Serum Visfatin as Predictor for Rheumatoid arthritis Severity: A Radiologically-controlled Comparative Study

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Objectives: To determine serum levels of visfatin in rheumatoid arthritis (RA) patients and evaluate its predictability for disease severity as judged by radiological severity scores.

Patients & Methods: The study included 70 patients; 41 patients had erosive lesions with a mean Larsen score of 32.1±7.3; range: 11-43 and 29 patients had mean Larson score of 7.3±1.3; range: 4-9. The study also included 20 cross-matched age and gender volunteers free of any form of joint affection as control group for laboratory data. All patients and controls gave blood samples for ELISA estimation of serum interleukin (IL)-6 and visfatin levels.

Results: Estimated serum levels of IL-6 and visfatin in studied patients were significantly higher, both as total and categorized according to Larson score, compared to control group. Moreover estimated serum levels of IL-6 and visfatin were significantly higher in patients had radiological evidence of presence erosion compared to those free of erosion. There was positive significant correlation between presence of radiological evidence of bone erosion and patients' age, clinical data and disease severity scores and serum levels of IL-6 and visfatin and ROC curve analysis showed that all of them could predict presence of bone erosion specifically. Regression analysis for verification of these parameters showed that serum visfatin was significant predictor in 3 models, IL-6 in 2 models and DAS-28 score in only one model, thus indicating that visfatin could be used as specific significant predictor for RA severity.

Conclusion: Serum visfatin level was elevated in RA patients and significantly correlated with severity scores. Regression analysis defined elevated serum visfatin as a specific predictor for erosion severity. However, wider scale studies are mandatory for evaluation of its prognostic value as a measure for remission and exacerbations of RA activity.

Keywords: Visfatin, interleukin-6, Radiological severity, Rheumatoid arthritis.

Introduction

Adipose tissue is not just related to body weight and appetite regulation. It is also implicated in obesity, a low-grade inflammatory state, as well as inflammatory conditions including rheumatoid arthritis which is an autoimmune disease where anti- and pro-inflammatory cytokine balance is critical. All major adipose derived products, simply termed adipokines, like leptin, adiponectin, visfatin and resistin, reportedly participate in inflammation and immunity, ***(Derdemezis et al., 2011, Krzystek 2011)***.

Visfatin; Pre-B cell colony-enhancing factor (PBEF), is a highly conserved, 52-kDa protein found in living species from bacteria to humans. It is a one of adipokine produced and secreted primarily by visceral white adipose tissue. Visfatin is also produced by endotoxin-stimulated neutrophils and inhibits neutrophil apoptosis through a mechanism mediated by caspase 3 and caspase 8, ***(Matsui et al., 2009)***.

Expression of PBEF is up-regulated in a variety of acute and chronic inflammatory diseases including sepsis, acute lung injury, rheumatoid arthritis (RA), inflammatory bowel disease, and myocardial infarction and plays a key role in the persistence of inflammation through its capacity to inhibit neutrophil apoptosis, ***(Popa et al., 2005, Neumann et al., 2007)***.

Visfatin exert three distinct activities of central importance to cellular energetics and innate immunity. Within the cell, PBEF functions as a nicotinamide phosphoribosyl transferase, the rate-limiting step in a salvage pathway of nicotinamide adenine dinucleotide (NAD) biosynthesis, so through regulation of cellular levels of NAD, visfatin impacts not only cellular energetics but also NAD-dependent enzymes. Although it lacks a signal peptide, PBEF is released by a variety

of cells, and elevated levels can be found in the systemic circulation of patients with a variety of inflammatory diseases. As an extracellular cytokine, PBEF can induce the cellular expression of inflammatory cytokines such as TNF-α, IL-1β, and IL-6. Finally, PBEF has been shown to be an adipokine expressed by fat cells that exerts a number of insulin mimetic and antagonistic effects ***(Sethi et al., 2005, Busso et al., 2008, Luk et al., 2008)***.

The current prospective comparative study aimed to determine serum levels of visfatin in patients with RA of varying duration of disease and to correlate it with serum IL-6 levels and clinical and radiological severity scores.

