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Biomarker Significance of Interleukin-18 in Juvenile Idiopathic Arthritis

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Abstract

Juvenile idiopathic arthritis (JIA) represents a group of multifactorial autoinflammatory arthritis diseases. A dysregulated production of pro-inflammatory cytokines is proposed to have a role in the pathogenesis of the disease. Interleukin-18 (IL-18) is one of these pro-inflammatory cytokines. Therefore, this study aimed to define the role of IL-18 in the pathogenesis of JIA. Accordingly, the serum level of IL-18 was determined in 59 Iraqi JIA patients and 58 matched controls. The results revealed a significantly increased median of IL-18 in the patients as compared to the control. A similar increased level was observed in subgroups of patients characterized according to gender, seropositivity for C-reactive protein and rheumatoid factors, juvenile arthritis disease activity score 27 (JADAS27), type of medication, and JIA subtypes. However, JADAS27 showed a significant positive correlation with IL-18 level. Receiver operating characteristic analysis revealed that IL-18 occupied a significant area under the curve, and therefore its significance as a biomarker was suggested. In conclusion, IL-18 is an important biomarker for JIA and may have a role in pathogenesis of disease.

Keywords: Juvenile idiopathic arthritis; Interleukin-18; Disease activity; Clinical subtype.

الاهمية الواسم-حياتية للبين ابيضاض-18 في التهاب المفاصل الرثوي لليافعين

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الخلاصه

يمثل التهاب المفاصل الرثوي لليافعين مجموعة من الالتهابات الذاتية الرثوية المتعددة الاسباب. ويفترض بان عدم انتظام انتاج الحركيات الخلوية البادئة للالتهاب يمتلك دورا في امراضية المرض. ويعد البين ابيضاض-18 واحد من هذه الحركيات الخلوية البادئة للالتهاب. لذلك هدفت هذه الدراسة الى تقصي دور البين ابيضاض-18 في امراضية التهاب المفاصل الرثوي لليافعين. وفي ضوء ذلك، تم قياس المستوى المصلي للبين ابيضاض-18 في 59 من مرضى التهاب المفاصل الرثوي لليافعين العراقيين و 58 من افراد سيطرة مطابقة. اظهرت النتائج زيادة معنوية بمتوسط البين ابيضاض-18 في المرضى مقارنة بالسيطرة. ولوحظت زيادة مماثلة في المجاميع الثانوية للمرض والمقسمة وفق الجنس وايجابية المصل لبروتين الطور

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الحاد–سي والعامل الرثوي وفعالية المرض ونوع العلاج والانواع السريرية. وقد اظهرت فعالية المرض ترابط ايجابي مع المستوى المصلي للبين ابيضاض–18. كما اظهر تحليل استقبال التشغيل المميز باحتلال البين ابيضاض–18 مساحة معنوية تحت القوس وهذا مايؤشر الاهمية الواسم–حياتية له. نستنتج من ذلك بان البين ابيضاض–18 هو واسم–حياتي مهم لالتهاب المفاصل الرثوي لليافعين ولربما يؤدي دورا مهما في امراضية المرض.

Introduction

Juvenile idiopathic arthritis (JIA) is a heterogeneous group of chronic arthritis that onsets before the age of 16 years and persists for at least 6 weeks [1]. Based on the Edmonton revised criteria of the International League of Associations for Rheumatology (ILAR), JIA is classified into seven subtypes; oligoarticular (OJIA), rheumatoid factor-negative polyarticular (RF-Poly), RF-positive polyarticular (RF+Poly), systemic-onset (sJIA), enthesitis-related arthritis (ERA), psoriatic arthritis and undifferentiated arthritis. OJIA is further classified into persistent (pOJIA) and extended (eOJIA) JIA [2]. Although JIA subtypes have heterogeneous clinical presentation and manifestations, as well as etiopathogenesis, they may share the same or different etiologies that implicate interaction with specific immunogenetic susceptibility [3]. However, the etiology of JIA is not well-defined but it is described as multifactorial with a prominent role of both genetic and environmental factors [4]. Data obtained from JIA patients propose mechanisms in which different external pathogens evoke multiple immune response pathways that include cytotoxic T lymphocytes and activation of complement components [5]. A further important immune pathway involves dysregulated production of pro-inflammatory cytokines, especially those of inerleukin-1 (IL-1) family, which are suggested to be responsible for at least part of the clinical symptoms in all JIA subtypes [6].

