Original Research Article

Serum Level of Calprotectin in Juvenile Idiopathic Arthritis and Its Correlation with Disease Activity Clinical and Ultrasonographic

Abstract

Background: Calprotectin is an essential proinflammatory component of the innate immune system. Musculoskeletal ultrasound (MSUS) was shown to be a relevant and accurate evaluation method for people with chronic inflammatory arthropathies. Therefore, it has been deemed promising for assessing the joints of children with Juvenile Idiopathic Arthritis (JIA). This research aims to determine the blood level of calprotectin in patients with juvenile idiopathic arthritis (JIA) and its connection with activity measures and ultrasonographically identified synovitis.

Methods: This study was carried out on 30 JIA patients, and 30 apparently healthy children as controls of matching age and sex. All patients were subjected to the following: history taking, complete clinical examination, disease activity assessment, full laboratory investigations, Ultrasound examination and enzyme-linked immunosorbent assay (ELISA) to assay the level of calprotectin in serum samples.

Results: Oligoarticular was the most common type of JIA. Methotrexate was the most common DMARD used either alone or with systemic corticosteroids. There was significant difference between JIA patients and controls as regard haemoglobin, ESR and CRP. JIA patients had significantly higher serum calprotectin levels when compared to controls. Systemic subtype was founded to have higher serum calprotectin levels. There was significant positive correlation between serum calprotectin and clinical parameters of disease activity (JADAS 27), CRP, ESR, serum ferritin and ultrasound score (EULAR-OMERACT

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combined scoring system for grading synovitis). There was insignificant correlation between serum calprotectin levels and RF, ANA and AntiCCP in patients' group. There was positive significant correlation between ultrasound score (EULAR-OMERACT combined scoring system for grading synovitis) and disease activity (JADAS 27).

Conclusion: Our research demonstrates a substantial correlation between serum calprotectin levels and clinical, laboratory, and ultrasound measures of joint inflammation. Serum In ordinary clinical practise, calprotectin is a helpful biomarker that, together with other indicators such as CRP and ESR and our clinical judgement, helps us make treatment choices. Indeed, this protein is very stable and readily detectable in serum.

Keywords: Calprotectin, Juvenile Idiopathic Arthritis.

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Introduction:

Juvenile idiopathic arthritis (JIA) is the most prevalent childhood chronic rheumatic illness and a leading source of acquired disability in children. [1] It is a diverse collection of various disease subtypes defined by the development of arthritis before the age of 16 and the persistence of symptoms for at least 6 weeks [2].

Calprotectin is a crucial proinflammatory element of innate immunity that functions as an endogenous damage-associated molecular pattern molecule upon activation of toll-like receptor 4 [3]. Fagerhol et al. [4] originally isolated calprotectin from granulocytes in 1980 and designated it L1 protein. Reflecting its protective function in epithelial defence and its fungicidal and bactericidal activities, the word calprotectin was subsequently coined. MRP8/14 is detectable in the plasma of all healthy individuals. The typical range is between 1 and 6 mg/l, and it rises in response to tissue damage and inflammation [5].

Calprotectin (S100A8/9 heterodimer) is found to be greater in individuals with active JIA compared to healthy controls, systemic infections, or malignancies; however, other indicators of inflammation such as ferritin, erythrocyte sedimentation rate and C-reactive protein are not specific for JIA [6, 7]. In adults with chronic inflammatory arthropathies, musculoskeletal ultrasonography (MSUS) has been shown to be a viable and accurate evaluation method [8-10]. As a result, it has been seen as particularly useful in evaluating the health of the joints in children with JIA [11-14].

MSUS was significant for its capacity to identify subclinical synovitis and enhance the categorization of patients into JIA subtypes, as a guide for intraarticular corticosteroid injections, and for detecting early articular injury. In addition, the ankle, the midfoot, the hip, the wrist, and the tiny joints of the hands and feet were determined to be the joints most suited for MSUS research [15].

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This research aimed to determine the blood level of calprotectin in JIA and its connection with activity measures and ultrasonographically diagnosed synovitis.

