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EVALUATION OF SERUM CALPROTECTIN AS A PROGNOSTIC MARKER OF DISEASE SEVERITY IN CHILDREN WITH JUVENILE IDIOPATHIC ARTHRITIS AT A TERTIARY CARE HOSPITAL IN BIHAR

Paediatrics

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ABSTRACT

Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease in children and one of the more common chronic illnesses during childhood. Calprotectin (S100A8/S100A9 protein) is known as a damage-associated molecular pattern (DAMP) protein and reflects neutrophil activation during inflammation. This study aims to evaluate the role of serum calprotectin as a marker to monitor the disease activity in children from Bihar, India with JIA.

107 children with JIA as per International League of Associations For Rheumatology (ILAR) criteria were recruited into the study, out of which 56 had active disease and 51 had inactive disease. Mean calprotectin value in children with active disease was found 2-fold higher than those with inactive disease. Also, calprotectin levels were analysed in 10 normal healthy children and it was observed that mean calprotectin value in children with active disease was 16-times higher than those who were normal healthy controls. Evidently, serum calprotectin levels were found to be a good marker of disease activity in children with JIA.

KEYWORDS

Juvenile idiopathic arthritis, Calprotectin, Disease activity.

I. INTRODUCTION

Juvenile idiopathic arthritis (JIA) represents a heterogeneous group of disorder and is divided into seven subgroups according to the International League of Associations for Rheumatology (ILAR) classification: oligoarthritis, rheumatoid factor (RF)-positive and RF-negative polyarthritis, enthesitis-related arthritis (ERA), psoriatic arthritis, systemic arthritis and undifferentiated arthritis[1,2,7]. It is the most common chronic rheumatic disorder during childhood and can be associated with significant morbidity and mortality representing an important cause of short-term and long-term disability. Immune mechanism of JIA is still not completely understood. Disease probably results, as with most auto-immune diseases, from a combination of genetic susceptibility (Human Leukocyte Antigen HLA) and non-HLA-related genes (polymorphisms in the genes encoding protein tyrosine phosphatase nonreceptor 22 (PTPN22), tumour necrosis factor (TNF)-α, macrophage inhibitory factor, interleukin (IL)-6 and its receptor, and IL-1α), environmental factors and over-activation of innate and adaptive (T-cell or B-cell) immunity[2,3]. The characteristic feature of JIA is joint inflammation that leads to synovitis resulting in synovial tissue thickening and accumulation of synovial fluid. This is manifested clinically as joint swelling, morning stiffness, joint pain, tenderness, and functional disability[3]. Joint involvement can be mild and self-limiting, and it can also be more severe causing joint destruction, disability, and loss of joint function. Extra-articular manifestations of JIA may cause significant morbidity. These include uveitis, fever, skin rash, hepatomegaly, splenomegaly, lymphadenopathy, and serositis[6]. Apart from uveitis, most of these extra-articular features occur in systemic onset JIA.

Even though, clinical criteria for JIA is fairly well defined, appropriate laboratory parameters for the diagnosis and assessment of disease activity is still lacking. Hence, various biomarkers, which serve as markers of inflammation, are an area of active research. Various biomarkers are currently under research in children with JIA and one such biomarker is serum calprotectin. Serum calprotectin (sCal), also known as myeloid-related protein 8/14, is part of the S100 proteins family (with proinflammatory effects) [8,9] and is formed by a stable heterodimer of S100A8 and S100A9 subunits[19]. It has been described in many studies involving numerous inflammatory diseases (Crohn's disease, cystic fibrosis, sepsis, etc) including various rheumatological diseases (rheumatoid arthritis, systemic lupus erythematosus, etc)[8-12] and, more recently, in severe forms of COVID-19[13]. It is released during the interaction of leucocytes with endothelium at the sites of inflammation as occurs in JIA. In recent years, few studies have reported the usefulness of calprotectin as a marker of disease activity in children with JIA[9,14,18,24,25]. But, most of these studies incorporated only 1 or few subtypes of JIA. There are few studies from India that looked at calprotectin levels in children with JIA[30,31,35]. We undertake this study to assess the usefulness of calprotectin as a marker of disease activity in children from Bihar, India with JIA incorporating all the disease subtypes and also to compare calprotectin with ESR and CRP for assessment of disease activity.