Seven pro-inflammatory cytokines (IL-1α, IL-1β, IL-18, IL-36α, IL-36β, IL-36γ, and IL-33) have been so far included in IL-1 family. Their upregulation is considered a hallmark of the autoimmune and inflammatory manifestations observed in JIA patients [6]. IL-18 is an important regulator of both innate and adaptive immune responses, being responsible for immune-mediated pathologies. It is probably one of the factors that contribute to pathogenesis of autoimmune diseases [7]. IL-18 is produced by monocytes, activated macrophages and dendritic cells, and is best known for its capability in inducing interferon-gamma (IFN-γ), stimulating inflammatory reactions and promoting the activation of T and natural killer (NK) cells [8]. Besides that, IL-18 inhibits osteoclast formation and down-regulates osteoclast functions. It is also indicated that IL-18 is involved in bone destruction. Further, IL-18 stimulates the differentiation of osteoclasts by upregulating RANKL (Receptor activator of nuclear factor kappa-B ligand) production from T cells in the synovium of rheumatoid arthritis (RA) patients [9, 10]. In sJIA patients, IL-18 serum level is extremely elevated compared to control or other JIA subtypes. It is also suggested to use IL-18 as a serum biomarker to distinguish sJIA from other febrile diseases [11].

In line with these developments, the present study investigated serum level of IL-18 in JIA patients. The level was correlated with clinical and laboratory profiles of disease. Type of medication and JIA subtypes were also considered in these correlations.

Materials and Methods

Patients and control

A case-control study was conducted during January-April, 2018. Fifty-nine Iraqi JIA patients were enrolled in the study. The patients were referred to the Rheumatology Unit at Baghdad Teaching Hospital (Baghdad, Iraq) for diagnosis and treatment. The diagnosis was made by rheumatologists at the clinic. It was based on the Edmonton revised criteria of ILRA for chronic JIA. The JIA subtypes were also classified according to these criteria [2]. Duration of disease, juvenile arthritis disease activity score 27 (JADAS27), and medication received were recorded for each patient. A control sample of 58 healthy age- and gender-matched children was also included. They were referred to the Healthcare Units in Baghdad for a routine health check. Based on a clinical evaluation of physicians, their health status was ascertained. The protocol of the study was approved by the Ethics Committee at Baghdad Teaching Hospital (Iraqi Ministry of Health). Written informed consent was obtained from the guardians of patients to participate in the study according to the 2008 Declaration of Helsinki.

Laboratory blood tests

All patients were assessed for hemoglobin (Hb), white blood cell (WBC) count and erythrocyte sedimentation rate (ESR). In addition, their sera were tested qualitatively for C-reactive protein (CRP) and rheumatoid factor (RF) using latex slide agglutination test kits (CRP-Latex, Spinreact Spain; Ref. ID: 1200305 and RF-Immuno-Latex, La Wama Diagnostica, Brazil; Ref. ID: 28100-L). Serum level of IL-18 was determined by ELISA kits (MyBioSource, USA; Catalogue N: MBS772103). Instructions of manufacture were followed.

Statistical analyses

Data of IL-18 serum level were tabulated in a data sheet of SPSS (version 19.0) and tested for normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). The tests revealed that the level did not follow a normal distribution. Therefore, IL-18 level was given as median and range. Significant differences were assessed by the non-parametric tests of Mann–Whitney *U* (for comparison between two groups) or Kruskal-Wallis (for comparison between more than two groups). The odds ratio (OR) was estimated in relation to IL-18 median (> and ≤ median) in patients and control. Spearman's coefficient (r) was used to express the correlation between IL-18 level, JADAS27 and disease duration. Receiver operating characteristic (ROC) analysis was employed to estimate area under curve (AUC), sensitivity and specificity of IL-18. False discovery rate (FDR) was applied to correct the *p*-value due to multiple comparisons. A corrected p (pc) ≤ 0.05 was considered significant. The statistical package SPSS (version 19.0) was used to carry out these analyses.