Patients and Methods:

Thirty individuals with JIA were analysed in this research. All of these patients met the criteria for inclusion in the International Classification of Rheumatic Diseases (ILAR) [16], and 30 seemingly healthy children as age- and gender-matched controls. Subjects were recruited from the outpatient clinics of Tanta University Hospitals' Physical Medicine, Rheumatology, and Rehabilitation Department and Paediatric Department. The Tanta University Faculty of Medicine Research Ethics Committee authorised the project. All individuals gave their informed, written consent to participate.

Exclusion criteria were other connective tissue illnesses like juvenile dermatomyositis, juvenile systemic lupus erythematosus or mixed connective tissue disease.

All patients were subjected to the following: History taking (Personal, present, past and family history), complete clinical examination with special attention to musculoskeletal system. The posture, overlaying skin, muscular atrophy, deformities, edoema, and soreness of the synovial joints were evaluated. For each joint, both active and passive motions were evaluated. Evaluation of disease activity using the Juvenile Arthritis Disease Activity Score 27 (JADAS 27) [17].

Complete laboratory tests: Complete blood count (CBC), Serum ferritin, Rheumatoid factor (RF), Erythrocyte sedimentation rate (ESR), Anti cyclic citrullinated peptide antibodies (anti-CCP), Antinuclear antibodies (ANA), C-reactive protein (CRP), and Serum calprotectin.

Using a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA), the kit measured the concentration of calprotectin in human blood samples.

Test principle: The serum Calprotectin (CALPRO) in samples is quantified using a double-antibody sandwich ELISA. Add CALPRO to a monoclonal antibody Enzyme well that has

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been pre-coated with a Human CALPRO monoclonal antibody; incubate; then, add CALPRO antibodies labelled with biotin and combined with Streptavidin-HRP to form an immune complex; incubate and wash again to remove any uncombined enzyme. The liquid's colour changes to blue at the addition of Chromogen Solutions A and B, and lastly to yellow upon the addition of an acid. The correlation between the chroma of colour and the concentration of Human Substance CALPRO in a sample was positive.

Sampling and sample storage: Blood samples were obtained using appropriate medical procedures to prevent haemolysis. Blood was allowed to coagulate at room temperature for 10-20 minutes before being centrifuged to extract the serum. At -20°C, specimens were kept. Avoiding repeated freeze-thaw cycles.

Ultrasound examination: All patients were evaluated in the ultrasound unit of Tanta University Educational Hospital's Physical medicine, Rehabilitation, and Rheumatology Department using SAMSUNG MEDISON (UGEO H60).

The child was advised to lay supine on the examination table for US evaluation of the majority of the joints. The smallest children (e.g., 5 years old) might sit on their parent's lap to remain quiet, but sedation was not necessary.

The 4 joint locations (knee, ankle, wrist, and 2nd MCP) were assessed for synovitis (synovial hypertrophy, joint effusion, and power Doppler (PD) signal) using B-mode and Doppler US in longitudinal and transverse planes with a linear multifrequency transducer (dynamic range 7-16 MHZ). Joint selection was mostly based on the fact that they are the peripheral joints most often affected by JIA. [18, 19] Specific anatomic landmarks required to be included in the images of all approved scans to guarantee that all tests done for the research used the identical scanning positions.

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Joint Scan approach [20]:

Knee: Knee The knees were bent 30 degrees on the examination table to expose the anterior suprapatellar recess. The transducer was positioned in the sagittal midline of the leg, with its distal end just cranial to the patella's superior border. Lateral parapatellar recesses: The transducer was positioned in the sagittal midline of the leg, just superior to the patella, and then rotated 90 degrees for transverse scanning.

Wrist: The wrist was flat and in a neutral posture with the palm pointing down (lateral/midline/medial dorsal). The proximal end of the transducer was positioned immediately distal to the radius diaphysis, while the distal end was placed longitudinally at the sagittal midline of the wrist.

2nd MCP: With the palm pointing downwards, laterally, or upwards, the neutral position of the finger was dorsal, lateral, or volar. The transducer was positioned longitudinally along the sagittal midline of the finger at the MCP (dorsal/lateral/volar) joint.

