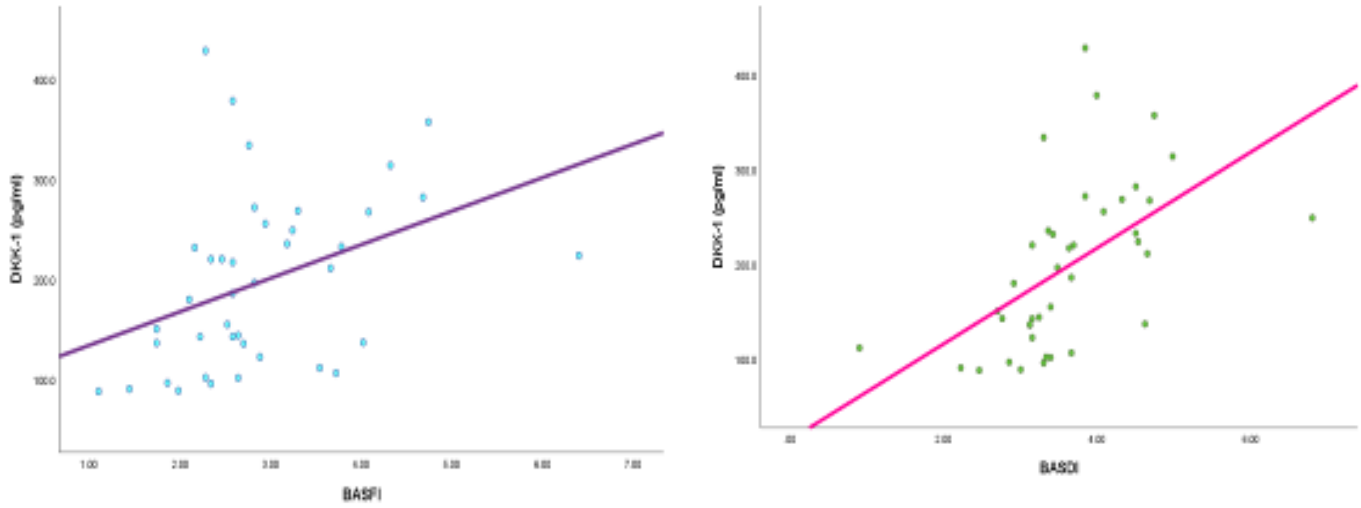

E F

Figure (2): Correlation between DKK-1 with (A) ESR at 1st hour (B) CRP, (C) MS, (D) ASDAS, (E) BASFI and (F) BASDI.

Moreover, there were positive significant association between DKK-1 and AS disease activity measures (p=0.024, 0.001 for ASDAS, BASDI respectively) **(Figure 3).**

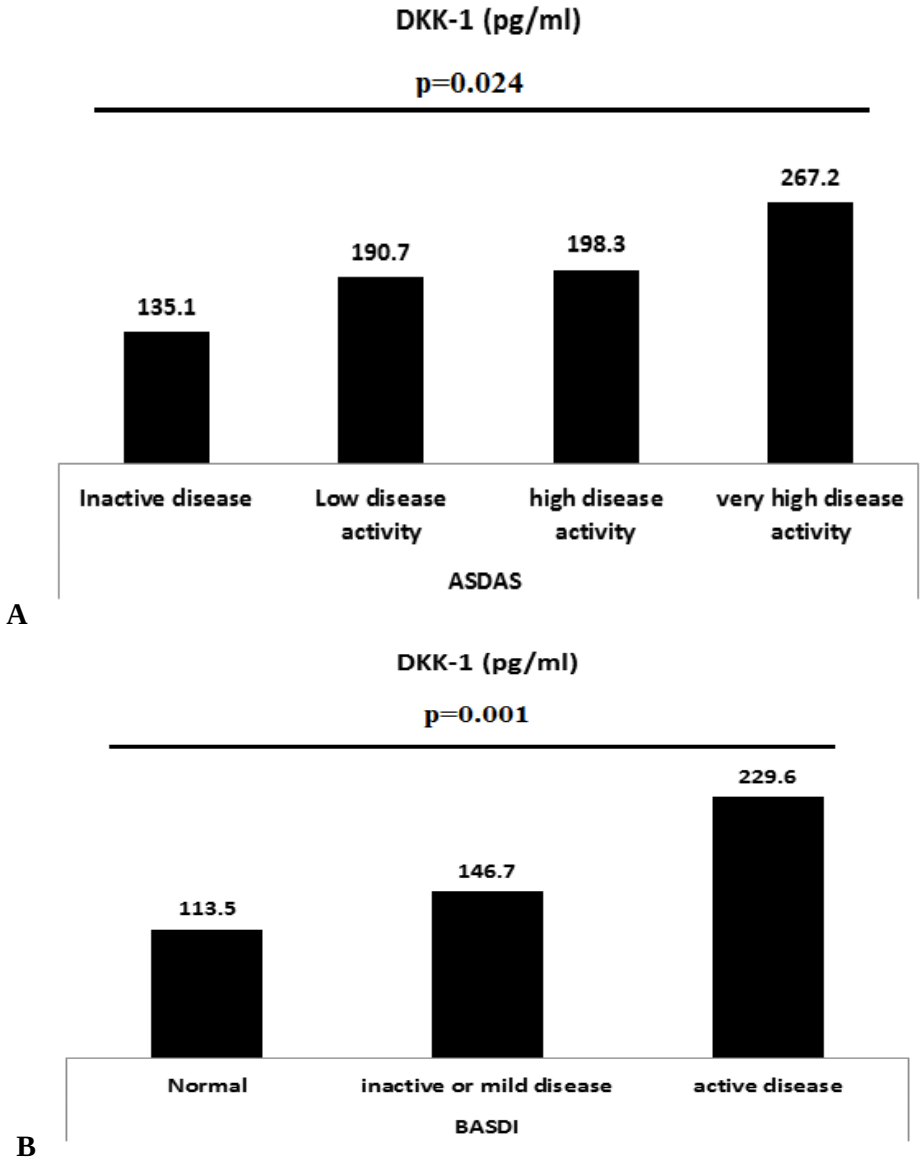

Figure (3): DKK-1 mean levels according to (A) ASDAS and (B) BASDI disease activity scores.