Patients & Methods

The present study was conducted at Rheumatology and Rehabilitation Department in conjunction with Clinical pathology Department, Benha University in conjunction with Medical Biochemistry departments at Benha and October 6 Universities since March 2010 till Sep 2011. After approval of the study by the Local Ethical Committee and obtaining patients' fully informed written consent, all patients had rheumatoid arthritis attending the outpatient clinic for first time or for follow-up were enrolled in the study so as to collect 70 patients with varied duration of disease. Only patients who fulfilled either four of seven ACR criteria or having morning stiffness ≥60 minutes, symmetrical arthritis and small joint arthritis (metacarpo-/metatarso-phalangeal joints/wrists) for at least 6 months were included in the study. Acute phase reactions were measured by erythrocyte sedimentation rate (ESR; mm/h) and C-reactive protein (mg/l) using standard laboratory methods and performed at hospital laboratory.

The study also included 20 cross-matched age and gender volunteers free of any form of joint affection chosen from those attending hospital blood bank for blood donation after passing the preliminary laboratory investigations required for blood donation according to hospital protocol to serve as control group.

Patients' data including age, gender, weight, height and calculation of body mass index (BMI) according to the equation: BMI= weight (kg)/height (m^2), ***(Garrow, 1990)*** were determined and duration of disease were determined.

Clinical evaluation included determination of disease activity using a 28 joint score (DAS-28), ***(Prevoo et al., 1995)*** and pain scores using a 100 mm horizontal visual analogue scale (VAS), with 0 indicates no pain and 100 indicates the worst intolerable pain, ***(Scott & Huskisson, 1976)***. Functional disability was evaluated using the Swedish version of the Stanford health assessment questionnaire (HAQ) to calculate the Disability Index (DI), ***(Ekdahl et al., 1988)***. Radiological evaluation using postero-anterior radiographs of hands, wrists, and forefeet was performed and verifired by comparison with standard reference films according to the Larsen–Dale index, ***(Larsen et al., 1977)***.

Whole blood sample (10 ml) was obtained from patients and controls and was collected in plain tube and allowed to clot and centrifuged at 5000 rpm for 10 minutes and serum was separated for quantitative ELISA estimation of serum IL-6 (ELISA kit from Pelikine™ Inc., Concord, USA), ***(Gaines-Das & Poole, 1993)*** and serum visfatin (Visfatin C-terminal ELISA kit, Phoenix Pharmaceuticals, Inc, Burlingame, CA, USA), ***(Fukuhara et al., 2005)***.

Statistical analysis

Obtained data were presented as mean±SD, ranges, numbers and ratios. Results were analyzed using Wilcoxon's Ranked test for unrelated data and Chi-

square test. Possible relationships were investigated using Pearson linear regression. Predictors for evaluation of radiological evidence of erosion were evaluated using the receiver operating characteristic (ROC) curve analysis judged by the area under the curve (AUC) and Regression analysis (Stepwise Method). Statistical analysis was conducted using the SPSS (Version 15, 2006) for Windows statistical package. P value <0.05 was considered statistically significant.

Results

The study included 70 patients; 53 females (75.7%) and 17 males (24.3%) with mean age of 54±5.6; range: 43-60 years. All patients had fulfilled the criteria of ACR with a mean duration of disease of 4.5±1.7; range: 2-8 years. Mean DAS-28 score for disease activity was 4.1±1.3; range: 1.4-6.9, mean VAS joint pain score was 38.8±14.7; range: 15-71 and mean DI was 12.7±5.6. Mean ESR level was 354±11.5; range: 11-55 mm/h and mean CRP level was 18.7±8.4; range: 6-72 mg/l.

Erosive lesions were identified in 41 patients (58.6%) with a mean Larsen score of 32.1±7.3; range: 11-43, while the remaining 29 patients (38.6%) with a mean Larson score of 7.3±1.3; range: 4-9 with a mean total Larsen score of 23.1±14.3; range: 4-45.

Estimated serum levels of IL-6 and visfatin in studied patients were significantly higher, both as total and categorized according to radiological evidence of presence of erosion, compared to control group. Moreover estimated serum levels of IL-6 and visfatin were significantly higher in patients had radiological evidence of presence erosion compared to those free of erosion, (Table 1).

There was positive significant correlation between presence of radiological evidence of bone erosion and patients' age, clinical data and disease severity scores and serum levels of IL-6 and visfatin, (Table 2). ROC curve analysis of correlated factors versus presence of radiological evidence of bone erosion showed that all of them could predict it specifically, (Table 3, Fig. 1).

Using Regression analysis for verification of clinical and laboratory findings as specific predictors for presence of joint erosion as a diagnostic finding for RA severity showed serum visfatin was significant predictor in 3 models, IL-6 in 2 models and DAS-28 score in only one model, (Table 4), thus indicating that visfatin could be used as specific significant predictor for RA severity.