Results

Baseline profiles

Juvenile idiopathic arthritis patients were tested for laboratory and clinical profiles of Hb, WBC, CRP, RF, disease duration (< year, 1-3 years and > 3 years), JADAS27, medication, and subtypes (Table-1). JADAS27 is an absolute disease activity measure that can be used to determine and evaluate disease activity status and course in individual patients. It consists of the following measures: physician's global assessment (score 0-10), parent's global assessment (0-10), active joint count (assessed in score of 27), and ESR (normalized to score 0-10). The total score ranges from 0 to 57, or can be simplified as low (score: 0-3), moderate (score: 4-10) and high (score: 11-57) [12]. Most JIA patients were classified as low JADAS27 (49.2%). With respect to medication, four patients were newly diagnosed cases and no medication was provided at the time of investigation (untreated), while 4, 20, 22 and 9 patients received methylprednisolone (MPS: 50 mg/day), methotrexate (MTX: 5-15 mg/week), etanercept (ETN: 25 mg/week), or adalimumab (ADM: 20 mg/week), respectively. There was no overlap in the medication received. As related to JIA subtypes, six subtypes were recognized (pOJIA, eOJIA, RF-Poly, RF+Poly, sJIA and ERA). pOJIA was the most frequent (28.8%), while ERA was less frequently observed (3.4%).

Table 1-Laboratory and clinical profiles of JIA patients

Profile	**N**	**Mean ± SD or %**	**Profile**	**N**	**%**
Age (year)	59	10.2 ± 4.2	*Medication*		
Hb (mg/dL)	59	11.2 ± 1.5	Untreated	4	6.8
WBC ($\times 10^9$/L)	59	10.0 ± 3.7	Methylprednisolone	4	6.8
ESR (mm/h)	59	39.5 ± 29.5	Methotrexate	20	33.9
CRP+	34	57.6	Etanercept	22	37.3
RF+	16	27.1	Adalimumab	9	15.2
CRP-,RF-	16	27.1	*JIA subtypes*		
CRP+,RF+	7	11.9	pOJIA	17	28.8
Disease duration (year)	59	1.9 ± 0.7	eOJIA	14	23.7
< year	19	32.2	RF-Poly	13	22.0
1-3 years	29	49.2	RF+Poly	7	11.9
> 3 year	11	18.6	sJIA	6	10.2
JADAS27	59	1.8 ± 0.9	ERA	2	3.4
Low	29	49.2			
Medium	14	23.7			
High	16	27.1			

Hb: Hemoglobin, WBC: White blood cell, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, RF: Rheumatoid factor, JADAS27: Juvenile arthritis disease activity score 27, JIA: Juvenile idiopathic arthritis, pOJIA: Persistent oligoarticular JIA, eOJIA: Extended oligoarticular JIA, RF-Poly: RF-negative polyarticular, RF+Poly: RF-positive polyarticular, sJIA: Systemic-onset JIA, ERA: Enthesitis-related arthritis, N: Absolute number, SD: Standard deviation.