II. METHODS

This was a cross-sectional study comprising of 107 children of age below 16 years who were recruited over a period of two years from April '19 to May '21 from the Paediatric Rheumatology Clinic of Darbhanga Medical College, Bihar and were diagnosed to have JIA as per the International League of Associations for Rheumatology (ILAR) criteria[7]. Children with inflammatory bowel disease, kawasaki disease, systemic lupus erythematosus and cystic fibrosis were excluded as they can also have high calprotectin levels.

Clinical and relevant demographic data were obtained from the participants and entered into the clinical proforma which contained details such as age, sex, disease duration, disease subtype, disease activity and laboratory parameters such as blood counts, *erythrocyte sedimentation rate* (ESR), C-reactive protein (CRP) and calprotectin levels. Blood samples were collected for CBC, ESR and serum calprotectin levels by venepuncture method after obtaining consent from parents of the participants. Samples for calprotectin analysis were stored in the Department of Clinical Microbiology in temperature of -20 degree Celsius and all calprotectin samples were analysed over a period of 2 days at the end of recruitment of all the study participants. The laboratory values of the participants were then obtained from the hospital patient information system. Patients were divided into active and inactive groups based on the disease activity at the end of the study. The laboratory personnel analysing the calprotectin level were blinded about the disease activity status of the patients.

Serum calprotectin levels were measured using a Human Calprotectin ELISA Kit (by Wuhan Fine Biological Technology Company limited). This kit is based on sandwich enzyme-linked immune-sorbent assay (ELISA) technology. Anti-calprotectin antibody was pre-coated onto 96-well plates. Biotin conjugated anti-calprotectin antibody was used as detection antibodies. The standards, test samples and biotin

conjugated detection antibody were added to the wells and washed with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalysed by HRP to produce a blue colour product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the calprotectin amount of sample captured in plates. The O.D. absorbance was read at 450nm in a microplate reader, and then the concentration of calprotectin was calculated. The coefficient of variation for duplicate measurements done by ELISA was < 6%.

Subsequently, statistical analyses were performed using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL, USA). Continuous variables such as ESR and CRP were described as mean with standard deviation or median and interquartile range (IQR), and categorical variables as frequencies with percentages. The diagnostic performance of calprotectin level was assessed by plotting receiver operating characteristic (ROC) curve for active and non-active cases. The correlations between serum calprotectin level and disease-related variables were evaluated using Spearman's correlation test. P value < 0.001 was considered as statistical significance. The study was approved by the ethics committee of Darbhanga Medical College & Hospital, Bihar.

III. RESULT

107 children were recruited during the study period. Baseline demographics and blood investigation records were analysed. At the end of the study, children with JIA were divided into two groups. Group I was JIA children with active disease (n = 56) and Group 2 was JIA children with inactive disease (n = 51) as assessed by Wallace criteria. Also, reported here are estimated calprotectin levels in 10 normal healthy children.

Table 1: Demographic characteristics of all children (n = 107)

Parameter	Number (n = 107)	(%)
Gender		
Male	61	57
Female	46	43
Mean age at diagnosis (years)	11.15 ± 3.75	
Diagnosis		
Oligoarticular JIA	7	6.6
Polyarticular RF pos	3	2.8
Polyarticular RF neg	33	30.8
Enthesitis related arthritis	16	15
SOJIA	48	44.8
Disease duration (years)	3.86 ± 2.64	
Disease status		
Active	56	52
Remission on drugs	50	47
Remission off drugs	1	1

