Pre-B Cell Colony-Enhancing Factor as a Marker of Erosions in Rheumatoid Arthritis Patients

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Abstract: Objective: Our objective in this study was to evaluate pre-B cell enhancing factor (PBEF)/ Visfatin as a disease marker of rheumatoid arthritis, studying its serum level, clinical significance, association with activity and radiographic joint damage. Methods: 28 patients diagnosed according to the 2010 American College of Rheumatology criteria for RA were enrolled in the study. They underwent clinical and laboratory assessment. Radiographic assessment was done and Larsen score was calculated. Anti-CCP3 IgG antibodies were determined using semiquantitative ELISA and Serum PBEF was determined using ELISA and statistical analysis of the results was performed. Results: There was a significant rise in both anti-CCP and serum PBEF levels in RA patients versus controls. PBEF serum levels were significantly higher among patients with erosions as compared to patients without detectable erosions. Serum PBEF showed a significant decrease among patients with severe activity as compared to patients with moderate activity. Using the Ranked Sperman Correlation test, we did not find any significant correlation between age of patients, disease duration, BMI, HAQ, VAS or the DAS 28 with the level of PBEF. The test validity characters for discrimination of erosions in RA for serum PBEF showed 53.8 % specificity, 86.7% sensitivity and 71.4% efficacy. Conclusion: PBEF has a role in the pathogenesis of RA and could be considered as a disease marker in RA and a marker of radiographic bone damage. Further studies are needed to determine the possibility of PBEF as a potential therapeutic target in early RA to prevent erosions.
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1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by systemic inflammatory and destructive joint lesions that are manifested by the involvement of joints, various organs and systems into the pathological process [1].

Failure to diagnose or treat a patient with RA at the early stages of the disease increases the risk of progression to persistent joint inflammation and damage. On the other hand, aggressively treating patients with mild arthritis, which probably will not evolve to erosive forms is also damaging. It exposes such patients to risk without proven benefits and represents the opposite of effective early treatment. Therefore, early diagnosis of those patients who will progress to more severe forms and consequently will require therapy that is more aggressive is essential [2].

It has been suggested that age at disease onset and/or patients' age have influence on disease activity and clinical outcome [3]. Furthermore, female gender, DRB1*04 alleles and the presence of anti-CCP antibodies at baseline were reported to be the most important predictors of radiographic progression. However, the utility of these parameters in clinical practice is limited by their relatively low positive predictive value [4].

Some authors stated that positive antibodies against cyclic citrullinated peptides (anti-CCP) correlated with both the radiological joint damage score and inflammatory parameters in early and established RA, indicating that anti-CCP could serve as a diagnostic tool and predict structural joint damage and thus they proposed that anti-CCP positive patients should receive aggressive therapeutic intervention [5].

Pre-B cell colony-enhancing factor (PBEF), also known as Visfatin, is a 52-kDa protein found in living species from bacteria to humans. PBEF has shown both nuclear and cytoplasmic expression. Within the cell, it functions as a nicotinamide phosphoribosyl transferase, the rate-limiting step in a salvage pathway of nicotinamide adenine dinucleotide (NAD) biosynthesis. By virtue of this role, it can regulate cellular levels of NAD and so affect not only cellular energetics but also NAD-dependent enzymes such as sirtuins. It has been shown to be an adipokine expressed by fat cells and exerts a number of insulin mimetic effects [6].

PBEF was added to a growing list of adipocytokines with potent effects on immunity and inflammation in addition to their metabolic activities [7]. In CD14 (+) monocytes, PBEF induces the production of IL-1beta, TNF-alpha, and IL-6. Moreover, it increases the surface expression of co-stimulatory molecules CD54, CD40 and CD80. PBEF-stimulated monocytes show augmented FITC-dextran uptake and an enhanced capacity to induce alloproliferative responses in human lymphocytes [8]. PBEF is induced by inflammation and immune activation. It enhances B cell differentiation, initiation of cytokines and matrix metalloproteinases and inhibits neutrophil apoptosis thus playing a key role in persistence of inflammation [9].

Though 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 various inflammatory diseases. Its expression is up regulated in a variety of acute and chronic inflammatory diseases including rheumatoid arthritis, sepsis, acute lung injury, inflammatory bowel disease, and myocardial infarction [6]. A study on experimental animals proved that inhibition of PBEF markedly reduced inflammation, arthritis severity and cartilage damage in a collagen-induced arthritis model. They postulated that pharmacologic inhibition of PBEF led to reduced levels of intracellular NAD in inflammatory cells and decreased production of TNF-alpha and IL-6 by such cells with clinical effects comparable to those of a TNF-alpha inhibitor in a murine arthritis model [10]. Information about the connection between PBEF and disease activity in RA patients is conflicting and little is known about its role in radiographic joint damage.