Table (1): Serum levels of IL-6 and visfatin estimated in studied patients categorized according to radiological presence of erosion compared versus control group

	Control (n=20)	Patients		
		Non-erosive (n=27)	Erosive (n=43)	Total (n=70)
IL-6 (ng/ml)	4.81±3.1 (1.9-11.9)	17.66±8.7* (5.4-36.9)	25.28±9.19*† (6.1-47.9)	22.12±9.7* (5.4-47.9)
Visfatin (ng/ml)	3.39±0.84 (2.3-5.7)	6.75±1.21* (4.9-11.3)	11.23±3.55*† (5.8-18.9)	9.37±3.62* (4.9-19.1)

Data are presented as mean±SD; ranges are in parenthesis

*: significant difference versus control group †: significant difference versus non-erosive group

Table (2): Correlation coefficient between radiological presence of erosion versus age, clinical data and serum IL-6 and visfatin levels

	Age	Duration of disease	DAS-28 score	Pain VAS score	DI	Serum IL-6	Serum visfatin
"r"	0.302	0.384	0.562	0.498	0.444	0.435	0.606
P	=0.011	=0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table (3): ROC curve analysis of age, clinical data and serum IL-6 and visfatin levels as predictors for presence of radiological evidence of erosion as judged by area under curve

	Area under curve
Serum IL-6	0.770
Serum visfatin	0.876
Age	0.669
Duration of disease	0.705
DAS-28 score	0.810
Pain VAS score	0.790
ID	0.772

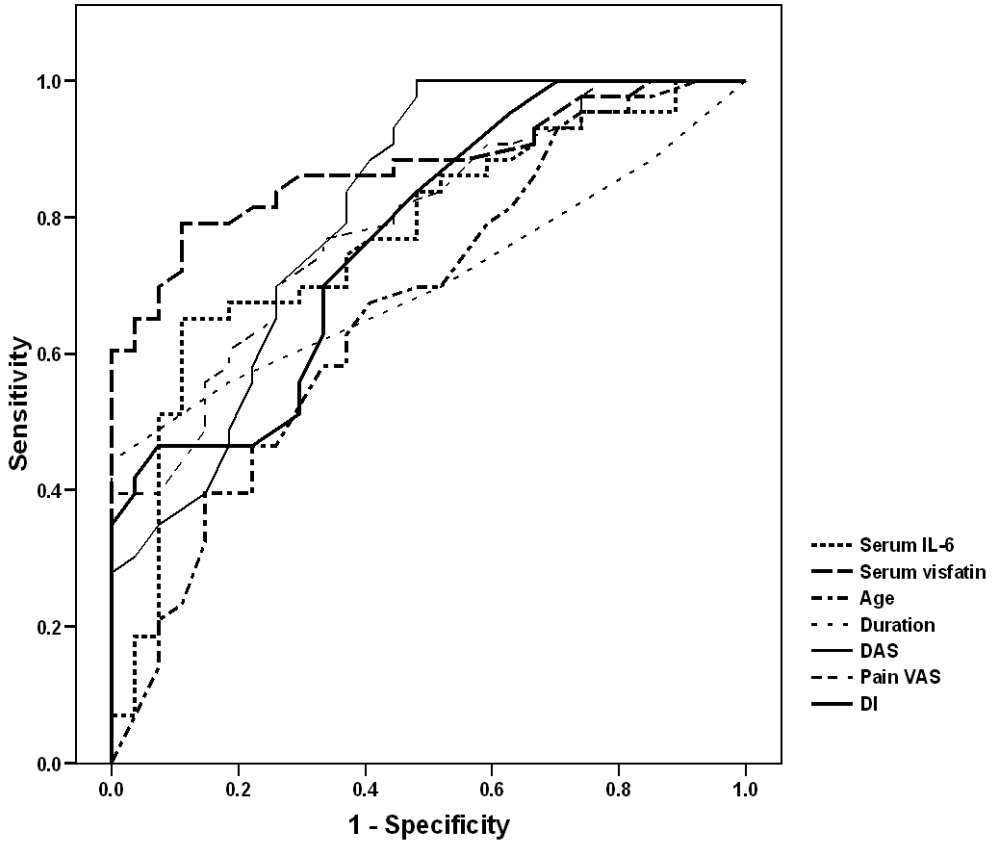

Fig. (1): ROC curve analysis of age, clinical data and laboratory findings as predictors of radiological presence of erosion

Table (4): Regression analysis models "Stepwise method" to identify the significant predictor for presence of radiological evidence of erosion