Ankle: ankle was dorsally extended, with the sole lying on the examination table, to provide medial, midline, lateral, and dorsal views. The talus dome served as the transducer's target since it was situated in the sagittal midline of the ankle. In youngsters, the volar approach is often omitted since the dorsal approach provides a superior view of the anechoic/hypoechoic profile of unossified epiphyseal cartilage of bones creating the dorsal recess of radiocarpal and midcarpal joints. Investigators were instructed to look for evidence of synovitis, which were assessed using a grey scale mode, by sweeping the transducer from the medial to lateral side of the joint during B-mode US scanning and similarly from the proximal to distal side during transverse scanning [21]: None (Grade 0): the absence of SH regardless of the presence of effusion. Minimal (Grade 1): SH with or without effusion up to the level of the horizontal line that connects the bone surfaces. SH with or without effusion extending beyond joint line but with convex (curved downwards) top surface, or hypertrophy extending beyond joint line

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but with flat upper surface. SH with or without effusion extending beyond the joint line, but with a flat or convex top surface (curved downwards).

By PD mode: There is no Doppler action (Grade 0). Minimal (Grade 1): up to three single Doppler spots or one confluent Doppler spot and two single Doppler spots or two confluent Doppler spots. Moderate (Grade 2): Doppler signals in the overall GS background are larger than Grade 1 but less than 50 percent. Severe (Grade 3): exceeding Grade 2 (>50% of the GS in the backdrop).

Classification based on the EULAR-OMERACT combined scoring system for rheumatoid arthritis synovitis (RA). Normal joint (Grade 0): no SH nor PD signal observed in greyscale (within the synovium). Minimal synovitis (Grade 1): SH of Grade 1 with PD indications of Grade 1. Grade 2 moderate synovitis: Grade 2 SH with Grade 2 PD signal or Grade 1 SH with Grade 2 PD signal. Severe synovitis (Grade 3): Grade 3 SH and Grade 3 PD signal or Grade 1-2 SH and Grade 3 PD signal.

Statistical analysis

SPSS v26 was used to do statistical analysis (IBM Inc., Chicago, IL, USA). Comparing the two groups using an unpaired Student's t- test, quantitative data were provided as mean and standard deviation (SD). When applicable, qualitative variables were given as frequency and percentage (%) and analysed using the Chi-square test or Fisher's exact test. A Spearman coefficient was calculated to determine the relationship between two irregularly distributed quantitative variables. A two-tailed P value < 0.05 was deemed statistically significant.

Results:

Regarding age and sex distribution of JIA patients and controls, there was insignificant variation between JIA patients and controls as regard age and sex. **Table 1**

Table 1: Age and sex distribution of JIA patients and controls

	Patients (n = 30)		Control (n = 30)		Test of Sig.	P
Sex	**No.**	**%**	**No.**	**%**		

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Male	14	46.7	14	46.7	χ^2= 0.000	1.000
Female	16	53.3	16	53.3		
Age (years)						
range.	4.0 – 15.0		4.0 – 15.0		t= 0.357	0.722
Mean ± SD.	9.97 ± 3.19		9.67 ± 3.31			

Regarding type of JIA, 46.7% of JIA cases were oligoarticular, 26.7% were polyarticular, 23.3%were systemic and enthesitis related JIA were 3.3%. **Table 2**

Table 2: The distribution of different subtypes of JIA in the studied group

Type of JIA	**Total (n= 30)**		**Male (n= 14)**		**Female (n= 16)**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Oligo persistent	9	30.0	3	21.4	6	37.5
Oligo extended	5	16.7	4	28.6	1	6.3
Polyarticular (RF- ve)	8	26.7	3	21.4	5	31.3
Polyarticular (RF+ ve)	0	0.0	0	0.0	0	0.0
Systemic	7	23.3	4	28.6	3	18.9
Enthesitis related JIA	1	3.3	0	0	1	6.3
Psoriatic	0	0.0	0	0.0	0	0.0