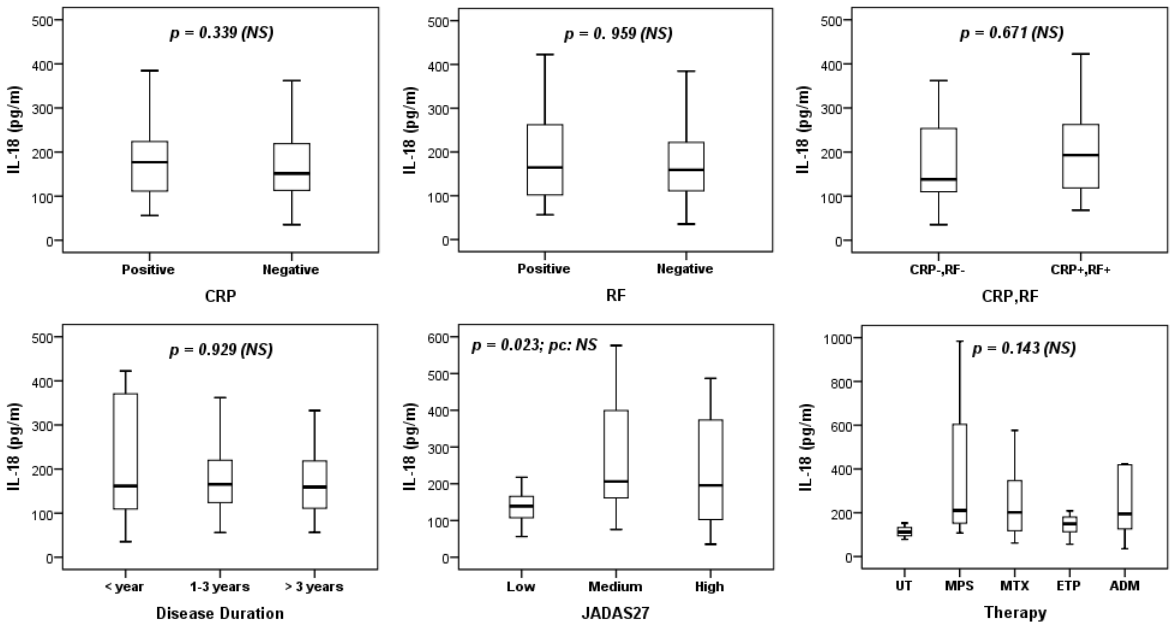
IL-18 serum level

A significantly increased level of IL-18 was recorded in JIA patients as compared to control ($pc < 0.001$). The same manner of a significantly increased level was observed in subgroups of patients characterized according to demographic, laboratory or clinical profile. Moreover, there were no significant differences between these subgroups. Medium and high JADAS27 patients were an exception; they showed an increased level of IL-18 compared to low JADAS27 patients, but the difference was significant before correcting the *p*-value ($p = 0.023$) (Table-2 and Figure-1). Estimating the OR value (based on > and ≤ median of IL-18) revealed that subgroups of JIA patients (males, age group < 12 years, medium-high JADAS27 and polyarticular subtype) were positively associated with an IL-18 level that is greater than the median (i.e. a higher OR was recorded in these subgroups) (Table-3). JADAS27 showed a significant positive correlation with IL-18 level (Figure-2). ROC analysis revealed that IL-18 occupied a significant AUC in JIA patients ($p < 0.001$). The AUC was 0.819 (95% CI: 0.739-0.898), and under a cut-off value of 107.6 pg/ml, the diagnostic sensitivity and specificity values of IL-18 were 78 and 73%, respectively (Figure-3).

Table 2-Serum level of IL-18 in JIA patients and control

Group		IL-18 Median (range); pg/ml				*p* (*pc*)
		N1	JIA	N2	Control	
Total patients		59	161.6 (35.2-1101.4)	58	42.9 (2.0-1086.9)	< 0.001 (S)
Gender	Male	19	165.5 (56.4-984.2)	24	43.9 (17.2-1086.9)	< 0.001 (S)
	Female	40	155.9 (35.2-1101.4)	34	33.3 (2.0-277-1)	< 0.001 (S)
p (*pc*)		0.733 (NS)		0.255 (NS)		
Age groups (year)	< 12	38	162.7 (61.4-984.2)	20	46.0 (15.7-152.7)	< 0.001 (S)
	12-16	21	159.1 (35.2-1101.4)	38	42.2 (2.0-1086.9	< 0.001 (S)
p (*pc*)		0.704 (NS)			0.793 (NS)	

JIA: Juvenile idiopathic arthritis, N1: Number of patients, N2: Number of control, *p*: Mann–Whitney *U* test probability, *pc*: Corrected *p*, NS: Not significant ($p > 0.05$), S: Significant ($p \leq 0.05$).



