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Acute phase reactant, Pentraxin 3, as a novel marker for the diagnosis of rheumatoid arthritis

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ABSTRACT

Introduction: Pentraxins are a group of highly conserved acute-phase reactant proteins and play crucial role as modulators of inflammatory processes. Pentraxin 3 (PTX3) is primarily produced and released by vascular cell wall, hence, we attempt to establish the role of PTX3 as a biomarker for Rheumatoid Arthritis (RA) compared to CRP.

Methods: Thirty patients having active RA as cases and 30 osteoarthritis (OA) patients as controls were recruited. Paired serum and synovial fluid samples were analysed for concentrations of both PTX3 and CRP by using high sensitivity ELISA kit and ROC curve was plotted.

Results: Concentrations of PTX3 and CRP were significantly higher in RA patient serum ($p < 0.0001$) as well as in synovial fluid ($p < 0.0001$) and correlated with disease severity. Upon correlation analysis, positive correlation was found between serum and synovial fluid concentrations of PTX3 and CRP. The diagnostic potential of PTX3 was observed in synovial fluid while combination of PTX3 and CRP showed better sensitivity in serum.

Conclusion: PTX3 found to be sensitive non-invasive indicator of clinical arthritic activity in RA patients when compared to traditional markers like CRP. Combination of PTX3 and CRP could serve as better differential diagnostic markers for RA after validation in larger patient cohort.

1. Introduction

Rheumatoid arthritis (RA) is a common systemic inflammatory disease characterized by the presence of destructive polyarthritis which affects the small joints of the hands and feet (though the disease process can virtually affect any synovial joint) [1]. The prevalence of RA ranges from 0.5% to 2% among the general population, mostly affecting women who are two times more susceptible than men. There is neither exact definition nor a pathognomonic test of RA. The diagnosis of RA, therefore, rests on a composite of clinical and laboratory observations [2,3].

The acute-phase response to tissue injury and inflammation is accompanied by the dramatic increase in hepatic synthesis of plasma proteins known as acute-phase reactants (APR). Therefore, characterization of APR responses in RA is essential to gain insights into the activity of this disease and to assess the degree of inflammation [4,5]. The important APR include serum C-reactive protein (CRP), amyloid A (SAA), haptoglobin (Hp), ferritin, and plasma fibrinogen. The main functions of APRs are to recognize a variety of pathogenic agents and then to either eliminate them or neutralize their harmful effects by utilizing the complement pathways and macrophages in the host [6]. Among the various APRs, CRP has been considered as the most useful biochemical marker for evaluating the disease activity of patients with RA [7]. C- reactive protein (CRP) binds to modified low-density lipoproteins, bacterial polysaccharides, apoptotic cells, and nuclear materials [8]. By virtue of these recognition functions, CRP participates in the resolution of cardiovascular, infectious, and autoimmune diseases [9].

The classical acute phase proteins include pentraxins which are a group of evolutionarily conserved ancient proteins. Depending on their structure, pentraxins are divided into short and long pentraxin families. Pentraxin 3 (PTX3) is the prototype of the long pentraxin group. PTX3 synthesis is stimulated by a variety of molecules involved in the inflammatory process. The inflammatory mediator is typically produced at inflammatory sites, however, it can also be released at the sites remote from the original inflammatory insult [10]. Due to the fact of PTX3 being primarily produced and released by vascular wall cells, it might be used as a sensitive and independent inflammatory marker. PTX3 plays an important role in innate immunity, inflammation, vascular integrity, fertility, pregnancy, and also in the central nervous

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system. In innate immunity, its normal function is to increase the immune response to selected pathogens while also exerting control over potential autoimmune reactions. PTX3 may play a role in inflammatory circuits of RA, and its relevance as a marker of disease activity deserves further study [11].

Studies have demonstrated the increased concentrations of PTX3 in patients of RA [12] in comparison to the non-specific traditional acute phase reactants like erythrocyte sedimentation rate (ESR) and CRP. No study has been reported yet to evaluate the concentrations of PTX3 in both serum and synovial fluid in Indian population of rheumatoid arthritis. Hence, in the present study, we hypothesized to measure the circulatory concentrations of PTX3 in serum and synovial fluid of patients with RA. The correlation analysis has been done of PTX3 concentrations with conventional parameters of disease activity i.e. CRP along with the correlation of PTX3 in serum and synovial fluid. The diagnostic potential of PTX3 alone or in combination with CRP was also determined for the better identification of rheumatoid arthritis.