The aim of this study was to evaluate pre-B cell enhancing factor (PBEF)/ Visfatin as a disease marker in RA by studying its serum level, clinical significance, association with activity and establishing it as a potential marker of radiographic joint damage in patients with rheumatoid arthritis. Consequently, we aimed at identifying PBEF as a potential therapeutic target in RA patients with erosive joint damage.

2. Patients and Methods:

Twenty-eight patients suffering from rheumatoid arthritis diagnosed according to the 2010 American College of Rheumatology criteria for RA [11], attending the Rheumatology and Rehabilitation outpatient clinic and the Rheumatology outpatient clinic of Internal Medicine Department of Ain Shams University Hospital were included in this study. Thirteen age-matched healthy individuals served as the control group. Patients gave informed consents to participate in the study. None of the patients and controls had diabetes mellitus.

Every patient included in this study was subjected to the following:

1. Full history taking with particular attention to disease duration, duration of morning stiffness, and activities of daily living.
2. Thorough clinical examination with particular attention paid to general examination and examination for extra-articular manifestations. As well as, complete musculoskeletal examination of all joints.
3. All patients underwent disease activity evaluation by the 28 joint Disease Activity Score (DAS-28). Patients with score $\leq$ 3.2 were classified as having inactive disease, scores of >3.2 - $\leq$ 5.1 were classified as having moderately active disease while > 5.1 denoted very active disease [12].
4. Pain was assessed by a 0-100 mm horizontal visual analogue scale (VAS). Zero indicated no pain while 100 indicated the worst intolerable pain.
5. Functional disability was evaluated using the Health Assessment Questionnaire (HAQ) to calculate the disability index (DI). The eight categories assessed by the DI are 1) dressing and grooming, 2) arising, 3) eating, 4) walking, 5) hygiene, 6) reach, 7) grip, and 8) common daily activities. The difficulty during each of these acts was assessed as follows: zero= without any difficulty, one=with some difficulty, two=with much difficulty and three=unable to do, then the sum of the categories scores is calculated and divided by the number of categories. This gives a score in the 0-3 range [13].
6. Laboratory investigations:

Sample collection:

- Two ml of venous blood were collected in EDTA-vacutainers for complete blood count.
- Two ml of blood were collected in plain vacutainers for analysis of CRP, Anti-CCP and PBEF.

Methods:

a. Complete blood picture (using Coulter counter).
b. Semi-quantitative measurement of C-reactive protein (CRP) by Latex agglutination assay (Teco Diagnostics, USA).
c. Erythrocyte sedimentation rate (ESR) by Westegren Blot technique.
d. Anti-CCP3 IgG antibodies (anti-Cyclic Citrullinated Peptide 3) were assessed using semiquantitative ELISA (INOVA DIAGNOSTICS, Inc. San Diego, USA). Validity of test results (Anti-CCP): High Positive control OD had to be > 1.0 while Negative control < 0.2 and low Positive

control OD had to be > 0.25 or more than twice the Negative control.

Calculation of Anti-CCP3 IgG: Sample value = sample OD/CCP3 IgG low positive OD x CCp3 IgG low positive control (units). Interpretation of results of anti –CCP: the sample is classified as negative if < 20 units and positive if ≥ 20 units.

e.Serum PBEF (Visfatin) assessment was determined using ELISA, based on the principle of Competitive Enzyme Immunoassay (RayBio,Inc. Norcross, GA). After incubation of the plate with anti-PBEF antibody, both biotinylated PBEF peptide and peptide standard or targeted peptide in samples interacted competitively with the PBEF antibody. Uncompeted (bound) biotinylated PBEF peptide then interacted with Streptavidin horse radish peroxidase (SA-HRP) which catalyzed a color development reaction that was measured at 450nm. The intensity of colorimetric signal was inversely proportional to the amount of PBEF peptide in the standard or samples.

7. Postero-anterior radiographs of hands, wrists, and forefeet were taken at inclusion in the study and on the day of sample collection. Joint destruction was classified by comparison with standard reference films according to the Larsen-Dale index [14]. The joints assessed for this index are the wrists, all metacarpo-phalangeal joints (=10), all proximal interphalangeal joints (=8), both first interphalangeal joints in the hands (=2), metatarso-phalangeal joints II-V (=8), and both first interphalangeal joints in the feet (=2). Thus, 32 joints are scored in all. The degree of erosive damage is the most decisive criterion in grading and the finding of at least one definite erosion in the radiographs was sufficient to consider the patient as having erosive disease. The Larsen score is the total sum of the grading in all 32 joints, with a range of 0-200. The x rays were read by two independent readers.

