Serum Visfatin levels in Ankylosing Spondylitis: Correlation with Disease Activity

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ABSTRACT

Background: Ankylosing spondylitis (AS) is a form of spondyloarthropathy (SPA) that is featured by sacroiliac joints (SIJ), axial skeleton, entheses and peripheral joints inflammation. Non-radiographic axial spondyloarthropathy or nr-axSPA is a common early sign of AS before developing radiographic sacroiliitis. Visfatin is an adipokine produced in fat tissue mainly, as well as in skeletal muscle, brain, and hepatocytes. Visfatin has been demonstrated to contribute to bone homeostasis and it can induce pro-inflammatory responses in different cell types.

Objective: This study aimed to investigate serum visfatin level among AS, nr-axSPA patients and controls and to elucidate its possible relationship with disease activity.

Patients and methods: This was a case-control study, which comprised 26 AS cases, 26 nr-axSPA cases and 27 healthy volunteers age- and sex-matched with the patient groups. ELISA was used in measuring visfatin concentration in the serum of studied subjects. **Results:** Serum visfatin level was significantly higher in AS and nr-axSPA cases groups in comparison with control group ($p<0.001$). There was positive correlation between visfatin with Bath AS Disease Activity Index (BASDI) ($P= 0.032$) and ($P=0.001$), and Bath AS Functional Index (BASFI) ($p=0.004$) and ($p=0.001$) in AS and nr-axSPA groups respectively. There were significant positive correlations between modified Stoke AS Spinal Score (mSASSS) and visfatin values in AS group ($p<0.001$). Visfatin concentrations didn't correlate with age, BMI, disease duration, ESR, CRP and ASDAS-CRP.

Conclusion: Serum visfatin concentration was elevated in cases with AS and nr-axSPA, and it was accompanied by disease activity, functional impairment and radiographic damage.

Keywords: AS, nr-axSPA, Visfatin, BASDAI, mSASSS.

INTRODUCTION

AS has been considered as a chronic immune-mediated arthritis that causes inflammation in the entheses, peripheral joints, and axial skeleton [(1)]. In more severe situations, inflammation can develop into calcification and fibrosis, which cause the spine to fuse together and lose its flexibility, giving it a "bamboo" look and static posture [(2)]. Visfatin is a beneficial protein with nicotinamide phosphoribosyl transferase (NAMPT) enzyme activity and has the capacity to enhance the differentiation of B cell [(3)]. Adipokines are released mainly by fat cells, they considered as an important element of the complicated network of soluble mediators comprised in the pathogenesis of chronic immune related and inflammatory disorders as rheumatic diseases [(4)].

Adipose tissue, along with other tissues like the liver, bone marrow, and muscles, produces visfatin [(5)]. Visfatin promotes the formation of proinflammatory and anti-inflammatory cytokines in human monocytes, including intrelukin-1(IL-1), IL-1Ra, IL-6, IL-8, IL-10, and or TNF-α [(6)]. It was discovered to be involved in bone homeostasis via promotion of the osteoblastic proliferation of osteoblasts [(7)], and hindering osteoclastogenesis [(8)]. The modified Stoke AS Spinal Score (mSASSS) is one of several scoring techniques accessible for assessing radiographic spinal destruction in AS [(9)]. This work aimed to evaluate serum visfatin level among patients with Magnetic Resonance Imaging (MRI) and plain x-ray of sacroiliitis (AS group), patients with MRI but without plain x-ray evidence of sacroiliitis (nr-axSPA group) and control individuals and to evaluate their possible correlation with disease activity. Of note, the present study is the first to measure AS Disease Activity Score with CRP (ASDAS-CRP) and to illustrate its correlation to serum visfatin levels in AS and nr-axSpA cases.

PATIENTS AND METHODS

This was a case-control study comprised 79 subjects divided into three groups: AS group (26 patients), nr-axSPA group (26 patients) and control group (27 healthy volunteers age- and sex-matched with the patients). The AS cases met the modified New York criteria for AS [(10)], while nr-axSPA cases met the Assessment of Spondyloarthritis International Society (ASAS) criteria [(11)]. Entire cases were 18 years old or above.