Table 2: Baseline characteristics of active and inactive disease

Baseline characteristics	JIA (n = 107)	
Disease status	Active disease (n=56)	Inactive disease (n= 51)
Age (years)	11.30 ± 3.88	11.00 ± 3.60
JIA Subtype		
Oligoarticular	3	4
RF + Polyarticular JIA	1	2
RF – Polyarticular JIA	12	21
Systemic JIA	33	15
ERA	7	9
Hemoglobin gm/dl	10.02 ± 1.46	12.04 ± 1.77
Platelet (cu mm)	453819 ± 146032	324034 ± 114691
Total Count	19782 ± 29612	9191 ± 2836
ESR (mm/hour)	46.71 ± 17.14	14.24 ± 8.07
CRP (mg/dl)	100.65 ± 69.83	3.07 ± 0.26

Table 3: Mean and standard deviation of calprotectin in various groups

Calprotectin (ng/ml)	**Normal children (n = 10)**	**ActiveDisease (n= 56)**	**Remission (n = 51)**	**P value**
Mean (ng/ml)	233 ± 220	3954 ± 3220	1899 ± 1765	< 0.001
Range (ng/ml)	86 - 764	651 - 14310	160 – 7780	

Mean values of calprotectin in children with active and inactive disease were 3954 ng/ml and 1899 ng/ml, respectively. For children, who were normal healthy controls, a mean calprotectin value of 233 ng/ml was observed. Calprotectin values in all groups (active disease, inactive disease and healthy controls) were statistically significant (p = < 0.001).

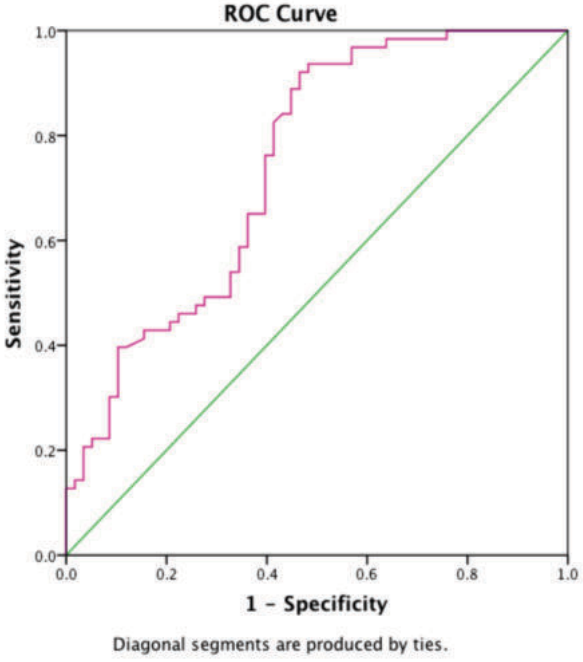

Fig 1: ROC curve analysis showing the diagnostic performance of calprotectin for discriminating patients with active JIA from patients with inactive disease

As shown in Figure 1, the area under the curve for calprotectin was 0.744. For a value of 1760 ng/ml, calprotectin had a sensitivity of 77 % and specificity of 61 %.