Regarding disease duration and activity, most of JIA patients (43.3%) had high disease activity and duration of the patients ranges from 1 to 7 years. Regarding medical history, Methotrexate was the most common DMARD used either alone or with systemic corticosteroids. **Table 3**

Table 3: Studied cases distribution according to disease activity, duration of disease and medical history in patients' group (n = 30)

Disease activity (JADAS27)	**No.**	**%**
Inactive	2	6.7
Mild	9	30.0
Moderate	6	20.0
High	13	43.3
Duration of disease(years)		
range.	1.0 – 7.0	
Mean ± SD.	2.63 ± 1.61	
Medical history	No.	%
Non-steroidal only	2	6.7
Arthfree	1	3.3
Mtx	26	86.7
Corticosteroid	21	70
Humira	1	3.3
Actemra	1	3.3
Salazopyrine	1	3.3

Regarding laboratory investigations, there was considerable variation between JIA patients and controls as regard haemoglobin, ESR and CRP. Regarding serum Calprotectin, there was

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significant elevation of serum calprotectin levels in patients' group as compared with controls. **Table 4**

Table 4: Comparison between the two studied groups according to laboratory investigations .

	Patients (n = 30)	**Control (n = 30)**	**Test of Sig.**	**P**
Hb				
Mean ± SD.	11.36 ± 0.94	12.27 ± 0.97	t= 3.648*	0.001*
WBCs				
Mean ± SD.	7320.0 ± 2440.24	6540.0 ± 1466.31	U= 377.50	0.277
PLT				
Mean ± SD.	318.10 ± 64.69	327.43 ± 61.42	t= 0.573	0.569
1st ESR				
Mean ± SD.	28.23 ± 14.25	8.73 ± 3.90	U= 50.0*	<0.001*
CRP				
Negative	15(50.0%)	30(100.0%)	χ^2=20.0*	<0.001*
Positive	15(50.0%)	0(0.0%)		
Mean ± SD.	8.97 ± 5.27	–		
Serum ferritin				
Mean ± SD.	63.78 ± 76.64	35.34 ± 17.65	U= 341.50	0.109
RF				
Negative	30(100.0%)	30(100.0%)	–	–
Positive	0(0.0%)	0(0.0%)		
ANA				
Negative	29(96.7%)	30(100.0%)	χ^2= 1.017	FEp= 1.000
Positive	1(3.3%)	0(0.0%)		
Anti CCP				
Negative	30(100.0%)	30(100.0%)	–	–
Positive	0(0.0%)	0(0.0%)		
Serum calprotectin (mg/l)				
range.	1.0 – 24.80	1.0 – 1.40	105.50*	<0.001*
Mean ± SD.	3.75 ± 6.0	1.09 ± 0.11		

Regarding correlation between subtype of JIA and Serum calprotectin, there is considerable correlation between serum calprotectin and subtyping of JIA. It was highly elevated in systemic JIA while regarding relation between serology and serum calprotectin, there was insignificant correlation between serum calprotectin levels and RF, ANA and AntiCCP in

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patients' group. Regarding Correlation between Serum calprotectin with disease activity, there was significant positive correlation between JADAS 27 and serum calprotectin. **Table 5**

Table 5: Correlation between subtype of JIA, serology and Serum calprotectin and correlation between Serum calprotectin with disease activity (JADAS27) in patients' group,