Exclusion criteria: Patients suffering from diabetes mellitus, osteoarthritis, obesity, cardiac diseases, malignant tumours or other autoimmune rheumatic diseases.

The following data was gathered: detailed history taking, general and systemic examination, locomotor examination of axial and peripheral joints, laboratory disease activity determinants including ESR and CRP, disease activity measures such as the Bath AS Disease Activity Index (BASDAI) [(12)], the ASDAS [(13)] and Bath AS Functional Index (BASFI) [(14)], and imaging including MRI of SIJ as well as X-ray of SIJ, cervical and lumbar spines were obtained.

Measurement of visfatin serum levels: Blood samples were obtained from all participants then centrifuged and serum was frozen at -80 ℃ till analysis. ELISA (The kit was Sunred Biological Technology Co, Catalog No.

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201-12- 0026, Shanghai, China) was used in measuring visfatin concentration in the serum.

Ethical approval: A written informed consents were obtained from all the included subjects. The study design was approved by The Ethical Committee Faculty of Medicine, Mansoura University (Code: MS.22.01.1821). The Helsinki Declaration was followed throughout the study's conduct.

Statistical Analysis: The data were analysed by SPSS software, version 25 (SPSS Inc., Chicago, IL, US). Numbers and percentages were utilized to evaluate qualitative data. Quantitative data were defined by utilizing median for non-normal distribution of data and mean ± SD for normal distribution of data following assessing normality by utilizing the Kolmogorov-Smirnov test. Significance of the results was judged at the ≤ 0.05 level. Chi-Square (X^2), Fischer exact test, Monte Carlo test were utilized for comparison of qualitative data between groups. Kruskall Wallis and Mann Whitney U test were utilized for comparison between 2 studied groups and more than 2 studied groups, correspondingly for non-normal distribution of data. Student t test was utilized to compare 2 independent groups for normal distribution of data. One Way ANOVA test was utilized for comparison of at least 2 independent groups. The Spearman's correlation is utilized to examine whether two variables are correlated with one another or not. ROC curve was utilized to measure validity of continuous variables.

RESULTS

The 26 AS cases, 26 nr-axSPA cases and 27 healthy controls (HC) were age and sex-matched. The mean age was 37.26, 34 and 34.37 years in AS, nr-axSPA and control groups respectively. No significant differences existed between the 3 groups as regards age, sex and body mass index (BMI). AS cases had significantly longer disease duration than nr-axSpA patients (4 years versus 0.5 year in both cases groups respectively, P<0.001). On clinical assessment, 5 cases (19.2%) of the AS group and 6 cases (23.1%) of the nr-axSpA group had peripheral arthritis, while 8 cases (30.7%) of the nr-axSpA group and 6 cases (23.1%) of the AS group had enthesitis with no significant differences between both groups.

Moreover, 3 AS cases (11.5%) and 2 of the nr-axSpA cases (7.7%) had uveitis with no significant differences between both groups. There were significant limitation of chest expansion, modified Schober test and occiput to wall test in AS in comparison with nr-axSPA group (p<0.001). On the other hand, there were non-significant differences between the two cases groups with regard to morning stiffness (P=0.628), enthesitis (P=0.475), peripheral arthritis (P=0.734), sacral compression (P= 0.577) and Patrick test (P= 0.375). As regard disease activity and functional status of our patients, ASDAS ranged from 1.1-3.8 and from 1.2-4, BASDAI ranged from 0.5-6.3 and from 1.4-6.5 while BASFI ranged from 2.7-9 and 1.2-7 in AS and nr-axSpA groups correspondingly with no significant differences between both cases groups (Table 1).