		ß	SE	t	p
Model 1	DAS-28 score	0.212	0.040	2.070	=0.042
	Serum IL-6	0.338	0.004	3.381	<0.001*
	Serum visfatin	0.470	0.013	4.803	<0.001*
Model 2	Serum IL-6	0.394	0.011	4.646	=0.002*
	Serum visfatin	0.578	0.004	6.825	<0.001*
Model 3	Serum visfatin	0.606	0.013	6.285	<0.001*

ß: standardized coefficient SE: Standard error *: significant parameter

Discussion

All enrolled patients fulfilled the ACR criteria with duration of manifestations of RA for more than 6 months and a mean DAS-28 score for disease activity of 3.9±0.8, mean VAS joint pain score was 60.2±5.2 and mean DI of 12.3±5.1. These data could be attributed to that previously reported by ***Gerber et al., (2003)*** and ***Scott et al., (2003)*** who found the active joint count predicts subsequent performance and function for patients with recent onset, inflammatory synovitis more effectively than whether patients met ACR criteria for RA. In hand on reliance on the predetermined scores, ***Klarenbeek et al., (2011)*** found clinical DAS and simplified DAI were the most stringent definitions of remission, DAS28 and DAS28-CRP had the highest proportions of remission and higher levels of disease activity were associated with decreased physical functioning and more radiological damage progression.

Serum levels of IL-6 and visfatin were significantly higher in patients compared to controls and in those had radiological evidence of erosion compared to patients free of erosion. Increased levels of both IL-6 and visfatin indicated pathogenic relation between both cytokines. In support of this assumption, there was a positive significant correlation between serum levels of both parameters on one side and the presence of radiological evidence of erosion on the other side.

The causal-result relationship between IL-6 and visfatin in RA is controversial as ***Nowell et al., (2006)*** experimentally found IL-6 trans-signaling regulated PBEF in a STAT-3-dependent manner, PBEF was regulated by the IL-6-related cytokine oncostatin M and that the involvement of PBEF in arthritis progression was confirmed in vivo, where induction of antigen-induced arthritis resulted in a 4-fold increase in the synovial expression of PBEF. On reverse, ***Brentano et al., (2007)*** found that in RA synovial fibroblasts, PBEF was up-regulated by Toll-like receptor ligands and PBEF itself activated the transcription factors NF-kB and activator protein 1 and induced IL-6, IL-8 and metalloproteinases 1 and 3 in RA synovial fibroblasts as well as IL-6 and TNF-α in monocytes.

The reported elevated serum visfatin levels go in hand with ***Otero et al., (2006)*** who found patients with RA showed considerably higher plasma levels of leptin, adiponectin and visfatin than healthy controls, but no marked difference was observed in resistin levels between patients and controls. ***Rho et al., (2009)*** who found visfatin concentrations were associated with higher Larsen scores, and this association remained significant after adjustment for age, race, sex, disease duration, BMI, and inflammation.

In hand with such correlation, ***Straburzyńska-Lupa et al., (2011)***, reported that the positive correlation between levels of visfatin and resistin and laboratory markers of inflammation may suggest that visfatin plays a role in inflammation in RA and such role was independent on presence of abdominal obesity. Also, ***Alkady et al., (2011)***, found serum and synovial adiponectin and visfatin were positively correlated with DAS28-ESR in RA patients with active disease. ***Senolt et al, (2011)*** found serum visfatin levels were significantly higher in patients with RA compared with healthy controls and significantly decreased following treatment with anti-B cell therapy.

Regression analysis of clinical and laboratory findings as predictors of the presence of radiological evidence of bone erosion defined serum levels visfatin as specific predictor that was persistently significant in all regression analysis models. In line with the specificity of visfatin for RA, ***Klein-Wieringa et al., (2011)*** found levels of IL-6, TNF-α, visfatin, and adiponectin were positively associated with radiographic

progression over 4 years and this association was independent of BMI and concluded that adipokines are predictors of radiographic progression in RA, possibly through distinct underlying biologic mechanisms. Also, ***Senolt et al., (2011)*** reported that lack of change in the serum visfatin levels between baseline and week 16 following treatment with rituximab predicted worsening disease activity between weeks 16 and 24.

It could be concluded that serum visfatin level was elevated in RA patients and significantly correlated with severity scores. Regression analysis defined elevated serum visfatin as a specific predictor for erosion severity. However, wider scale studies are mandatory for evaluation of its prognostic value as a measure for remission and exacerbations of RA activity.

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