8. Statistical analysis:

IBM SPSS statistics (V. 19.0, IBM Corp., USA, 2010) was used for data analysis. Data were expressed as Mean ± SD for quantitative parametric measures.

The following tests were done:

1. Comparison between two independent mean groups for parametric data using Student t test.
2. Comparison between two independent groups for non-parametric data using Wilcoxon Rank Sum test.
3. Ranked Sperman correlation test to study the possible association between each two variables among each group for non-parameteric data. The probability of error at 0.05 was considered sig., while at 0.01 and 0.001 are highly sig. Diagnostic validity test including sensitivity, specificity, negative and positive predictive values were calculated.

3. Results

This study included 28 patients; 23 females (82.1%) and five males (17.9%). All patients fulfilled the ACR criteria for diagnosis of RA [11].

There was no significant difference between patients and controls as regards age, sex ratio or BMI. All patients were receiving methotrexate treatment in the range of 15-22 mg/ week as well as folic acid and NSAIDs upon need. Rheumatoid factor was positive in all 28 patients. The mean disability index as determined by the HAQ ranged from 0.25 to 2.88 with a mean of 1.15 ± 0.7.The outcome of clinical and laboratory evaluation of studied patients is shown in table 1.

Table (1): clinical and laboratory data of RA patients included in the study

Variable	Min.	Max.	Mean	SD
Age (years)	22	60	42.3	9.4
Disease duration (years)	0.17	24	6.6	5.57
Morning stiffness duration (minutes)	5	60	30.7	19.7
BMI (kg/m^2)	18	30	26.7	3.03
VAS pain score	10	90	46.4	21.6
Number of swollen joints	0	10	2.8	3.3
Number of tender joints	0	24	10.6	6.5
ESR 1st hr (mm/hr)	20	85	48.4	14.3
CRP(mg/dl)	3	96	49.4	38.9

*ESR: Erythrocyte sedimentation rate *CRP: C-reactive protein *SD: Standard deviation

Evaluation of disease activity using the DAS-28 score revealed a mean score of 5.35 ± 1.19. There were thirteen patients (46.4%) with moderately active disease with a mean DAS-28 score of 4.3 ± 0.5 and fifteen patients (53.6%) had very active disease with a mean DAS-28 score of 6.28 ± 0.7.

Radiological evaluation of patients revealed Larsen score ranging from 6 to 129 with a mean of 59.17 ± 32.8. Patients without erosions evident in their x-rays were 13 (46.4%) with a Larsen score ranging from 6 to 38, the mean being 34.5 ± 9.0. Patients with erosive disease were 15 (53.6%) with

Larsen score ranging from 40 to 129, the mean being 80.6 ± 30.7.

In the healthy controls, the anti-CCP ranged from 5.2-10.6 IU/ml with a mean of 8.12± 2.13.The mean level of anti-CCP antibodies in the serum of RA patients was 92.69 ± 112.9 IU/ml. The mean level of PBEF in the control group was 5.8 ± 8.8 pg/ml, while in rheumatoid patients it was 59.8 ± 109.2 pg/ml. There was a significant difference between both anti-CCP and serum PBEF levels in patients and controls (Table 2).

Table (2): Comparison between the RA patients and the control group as regard the serum anti-CCP and serum PBEF level.

		No.	Mean	SD	z	p	Sig.
Anti-CCP IU/ml	Controls	13	8.12	2.13	-0.9	<0.05	S
	Patients	28	92.68	112.9			
PBEF pg/ml	Controls	13	5.8	8.8	-3.188	< 0.001	HS
	Patients	28	59.8	109.2			

*CCP: Cyclic citrullinated peptides *PBEF: pre-B cell enhancing factor *sig.: Significance

Comparison between patients with erosions (15 patients), and patients with no evidence of erosions (13 patients) as regards age, BMI, ESR, DAS and anti-CCP levels revealed no significant difference. On the other hand, PBEF serum levels showed a significant difference between the two groups (Table3).

Table (3): Serum anti-CCP and PBEF levels in RA patients with and without erosive lesions.