Table (1): Sociodemographic and manifestations of the studied groups

Characteristic		AS group	nr-axSPA group	Control group	significance
Age/years		37.26±7.87	34.0±5.95	34.37±5.53	P=0.147
Sex N (%)	Male Female	19(73.1%) 7(26.9%)	14(53.8%) 12(46.2%)	17(63%) 10(37%)	P=0.355
BMI(Kg/m^2)		24.42±2.21	23.24±2.31	23.96±2.24	P=0.176
Disease duration (years)		4.0(0.17-15.0)	0.5(0.25-4.0)	-	P<0.001*
Morning stiffness (min)		22.5(0 -120)	15(0 -120)	-	P=0.628
Enthesitis		8(30.7%)	6(23.1%)	-	P=0.475
Peripheral arthritis		5(19.2%)	6(23.1%)	-	P=0.734
Uveitis		3(11.5%)	2(7.7%)		p=0.509
Spinal mobility	**Modified Shober test (cm)**	12.57±1.58	15.17±0.37	-	P<0.001*
	Occiput to wall: (n,%) ▪ Limited ▪ Normal	 17(65.3%) 9(34.6%)	 0 26(100%)	-	P<0.001*
	Sacral compression test (n,%) ▪ Positive ▪ Negative	 13(50%) 13(50%)	 15(57.7%) 11(42.3%)	-	P= 0.577
	Patrick test: Positive Negative	7(26.9%) 19(73.1%)	10(38.5%) 16(61.5%)	-	P= 0.375
	Chest expansion (cm)	2.0(1.5-3.0)	3.2(2.3-5)	-	P<0.001*
Activity	**ASDAS**	1.8(1.1-3.8)	2.09(1.2-4.5)	-	P=0.09
	BASFI	4.4(2.7-9)	3.7(1.2-7)	-	P=0.509
	BASDI	3.5(0.5-6.3)	4(1.4-6.5)	-	P=0.583
	High active (n,%) **Low active (n,%)**	15(57.7%) 11(42.3%)	14(53.8%) 12(46.2%)	-	P=0.780

BMI: body mass index, * statically significant, ASDAS: Ankylosing spondylitis disease activity score, BASDAI: Bath Ankylosing Spondylitis Disease Activity Index, BASFAI: Bath Ankylosing spondylitis Functional Index.

AS and nr-axSPA cases demonstrated significantly higher visfatin levels in comparison with control group, (median 16.95 ng/ml and12.18 ng/ml Vs 7.5ng/ml correspondingly, p<0.001) (as demonstrated in Figure 1).