Table 2 demonstrates that; there were significant positive correlations between ESR1, CRP, MS, ASDAS CRP, BASFI, BASDI and DKK-1 levels.

Table (2): Correlations between DKK-1 and ESR, CRP, MS and disease activity measures.

	DKK-1	
	rs	***p***
ESR1	0.460	<0.001
CRP	0.320	0.039
MS	0.313	0.049
ASDAS	0.439	0.005
BASFI	0.389	0.013
BASDI	0.551	<0.001

Table (2) demonstrates that; mean ASDAS was 2.4±0.7, ranged from 0.8-4.1, mean BASFI was 3±1.9, ranged from 0.8-6.8, mean BASDI was 3.6±1.3, ranged from 0.9-6.8.

Table (2): Disease activity among AS patients.

		Patients
		N=40
ASDAS -CRP	**mean±SD**	2.4±0.7
	Range	0.8-4.1
BASFI	**mean±SD**	3±1.9
	Range	1.1-6.4
BASDI	**mean±SD**	3.6±1.3
	Range	0.9-6.8

DISCUSSION

AS is a chronic, inflammatory disorder that affects the axial spine. AS can manifest with a variety of clinical signs and symptoms. Chronic back pain and marked spinal stiffness are the commonest characteristics of AS [(12)]. The Wnt/β-catenin pathway is important in the context of the regulation of osteoblasts' proliferation, maturation, differentiation, and function. Dkk-1 is an inhibitory factor of the Wnt/β-catenin pathway. Dkk-1 promotes β-catenin phosphorylation and degradation, thus reducing bone-forming osteoblasts and increasing bone-resorbing osteoclasts, leading to a bias towards bone erosions [(13)].

The current study found that AS cases had significantly greater DKK-1 concentrations in comparison with control subjects. According to Ahmed and colleagues, DKK-1 upregulated the macrophage colony-stimulating factor, which would promote the synthesis of osteoclast differentiation factor and increase osteoclasts' quantity and activity and thereby bone resorption [(14)]. Remarkably, Zhang et al. indicated that DKK-1 is potentially responsible for AS development through Wnt signaling pathway [(15)].

Dkk-1 concentrations in AS cases were significantly high and depended upon the disease stage, however not on the existence/nonexistence of osteoporosis. Therefore, Dkk-1 can be a helpful biomarker of the progression of AS which reflects a liability for its progression before the appearance of radiological alterations [(16)]. While 6 studies assessed the association between DKK-1 values and AS, meta-analysis outcomes concluded that DKK-1 concentrations were significantly greater among AS cases as compared with control subjects signifying that increased DKK-1 concentrations might be a risk factor for AS. DKK-1 concentrations might also serve as a potential marker for AS diagnosis [(4,5, 17,18,19, 20)].

On the other hand, Taylan and co-workers found no difference in serum DKK1 concentrations between AS cases and control subjects, [(21)], whereas Rossini and co-workers revealed that serum DKK1 concentrations were significantly lower among AS cases as compared with control subjects [(22)]. Our study revealed that higher DKK-1 concentration was significantly associated with higher ASDAS and BASDI grades, as well as DKK-1 showed significant positive correlations with ESR1, ESR2, CRP, MS, BASMI, ASDAS CRP, BASFI and BASDI. In agreement with a study carried out by **Labeeb *et al.*** [(23)] who confirmed these results. This comes in accordance with **Elshishtawy *et al.*** [(19)] **Klingberg *et al.*** [(24)]**; Nocturne *et al.*** [(25)]**; Rossini *et al.*** [(22)]**; Xiong *et al.*** [(26)]**.** However, other reports have proposed that DKK1 has a dual role in osteoblast differentiation. Certain reports showed that DKK1 reduced in extracellular matrix maturation but increased in the mineralization stage of osteoblasts differentiation; thus, it is included in mineralization-associated physiologic alterations [(27, 28)].

The discrepancy in results between studies might be due to a change between experimentation and reality, or between animals and human subjects. Also, the difference might reflect a dual role of DKK1 or dissimilar activities between functional and circulating DKK1. Regardless, further studies are required to close such gaps.

Limitations of the present study include a small sample size and as a result, we have no follow-up data to assess AS prognosis. Thus, major studies are needed to more precisely explore the diagnosis and prognosis of AS and the pathophysiologic role of DKK-1. Likewise, no more studies performed on the association between DKK-1 and AS disease activity, so more studies have to be performed to confirm our results.

CONCLUSION

Serum DKK-1 concentrations are increased in AS cases compared to control subjects as well as correlated positively with disease activity. Our results can ultimately participate in the development of new therapies that specially target bone development between cases with AS. Further investigations of the function of DKK-1 in AS cases are also important.

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Competing interests: Nil.

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