2. Materials and methods

The study was conducted in the Department of Medicine & Orthopaedics OPD, IPD, Department of Medicine, Jawaharlal Nehru Medical College, A.M.U., Aligarh in collaboration with Department of Biochemistry, All India Institute of Medical Sciences (AIIMS), New Delhi. The permission of the Institutional Ethical Committee was obtained prior to the study. Informed consent was taken from the subjects participated in the study.

2.1. Sample size distribution

Thirty patients of Rheumatoid Arthritis (i.e., RA group) and 30 Osteoarthritis (OA) patients as controls (i.e., OA group) were recruited. All patients in RA group fulfilled the Revised ARA Criteria for the Classification of RA [7] and all patients in OA group fulfilled the clinical and radiological features of OA [8]. A detailed history of the present illness, past history, personal history and family history was elicited. Simple disease activity index (SDAI) was used to score the disease activity.

2.2. Blood and synovial fluid collection

Blood was collected from the study subjects by venipuncture with aseptic precautions and transferred immediately to a sterile plain vial and left as such to clot at room temperature. The serum was separated by centrifugation at 3000 rpm for 3–5 min and stored at −80 °C for PTX3 and CRP estimation. Blood was also collected in EDTA vial for other routine investigations. Synovial fluid was aspirated from the Knee joint under aseptic precautions and stored at (−80 °C) for PTX3 and CRP estimation and cytopathology [total cell count and differential cell count, by visual counting method].

2.3. ELISA for determination of PTX3 and CRP concentrations

High-sensitivity commercially available ELISA kits were used for determining the concentrations of PTX3 and CRP in serum and synovial fluid of the subjects. PTX3 and CRP ELISA kits were supplied by Hycult Biotech. This test is based on the double antibody sandwich immunoassay principle. A monoclonal antibody against the antigens (PTX3 and CRP) had been pre-coated onto the wells of the microtiter strips provided. Antigens present in the serum sample or synovial fluid or standard was incubated with the plates to allow the binding of antigens to the antibody. This is followed by the addition of a primary monoclonal anti- PTX3 and CRP antibody respectively conjugated to biotin in respective microtiter plates. Following incubation, washing was done to remove the unbound antigen. An avidin-HRP conjugated antibody specific for primary antibody was then added to the wells followed by washing to remove any unbound antibody enzyme reagent. Then, a TMB one-step substrate reagent reactive with HRP was added to the wells. The color development was terminated by adding acid and absorbance was measured at 450 nm. A reference curve was obtained by plotting the concentration of antigen of several dilutions of standard samples versus absorbance and concentrations of the antigens (PTX3 and CRP) in samples tested were calculated by its standard plot.

2.4. Statistical analysis

Patients were classified into groups according to various scores of disease activity. Analysis was performed using SPSS version 22.0. Data were presented as mean ± SD. Student's *t*-test was used for comparing continuous data between 2 groups. A $p < 0.05$ with 95% confidence interval was considered statistically significant.

2.4.1. Correlation analysis

Linear relationship was analysed using Pearson's correlation coefficient. Correlation analysis was done between serum and synovial fluid concentrations of PTX3 and CRP. In addition, concentrations of CRP and PTX3 in both serum and synovial fluid were correlated.

2.4.2. Receiver operating characteristic curve

Serum and synovial fluid concentrations of PTX3 and CRP were analysed for determining the receiver operating characteristic (ROC) curve to identify their utility as diagnostic markers along with the evaluation of optimal cut-points and their associated sensitivity and specificity.

3. Results

3.1. Study subjects

A total of 60 patients of RA and OA (n = 30 each) were recruited. The demographic details of study subjects were shown in Table 1. Out of 60 cases studied, 24 were males and 36 were females. In RA, there were 22 females and 8 males. The mean age of RA patients and OA

Table 1
Demographic details of rheumatoid arthritis and osteoarthritis patients. [ESR: Erythrocyte Sedimentation Rate].

Parameters		Total patients	Rheumatoid arthritis (RA)	Osteoarthritis (OA)
Total no. (n)		60	30	30
Male/female		24/36	8/22	16/14
Age (Mean ± S.D.) years		43.67 ± 14.67	41.8 ± 15.11	45.53 ± 14.23
ESR (mm/h; Mean ± S.D.)		29.72 ± 12.1	41.83 ± 20.9	17.6 ± 13.2
Rheumatic factor positivity (n)		–	20	–
RA patients according to Stage	Stage 1	–	5	–
	Stage 2	–	9	–
	Stage 3	–	9	–
	Stage 4	–	7	–