	EROSION	n	Mean	SD	Z	p	Sig.
Anti_CCP IU/ml	Negative	13	89.3	116.1	0	>0.05	NS
	Positive	15	95.8	114.1			
PBEF pg/ml	Negative	13	20.8	23.9	-2.22	<0.05	S
	Positive	15	93.5	140.9			

*CCP: Cyclic citrullinated peptides *PBEF: pre-B cell enhancing factor *sig.: Significance

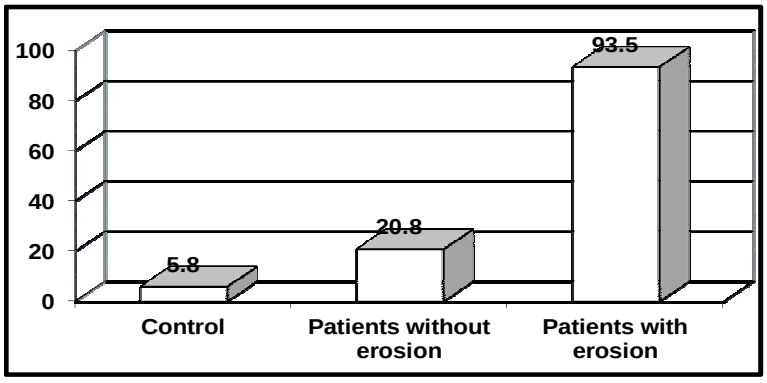
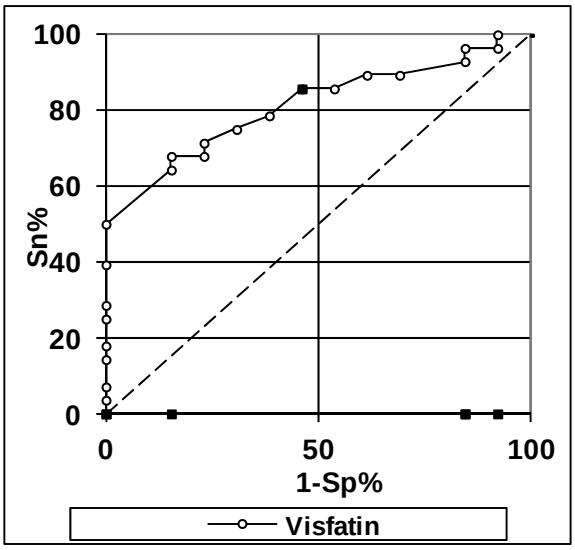


Figure (1): Comparison between control, RA patients without and with erosion as regards mean values of PBEF (pg/ml)

The HAQ score revealed a highly significant rise among patients with severe activity (p= 0.00). Serum PBEF showed a significant decrease (p=0.03) among patients with severe activity (22.7 ± 20.8 pg/ml) as compared to patients with moderate activity (102.5 ± 150.2 pg/ml).

Using the Ranked Sperman Correlation test, we did not find any significant correlation between age of patients, disease duration, BMI, HAQ, VAS or the DAS 28 with the level of PBEF.

The test validity characters for discrimination of RA patients from controls for serum PBEF at the best cut off value of 1.8 pg/ml showed 53.8 % specificity, 85.7% sensitivity and 75.6% efficacy (Fig. 2).

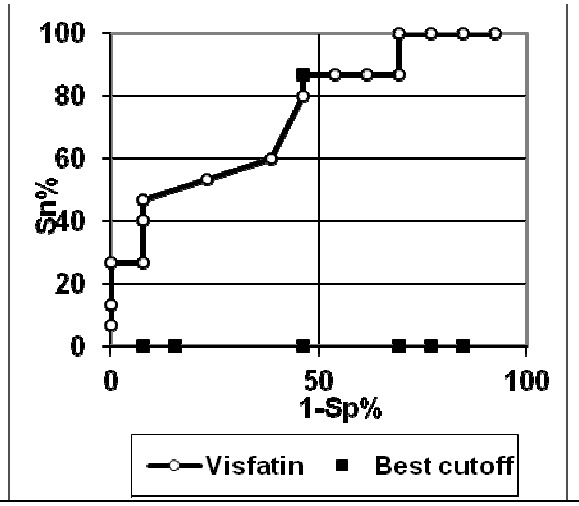


AUC	
PBEF	0.836

*AUC: Area under the curve

Figure (2): ROC curve analysis showing the diagnostic performance of PBEF in discriminating patients with RA from controls

The test validity characters for discrimination of erosions in RA for serum PBEF at the best cut off value of 8 pg/ml showed 53.8 % specificity, 86.7% sensitivity and 71.4% efficacy (Fig. 3).



AUC	
PBEF	0.675
PBEF	0.496

Figure (3): ROC curve analysis showing the diagnostic performance of PBEF in discriminating RA patients with erosion from those without erosions

4. Discussion

As current predictors of joint destruction in rheumatoid arthritis have low specificity, serological biomarkers reflecting bone and cartilage destruction have been proposed as tools in assessing prognosis of this disease [15].

