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CERULOPLASMIN AND ADENOSINE DEAMINASE AS SERUM BIOMARKERS IN RHEUMATOID ARTHRITIS

BIOCHEMISTRY

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ABSTRACT

Rheumatoid Arthritis is a chronic inflammatory disease. Several inflammatory biochemical markers have been associated with the disease. We evaluated the role of Ceruloplasmin and Adenosine deaminase as potential serum biomarkers in Rheumatoid Arthritis. Fifty consecutive cases of Rheumatoid Arthritis along with age and sex matched 50 controls were included in the study. Paired serum Ceruloplasmin and Adenosine deaminase were measured in all cases after diagnosis of Rheumatoid Arthritis was confirmed as well as eight weeks after the institution of Disease Modifying Antirheumatic Drug therapy. Results were analysed using Students't'test. Ceruloplasmin and Adenosine deaminase were found significantly raised compared to controls indicating their possible role as adjuncts in diagnosis of Rheumatoid Arthritis, however their role as potential follow up tool needs to be elucidated by further larger studies .

KEYWORDS

Inflammatory biomarkers, inflammatory arthritis

Introduction

Rheumatoid arthritis (RA) is the commonest cause of chronic inflammatory arthritis all over the world including India. The worldwide prevalence of rheumatoid arthritis is approximately 0.8%. Prevalence in Indian population is ranges from 0.28% to 0.7%. Women are three times more commonly affected than men [1].

RA is a chronic systemic disease of unknown etiology manifested primarily by inflammatory arthritis of the peripheral joints, usually in a symmetrical distribution. Systemic manifestations include hematological, pulmonary, neurological and cardiovascular abnormalities. Joint inflammation is characteristic of rheumatoid arthritis leading to progressive joint damage by destruction of articular cartilage and subchondral bones. Course of rheumatoid arthritis is highly variable and unpredictable. Spontaneous remission and exacerbations are characteristic with an increase in serum acute phase proteins. ESR and CRP are commonly elevated acute phase reactants and can be used as indices for diagnosis and following up disease activity. However they may not always reflect the activity of disease. Several inflammatory biomarkers such as cytokines interleukin (IL)-6 and tumour necrosis factor (TNF)-α have also been evaluated for their role in diagnosis and monitoring of the disease [2-3].

Ceruloplasmin (Cp) and Adenosine deaminase (ADA) are two serum biomarkers which have intrigued workers in RA. Cp caught the eye of researchers in this field with the finding of abnormally high serum copper concentration in patients with rheumatoid arthritis. It is likely that raised serum copper is secondary to the high concentration of Cp being acute phase reactant rather than a primary disturbance of copper metabolism [4].

Likewise presence of T-lymphocytes in synovium triggered research on ADA as a biomarker of inflammation. The exact cause of the elevated serum ADA in RA has not been established as yet, but the possibility exists that the enzymes released into the circulation from damaged cells are associated with cellular proliferation and increased turnover of cells. Some studies showed raised value in RA [5].

The current study was undertaken to evaluate the diagnostic and follow up role of serum Cp and ADA in RA

Materials & Methods

50 consecutive cases of 'RA factor' positive patients diagnosed as Rheumatoid Arthritis, based on 2010 Rheumatoid arthritis classification criteria at Rheumatology Department of tertiary care center, were included in the study after obtaining written informed consent, following institutional ethics of research [6].

The cases were in the age range of 21-45 yrs with 5 males and rest all females. 50 age and sex matched healthy individuals were also included in the study as control group. Blood was collected from all cases and controls after obtaining written informed consent, by venipunture from anticubtal vein, in labeled red top vacutainers. Paired samples were obtained from cases; first sample at diagnosis and second at 08 weeks after institution of Disease Modifying Antirheumatic Drug (DMARD) therapy [7].

Serum was separated by centrifugation and was stored in labeled sterile vials at -20ºC until analyzed. Hemolysis free sera were used, because ADA activity from erythrocyte could falsely increase the ADA results for serum. Both ADA and Cp are stable in serum for at least 24h at 25ºC, 7days at 4ºC and 3 month at -20ºC.During analysis, serum was allowed to come to room temperature, gently mixed and used for ADA and Cp estimations. Serum ADA was estimated by Galanti and Guisti method [8] and Cp was estimated by kinetic method based on Ferroxidase activity [9]. Statistical analysis was carried out applying Students 't' testing with point of significance set at $P<0.05$.