Serum calprotectin (µg/ml)	Type of JIA					H	P
	Oligo extended (n= 5)	**Enthesitis related JIA (n= 1#)**	**Oligopersistent (n= 9)**	**Polyarticular (n= 8)**	**Systemic (n= 7)**		
Range.	1.10 – 1.60		1.0 – 2.0	1.10 – 2.0	1.20 – 24.80	9.114*	0.028*
Mean ± SD.	1.26 ± 0.19	2.0	1.34 ± 0.28	1.36 ± 0.28	11.60 ± 8.93		
serology	N	Serum calprotectin **Mean ± SD.**				U	P
RF							
Negative	**30**	3.75 ± 6.00				–	–
Positive	**0**	–					
ANA							
Negative	**29**	3.20 ± 5.28				–	–
Positive	**1#**	19.70					
Anti CCP							
Negative	**30**	3.75 ± 6.00				–	–
Positive	**–**	–					
Disease activity (JADAS 27)	N	Serum calprotectin **Range**		**Mean ± SD.**		H	P
Inactive	**2**	1.10 – 1.20		1.15 ± 0.07		9.139*	0.027*
Mild	**9**	1.0 – 1.40		1.26 ± 0.12			
Moderate	**6**	1.10 – 2.0		1.40 ± 0.33			
High	**13**	1.10 – 24.80		6.96 ± 8.19			

Regarding Correlation between serum calprotectin and both laboratory investigations and Ultrasound findings, there was positive significant corelation between serum calprotectin and ESR, CRP, serum ferritin and ultrasound score (EULAR-OMERACT combined scoring system for grading synovitis). Regarding Relation between disease activity and Ultrasound findings, there was significant positive correlation between ultrasound score (EULAR-OMERACT combined scoring system for grading synovitis) and disease activity (JADAS 27). **Table 6**

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Table 6: Correlation between serum calprotectin and laboratory investigations, Ultrasound findings and Relation between disease activity and Ultrasound findings in patients' group (n= 30)

	Serum calprotectin	
	r_s	P
Hb	0.043	0.820
RBCs	0.002	0.992
WBCs	0.177	0.350
PLT	0.353	0.055
1st ESR	0.616	<0.001*
Serum ferritin	0.544	0.002*
CRP	0.545	0.002*
Ultrasound findings	**Serum calprotectin**	
	r_s	P
Wrist synovial hypertrophy	0.650	<0.001*
Wrist effusion	0.650	<0.001*
Wrist PDS	0.678	<0.001*
2nd MCP synovial hypertrophy	0.561	0.001*
2nd MCP effusion	0.561	0.001*
2nd MCP PDS	0.442	0.014*
Knee effusion	0.247	0.189
Knee synovial hypertrophy	0.247	0.189
Knee PDS	0.271	0.147
Ankle effusion	0.464	0.010*
Ankle synovial hypertrophy	0.464	0.010*
Ankle PDS	0.078	0.682
Ultrasound findings	**Disease activity**	
	r_s	P
Wrist synovial hypertrophy	0.522	0.003*
Wrist effusion	0.522	0.003*
Wrist PDS	0.517	0.003*
2nd MCP synovial hypertrophy	0.558	0.001*
2nd MCP effusion	0.558	0.001*
2nd MCP PDS	0.409	0.025*
Knee effusion	0.412	0.024*
Knee synovial hypertrophy	0.412	0.024*
Knee PDS	0.292	0.118
Ankle effusion	0.429	0.018*
Ankle synovial hypertrophy	0.429	0.018*
Ankle PDS	0.000	1.000

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Discussion:

JIA refers to all kinds of arthritis that begin before the age of 16, continue longer than six weeks, and have an unknown cause [1, 22]. It is a broad set of conditions that all present with joint inflammation, but have unique clinical symptoms, history, genetic basis, and pathogenesis.

Current International League of Associations of Rheumatology (ILAR) classification of JIA includes seven disease categories: systemic arthritis, oligoarthritis, RF-positive polyarthritis, RF-negative polyarthritis, Enthesitis-related arthritis (ERA), psoriatic arthritis, and undifferentiated arthritis [16]. JIA is characterised by joint inflammation similar to that of adult RA. Without appropriate therapy, JIA may worsen and destroy cartilage, bone, and soft tissues, leading to severe impairment and loss of function, and in rare cases, organ failure and death [23].

In agreement with Kahn [25], who found that oligoarticular JIA is the most prevalent subgroup of JIA, comprising 50-60% of most JIA cohorts. Polyarticular JIA constitutes 25-40% of JIA, while systemic JIA accounts for just 10-20% of JIA. Abou El-Soud et al [26] oligoarticular JIA was observed to be the most prevalent subgroup of JIA (52.2%), next, polyarticular JIA (29.5%), systemic JIA (13.6%), and ERA (4.5%) of the total JIA cases.