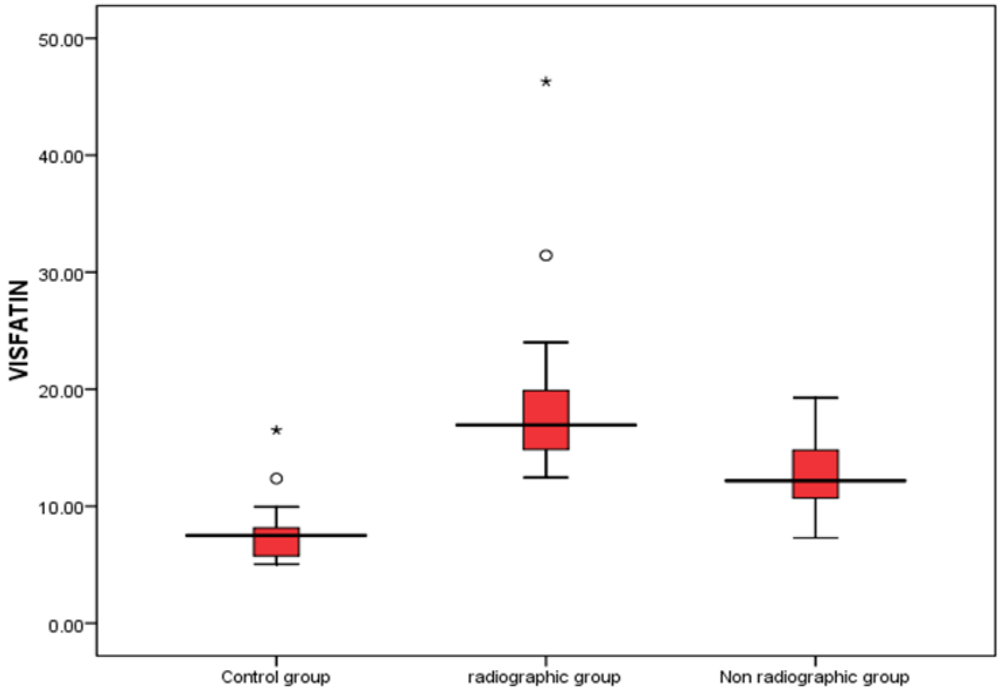

Figure (1): Visfatin levels among studied groups.

There were no statistically significant differences regarding activity markers including ESR and CRP between both cases groups (P=0.592 and P=0.359 for ESR and CRP respectively). Regarding X-ray in AS group: 100% had sacroiliitis, 23.1% had syndysmophytes, 26.9% had squaring of vertebral body, 30.8% had vertebral sclerosis and 26.9% had bamboo spine, while 26.9% had no abnormality in spines. As regard mSASSS, there were 7 cases (26.9%) with mSASSS= 0, and 19 cases (73.1%) with mSASSS ≥ 1. X-ray of SIJ and spine were free in nr-axSPA group. Regarding MRI, all studied cases had sacroiliitis (Table 2).

Table (2): Laboratory and radiological findings in AS and nr-axSPA patients

			AS group	nr-axSPA group	significance
Activity markers		**ESR1 (mm/1st hr)**	25(5-102)	21(10-100)	P=0.592
		CRP (mg/dl)	6(2-96)	12(1.13-92)	P=0.359
X ray (n, %)	**SIJ**	**Sacroillitis**	26 (100%)	0	-
	Spine	**No abnormality**	7(26.9%)	26 (100%)	
		Syndysmophyte	6(23.1%)	0	
		Sclerosis **Squaring**	8(30.8%) 7 (26.9%)	0	
		bamboo spine	7 (26.9%)	0	
		mSASSS=0 **mSASSS≥ 1**	7(26.9%) 19(73.1%)	26(100%)	
M R I (n, %)	**SIJ**	**Sacroillitis**	26(100%)	26(100%)	

SIJ: sacroiliac joint, mSASSS: modified Stoke Ankylosing Spondylitis Spinal Score.

Serum level of visfatin in AS and nr-axSPA groups positively correlates with BASDAI (P= .032 and P=.001) and BASFAI (p=.004 and p=.001) respectively. Also, in AS group, serum level of visfatin showed positive correlation with mSASSS (p<0.001). However, no significant correlations were demonstrated between serum visfatin values and age, BMI, disease duration, morning stiffness, chest expansion, modified Schober test, ESR and CRP in both cases groups (Table 3).

Table (3): Correlations between visfatin and age, BMI, disease duration, ESR, CRP,disease activity measures and mSASSS

	AS group		nr-axSPA group	
	Visfatin			
	r	***p***	***r***	***p***
Age	0.189	0.345	-0.310	0.124
BMI	-0.357	0.073	0.138	0.510
Disease duration	-0.101	0.624	-0.175	0.391
ESR1	0.090	0.663	-0.183	0.370
CRP	0.320	0.039	-0.003	0.990
ASDAS-CRP	0.040	0.847	-0.223	0.274
BASFI	0.543	0.004*	0.801	0.001*
BASDI	0.421	0.032*	0.780	0.001*
mSASSS	0.658	<0.001*	-	-

r:Spearman correlation coefficient.

The receiver operating curve (ROC) demonstrated that serum visfatin at a cutoff point of 12.85 ng/ml can significantly differentiate between AS cases and control group (AUC= 0.986; P<0.001) yielding sensitivity of 96.2% and specificity of 96.3% (Figure 2). The ROC curve revealed that serum visfatin at a cutoff point of 9.155 ng/ml can significantly differentiate between nr-axSPA patients and control group (AUC=0.925; P<0.001) yielding sensitivity of 92.3% and specificity of 88.9% (Figure 3).