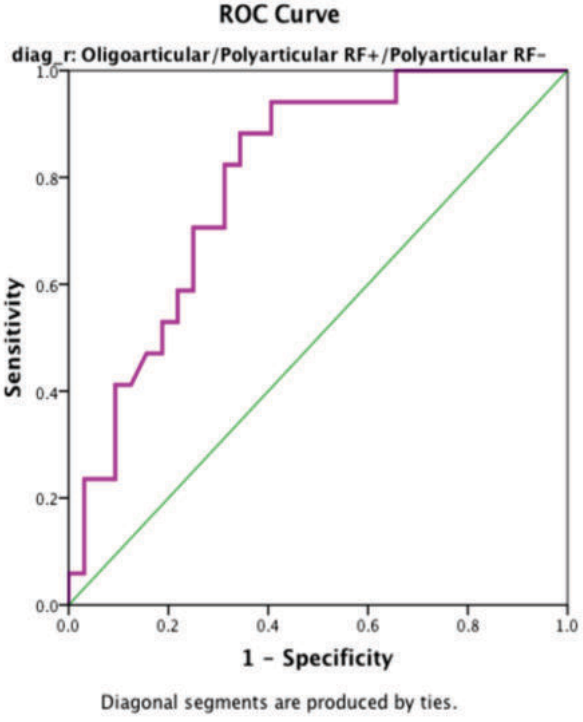

Fig 2: ROC curve of calprotectin in children with oligoarticular and polyarticular JIA

Area under the curve for calprotectin in children with oligoarticular and polyarticular JIA(RF +ve and RF –ve) was 0.797. In children with the above mentioned subtype, a calprotectin value of 1468 ng/ml had a sensitivity of 82 % and specificity of 69 %.

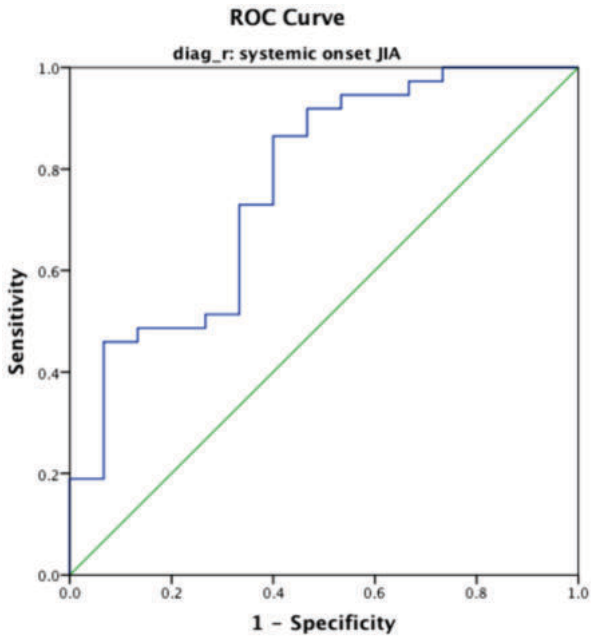

Fig 3: ROC curve of calprotectin in children with systemic onset JIA

Area under the curve for calprotectin in children with systemic onset JIA was 0.768. In children with the above mentioned subtype, a calprotectin value of 1111ng/ml had a sensitivity of 86 % and specificity of 60 %.

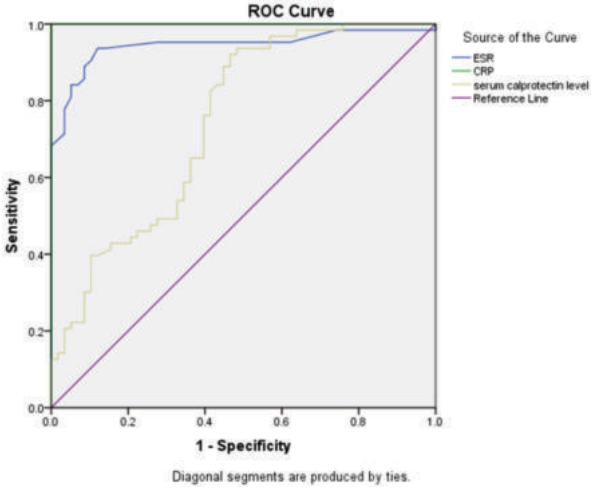

Fig 4: Comparison of ROC curve of ESR, CRP and calprotectin

The area under the curve for CRP, ESR and calprotectin were 1.0, 0.944 and 0.744 respectively. Hence, based on the ROC curve, it can be inferred that ESR and CRP are better markers of disease activity as compared to calprotectin.