Observations

The serum ADA and Cp values in disease group at diagnosis as well as eight weeks after institution of therapy when compared with control ADA and Cp values respectively are summarized in Table 1.

Table 1 Mean serum ADA & Cp ± SD in cases and controls

Parameters	Controls	Cases (Rheumatoid Arthritis)	
		Pre-treatment	Post-treatment
Serum ADA(U/L)	18.84± 6.10	30.76±12.33	26.98±12.94
Serum Ceruloplasmin (IU/L)	644.90±86.20	1470.04±331.14	1451±233.13

P<0.001 for difference between controls and pre-treatment samples for both the parameters.
P>0.05 for difference between pre-treatment and post-treatment samples for both the parameters.

The distribution of both the biochemical parameters for paired samples of all cases compared with the healthy controls is plotted in Box and Whiskers plot [Figure 1 & 2] which reveals overall no significant difference in median values of pre-treatment and post-treatment samples.

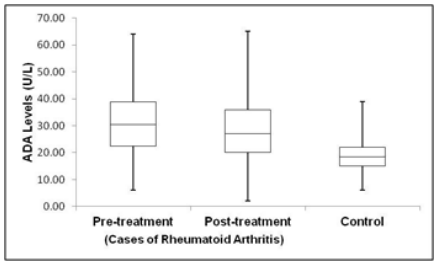

Figure1. Box & Whiskers plot of serum ADA levels of Rheumatoid Arthritis cases and controls

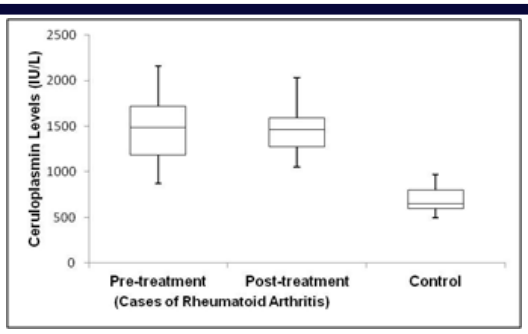

Figure 2. Box & Whiskers plot of serum Cp levels of Rheumatoid Arthritis cases and controls

Discussion

In this study initial values of serum ADA at diagnosis were one and a half to two times of mean control values. The rise was found to be statistically highly significant with p value of <0.001. There was a decrease in levels of serum ADA, 08 weeks after institution of therapy and one-third cases even showed normalization of serum values but overall fall in level was not found significant (p>0.05) *[Table1, Figure1]*. Demir et al. (2014) got higher in serum ADA levels in RA patients than in controls but were not related with any of the disease activity markers [10]. Salesi et al. (2012) found that ADA may be considered useful as a marker in diagnosis, prognosis, and monitoring of treatment with Methotrexate in RA[11].

Similarly, initial serum Cp values were found to be raised. The mean values were two times of control values (p<0.001). Serum Cp levels after institution of therapy however showed fluctuation. Although mean Cp values did show decrease after starting of therapy but were not found to be statistically significant (p>0.05) *[Table1, Figure2]*.Mohamed et al. (2017) found no difference between cases and controls for levels of Cp [12].

However, Strecker et al. (2013) did find statistically significant higher serum levels which positively correlated with ESR values but with no influence of treatment [13].

The fluctuation of serum values, eight weeks after the institution of treatment possibly indicates the nature of disease process which includes acute exacerbations and remmissions. Further study in this field with long duration of follow up is required to elucidate this finding.Serum ADA and Cp values in cases of rheumatoid arthritis after institution of DMARD have shown fluctuation of serum values after institution of therapy, possibly relating to the course of disease i.e. remission and exacerbation.

Conclusion

This study indicates that serum ADA and Cp has a potential role as inflammatory diagnostic markers in Rheumatoid Arthritis. However, their role in monitoring or follow up of therapy needs further substantiation by study involving longer duration of post treatment follow up on a larger sample size.

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