Anti-citrullinated peptide antibodies (ACPAs) are established as useful predictors of radiographic

progression in rheumatoid arthritis (RA). Other markers as anti-mutated citrullinated vimentin (anti-MCV), increased urinary levels of C-terminal cross-linked telopeptide of type II collagen (U-CTX-II), an increased urinary total pyridinoline/total deoxypyridinoline (U-PYD/DPD) ratio were independently associated with a higher risk for progression of bone erosion in RA [15, 16].

Pre B-cell colony enhancing factor (PBEF)/ Visfatin was reported to be an adipocytokine having proinflammatory and immunomodulating properties**.** However, the pathological role and clinical relevance of PBEF in the setting of RA are still unclear [17].

In this study, serum PBEF levels in RA patients were significantly higher than controls (z= -3.19, $p< 0.001$). Similar results were reported by several authors [9,18, 19]. Moreover, further research added that this adipocytokine was up regulated in synovial fluid of RA patients and demonstrated synovial fibroblasts as the major PBEF-producing cells in the rheumatoid synovium [17, 20].

No significant correlations between PBEF concentrations and the age of patients at the time of the study or disease duration were detected. These results were consistent with what was previously reported by some authors [21]. In disagreement with our findings, a recent study reported that PBEF concentrations correlated significantly with disease duration, which in their case could be due to progression of the disease over the time with increased bone erosions [9].

Serum levels of PBEF did not correlate with body mass index (BMI) in our series of patients with active RA in contrast to the case in non-RA subjects with wide range of BMI. These findings were in agreement with other research on RA patients [9, 19, 21]. This denotes that PBEF is an adipocytokine whose production in RA patients does not solely relate to the BMI but also to the disease process as part of the systemic inflammation and bone destruction suggesting a role for PBEF in the pathogenesis of RA.

In this study, we found that serum levels of PBEF did not correlate with parameters of disease activity whether clinical or laboratory markers of inflammation such as CRP or ESR levels. These findings differ from another study that reported that PBEF was correlated with serum markers of inflammation as well as clinical disease activity scores [17]. On the other hand, our results were in agreement with other groups reporting no significant correlation between PBEF levels and ESR, CRP levels or DAS 28 [19, 21]. Interestingly, our results showed a significant decrease of serum levels of PBEF in the group of RA patients with severe activity (mean $\pm$ SD = 22.7 $\pm$ 20.8 pg/ml) in comparison to the group of patients with moderate activity (mean $\pm$ SD= 102.5 $\pm$ 150.2 pg/ml). Such findings are somehow unexpected. However, they may be explained by depletion of PBEF in patients' sera due to its accumulation in the active joints synovial fluid and its predominant expression at sites of invasion into cartilage [17]. It is also possible that in patients with RA in the presence of the high inflammatory burden, PBEF secretion might be negatively influenced by other adipokines or other factors as part of a compensatory mechanism that aims to maintain a normal homeostasis in these patients.

To establish if RA patients with radiographic damage had greater concentrations of PBEF than those without erosions, we divided patients into two groups; group I included patients with erosions while group II included those without erosions according to Larsen score. A significant increase in PBEF serum levels was noticed in patients with erosive disease in comparison to those without (mean $\pm$ SD = 93.5 $\pm$ 140.9 and 20.8 $\pm$ 23.9 pg/ml respectively). In addition, a sensitivity analysis revealed 86.7% sensitivity of PBEF for discrimination of erosions. Influenced by this high sensitivity, we can speculate that PBEF is associated with radiographic damage in RA patients. Such results were in accordance with the findings reported in 2009 that PBEF concentrations were associated with higher Larsen scores, and this association remained significant after adjustment for age, race, sex, disease duration, BMI, and inflammation [9]. These findings suggest a role for PBEF as a mediator of joint damage in RA. Such results are supported by experimental animal research that suggested that PBEF modulated inflammatory responses and radiographic joint damage in animal models [10]. The mechanism by which PBEF plays a destructive role in joints of RA patients is through activation of the transcription factors NF-kB and activator protein 1 and induction of IL-6, IL-8, MMP-1 and MMP-3 in RA synovial fibroblasts (RASFs) as well as IL-6 and TNF alpha in monocytes of these patients [17].

Conclusion:

Due to its evident role in the pathogenesis of RA, Pre-B cell colony-enhancing factor/ Visfatin could be considered a useful disease marker in RA and a marker of bone destruction and radiographic damage. Further studies are needed to determine the possibility of PBEF as a potential therapeutic target in early RA to prevent erosions.

Conflict of interest statement

The authors have no conflict of interest to declare.

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