IV. DISCUSSION

Evaluation of inflammatory activity is quit essential in the management of patients with JIA. Various biomarkers which can reliably predict disease activity are currently under research. Calprotectin is one such marker which is currently under extensive research. During inflammation, activated macrophages and neutrophils secrete calprotectin in significant quantities, which in diseases states such as Crohn's disease, ulcerative colitis, acute pancreatitis, cystic fibrosis, and bacterial pneumonia can be detected in the serum, urine, or feces[20-23]. Calprotectin secretion is also associated with RA, systemic lupus erythematosus, juvenile idiopathic arthritis, and gout[16,17,18,19]. Particularly in patients with RA, calprotectin levels increase in the serum and synovial fluid and show a close correlation with the severity of joint damage[23]. Calprotectin may be used as a reliable marker in accessment of inflammatory activity.

We studied a total of 107 children with JIA. In our study, there was a slight preponderance of males who constituted 57 % of the study population as compared to females who constituted 43 % of study population. This was in consistence with the results published by Porkodi et al and Anish et al. who studied clinical profile of JIA in children from South India and showed a male preponderance of 62 % and 56 %, respectively[30,35]. Singh et al. studied the clinic immunological profile of children with JIA in North India (Chandigarh) and found a male preponderance of 64 %[31]. The mean age at diagnosis in our study, taking into account all 107 children was 11.15 ± 3.75 years.

In our study, 48 (44.8 %) children had systemic onset JIA constituting the maximum number. Children with polyarticular, oligoarticular and enthesitis related arthritis constituted 33.6 %, 6.6 % and 15 % of our study population, respectively. Maximum number of children in our study had systemic onset JIA and this might have been because of the fact that, children with systemic onset JIA have more frequent follow ups and hospital visits as part of our treatment protocol compared to children with other subtypes because of their disease severity.

The mean value of calprotectin in children with active disease (all disease subtypes included) was 3954 ng/ml and that in inactive disease was 1899 ng/ml. This difference was statistically significant (p =< 0.001). The mean value of calprotectin levels in normal healthy controls was 233 ng/ml. Thus, calprotectin levels in patients with inactive disease were 8-times more than in normal healthy controls. Children with active disease had 2-fold increased levels of calprotectin as compared to children with inactive disease.

In our study, calprotectin was not useful as a good marker of disease activity in children with ERA. The median calprotectin level in active disease was 2466 ng/ml and median calprotectin level in inactive disease was 2710 ng/ml. There is no logical explanation as to why median levels of calprotectin were lower in the active disease group as compared to inactive disease group in our study.

Apart from analyzing the values of calprotectin with regard to disease activity, we also compared calprotectin with ESR and CRP which are the current biomarkers being used for assessment of disease activity. There was a weak correlation between ESR and calprotectin with r value of 0.295 and p value was 0.001. Similarly, weak correlation was noted between CRP and calprotectin with r value of 0.426 and p value 0.001. Thus, our study proves the usefulness of serum calprotectin levels as a good marker of disease activity in children with JIA except in the ERA subgroup. However, we could not prove that calprotectin was better than ESR and CRP as a disease activity biomarker as claimed by other studies from various parts of the world.

V. CONCLUSION

We conclude that calprotectin levels are increased in the plasma of JIA children and plasma calprotectin correlates with disease activity[23-27].

However, it was not found to be superior to ESR and CRP. But, it seems logical to suspect that calprotectin will be a better marker for follow up of disease activity in patients receiving biological therapy as these drugs act against cytokines such as TNF-α, Interleukin-1 and Interleukin-16 which will reduce the circulating cytokines and thus, levels of acute phase reactants such as ESR and CRP[28,29,34]. Thus, more studies are needed involving Indian population that include sequential sampling of the same study population in their various phases of disease activity and look at the effect of biological agents and other medications on the levels of these biomarkers to precisely understand the accuracy of these biomarkers. A prospective study with a longer follow-up will help establish the role of calprotectin in monitoring disease activity and predicting flares.

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