In contrast, Al-Hemairi et al. [27] systemic JIA was found to be the most common onset type (36.5%), secondly the polyarticular (29.2%) then oligoarticular (28%). Also, Abdwani et al [28] The most prevalent subtype among patients in Oman was polyarticular JIA RF negative (39.2%), followed by oligoarticular JIA (31.8%), systemic JIA (17.8%), polyarticular JIA RF positive (7.5%), and psoriatic arthritis (0.9%).

This global diversity in JIA frequency and subtype distribution may represent variations in ethnicity, environmental variables, or immunogenic vulnerability, or it may be the consequence of underreporting in certain nations [29].

In agreement with Beukelman et al. [30], Blazina et al [31] and Fráňová et al [32] who noted that NSAIDs have been the major therapy for all types of JIA historically. If arthritis is still active, their usage as monotherapy for more than two months is discouraged.

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Methotrexate continues to be the most extensively used traditional DMARD for the treatment of JIA due to its efficacy in disease control and tolerable toxicity.

In agreement with La Céline et al. [35] and Frosch et al. [6] who revealed that the blood level of calprotectin in JIA patients was significantly higher than in the control group. These findings are consistent with the physiological explanation that calprotectin is an acute phase reactant that is typically expressed by monocytes, granulocytes, and macrophages in an early differentiation stage, following their activation by damage-associated molecular patterns (DAMPs) as a result of inflammation [36, 37]. CLP is accountable for leukocyte motility and trafficking, as well as inflammation amplification. CLP is a component of the innate immune system. As an endogenous ligand for the toll-like receptor 4TLR, it is identified as a DAMP. TLR4 is a member of the family of pattern recognition receptors; after engaging with DAMPs, it transduces the danger signal. It participates in the inflammatory cascade upstream of tumour necrosis factor (TNF)- and, like TNF-, is essential for LPS toxicity. As with other DAMP molecules, calprotectin has a dual function in phagocyte homeostasis. It contributes to the control of the cytoskeleton under normal conditions and is released as a warning signal when phagocytes are activated [38].

In a study by Collado et al. [40] Out of 37 joints presenting gray scale ultrasound determined synovitis, only 18 joints (48.65%) showed PD signal. Collado et al [40] revealed that ultrasonography is better for identifying subclinical disease activity by the existence of positive PD signals in individuals with inactive illness on and off treatment. In a recent study by Darwish et al. [41] They observed that MSUS is extremely sensitive for early diagnosis of joint involvement in JIA when compared to physical examination, since MSUS identified more synovitis than clinical examination (subclinical synovitis).

In agreement with Céline La et al. [35] active illness was associated with serum calprotectin levels that were more than twice as high as those seen in individuals whose

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disease was in remission (6555ng/mL vs. 11403ng/mL), demonstrating a strong correlation between disease activity and calprotectin levels. Additionally, it was assessed to be 1737 ng/mL, which is much higher than the levels seen in healthy controls. Additionally, several clinical (CHAQ and tender joint counts (TJC)) and biological (CRP and ESR) indices of disease activity are correlated with serum calprotectin. A stronger association is seen with ESR than CRP, and a less correlation is found between sCal and the JADAS10-CRP score. However, there was a strong correlation between how serum calprotectin levels changed over time and how JADAS10-CRP levels changed. Additionally, there was a high correlation between the change in DAS28 score and the change in serum calprotectin during the same time period, but not the CRP.

The research has certain caveats, such as its small sample size and the fact that it was conducted at a single location. Our findings still need to be confirmed by further researches.

Conclusions:

Serum calprotectin levels were significantly linked to joint inflammation as measured by clinical, laboratory, and ultrasound criteria. Serum Together with other indicators like CRP and ESR and our clinical judgement, calprotectin is a helpful biomarker in ordinary clinical practise that aids in determining the best course of treatment. In fact, this protein is detectable and relatively persistent in blood serum.

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