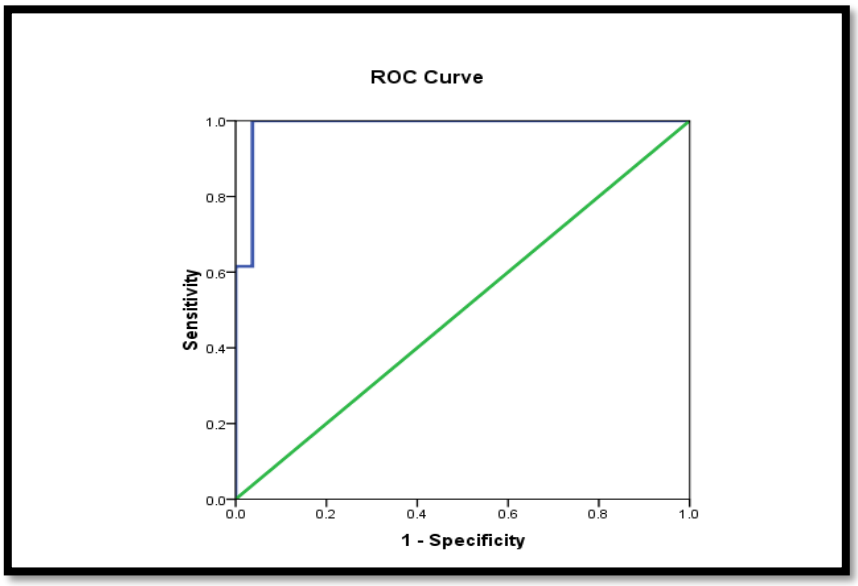

Figure (2): ROC curve of visfatin in differentiating between AS and control group

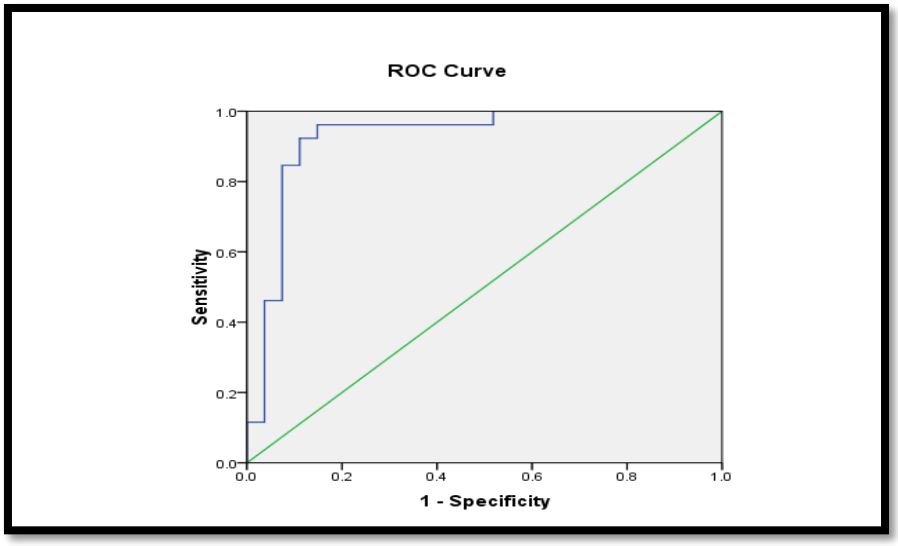

Figure (3): ROC curve of visfatin in differentiating between nr-axSPA and control group

DISCUSSION

Our study revealed that, there were increased visfatin levels among cases with AS and nr-axSpA when compared to control persons ($p < 0.001$). This partially agrees with few studies that demonstrated significantly greater serum visfatin levels in AS cases than in healthy control (HC) [15, 16, 17]. On the other hand, only one study had measured visfatin serum level in nr-axSpA patients and reported that there were non-significant higher visfatin levels in nr-axSpA patients than in HC [16].

In our study, both groups were age- & sex-matched, showing no statistical significant difference. The majority of the included cases were males in AS group, however no difference was found between both genders in nr-axSPA group. This comes in the same line with the outcomes of **Tournadre *et al.*** [18] who demonstrated that nr-axSpA has an equal sex distribution but AS or r-axSpA is more commonly diagnosed in males than in females (3:1). In contrast, **Li *et al.*** [19] revealed significant higher percentage of female patients in nr-axSpA patients than that in AS patients. This discrepancy can be ascribed to the variations in disease course between the both sexes as well as the persistent under-representation of female cases in clinical studies.

We reported that AS patients had longer disease duration than nr-axSpA patients (4 years versus 0.5 year). This agrees with some studies that found nr-axSpA patients have shorter mean disease duration than AS patients [19, 20].

Moreover, we showed that uveitis was present in only 3 of our AS cases (11.5%) and 2 of the nr-axSpA patients (7.7%) with no significant differences. This is consistent with the findings of **de Winter and his colleagues** [21] who found that uveitis is marginally more common in AS. However, previous researches showed that at least 30% of cases of SpA have been reported to have anterior uveitis [22, 23]**.** This discrepancy may be explained by the current treatment of AS involving early biological intervention or the smaller sample size in our study.

Our results revealed that serum level of visfatin didn't correlate with activity markers including ESR and CRP. This comes in the same line with **Syrbe *et al.*** [15] and **Miranda-Fiolly *et al.*** [24] who recorded that there was no statistically significant relationship between visfatin and both ESR and CRP.

Our results declared that there were no differences between the two cases group as regards disease activity scores (BASDAI and ASDAS-CRP) and functional scores (BASFI). This agrees with **Benchérifa and co-workers** [25] who found similar functional scores and disease activity between r-axSpA and nr-axSpA groups**.**

There was a positive association between visfatin value with BADAI and BASFI in both cases groups. These findings partially agree with **Baykara *et al*.** [17] who found positive relationship between visfatin and BADAI and BASFI in AS cases. Also, partially agree with **Hulejová *et al.*** [16] who showed that visfatin levels had positive correlation with BADAI in nr-axSpA cases not in AS cases, which can be explained by the different study design. In Contrast, **Miranda-Fiolly *et al.*** [24] reported that visfatin serum level didn't correlate with the BADAI and VAS spinal pain in non-diabetic AS cases receiving anti-TNF therapy. This discrepancy could be explained by slightly different disease activity (mean BASDAI) or smaller sample size in both studies.

Our results revealed no significant correlation between visfatin and ASDAS-CRP. Our study is the first to calculate ASDAS and illustrates its correlation to visfatin levels. In spite of that serum value of visfatin was correlated to BADAI and BASFI, it did not correlate with ASDAS-CRP. This could be clarified by the fact that BASDAI and BASFI scores include subjective parameters only without objective laboratory variables (CRP) that are involved in ASDAS.

We documented significant positive relationship between visfatin level and radiographic spinal injury evaluated by mSASSS. In the same line, **Hulejová *et al.*** [16] revealed a positive association between visfatin values and spinal radiological deterioration evaluated by the mSASSS among cases with axSpA and there were considerably greater visfatin concentrations among cases with spinal affection compared to cases with no spinal radiological alterations. In contrast, our results are in disagreement with **Hartl *et al.*** [26] who revealed no relationship between visfatin values and either radiological spinal deterioration or recent syndesmophyte development.

Despite the promising outcomes, small sample size has been considered the main limitation. Thus, additional long-term prospective researches are needed to properly assess the visfatin role in spinal radiographic deterioration in AS.

CONCLUSION

Serum visfatin concentrations were increased in AS and nr-axSPA cases compared to control subjects. Also, it correlated positively with disease activity and radiographic spinal damage assessed by mSASSS. Thus, it is valuable in evaluating disease activity and severity of AS and it can be a good indicator of radiological progression. Further studies of the function of visfatin in AS are also important to confirm our results.

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