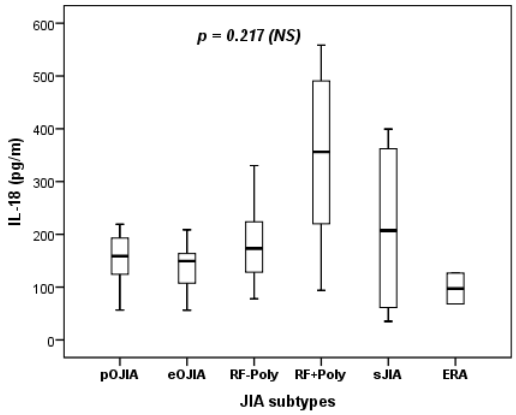


Figure 1: Boxplot presentation of IL-18 serum level in juvenile idiopathic arthritis (JIA) patients distributed according to laboratory and clinical profiles. CRP: C-reactive protein, RF: Rheumatoid factor, JADAS: Juvenile arthritis disease activity score, UT: Untreated, MPS: Methylprednisolone, MTX: Methotrexate, ETP: Etanercept, ADM: Adalimumab, pOJIA: Persistent oligoarticular JIA, eOJIA: Extended oligoarticular JIA, RF-Poly: RF-negative polyarticular, RF+Poly: RF-positive polyarticular, sJIA: Systemic-onset JIA, ERA: Enthesitis-related arthritis, *p*: Mann–Whitney *U* or Kruskal-Wallis test probability, *pc*: Corrected *p*, NS: Not significant ($p > 0.05$).

Table 3-Distribution of IL-18 level (> and ≤ median) in JIA patients and control

Group		Patients (N = 59)				Control (N = 58)				OR (95% CI)*	*p* (*pc*)
		> Median		≤ Median		> Median		≤ Median			
		N	%	N	%	N	%	N	%		
Total patients		42	71.2	17	28.8	16	27.6	42	72.4	6.49 (2.92 - 14.41)	< 0.001 (S)
Gender	Male	15	78.9	4	21.1	8	23.5	26	76.5	12.19 (3.23 - 45.96)	< 0.001 (S)
	Female	27	67.5	13	32.5	8	33.3	16	66.7	4.15 (1.44 to 11.95)	0.01 (NS)
Age group (year)	< 12	28	73.7	10	26.3	4	20.0	16	80.0	11.20 (3.10 - 40.43)	< 0.001 (S)
	12-16	14	66.7	7	33.3	12	31.6	26	68.4	4.33 (1.42 - 13.19)	0.014 (NS)
CRP	+	24	70.6	10	29.4	-	-	-	-	6.30 (2.50 - 15.87)	< 0.001 (S)
	-	18	72.0	7	28.0	-	-	-	-	6.75 (2.41 - 18.88)	< 0.001 (S)
RF	+	12	75.0	4	25.0	-	-	-	-	7.88 (2.29 - 27.09)	< 0.001 (S)
	-	30	69.8	13	30.2	-	-	-	-	6.06 (2.56 - 14.31)	< 0.001 (S)
JADAS27	Low	19	65.5	10	34.5	-	-	-	-	4.99 (1.94 - 12.82)	0.001 (S)
	M-H	23	76.7	7	23.3	-	-	-	-	8.63 (3.15 - 23.65)	< 0.001 (S)
JIA Subtypes	OJIA	21	67.7	10	32.3	-	-	-	-	5.51 (2.16 - 14.04)	< 0.001 (S)
	Poly	16	80.0	4	20.0	-	-	-	-	10.50 (3.13 - 35.24)	< 0.001 (S)

JIA: Juvenile idiopathic arthritis, CRP: C-reactive protein, RF: Rheumatoid factor, JADAS: Juvenile arthritis disease activity score, M-H: Medium-High, OJIA: Oligoarticular JIA, Poly: Polyarticular, N:

Absolute number, %: Percentage, OR: Odds ratio, CI: Confidence interval, *p*: Two-tailed Fisher's exact probability, *pc*: Corrected *p*, NS: Not significant ($p > 0.05$), S: Significant ($p \leq 0.05$), +: Positive, -: Negative. *OR was estimated compared to control.

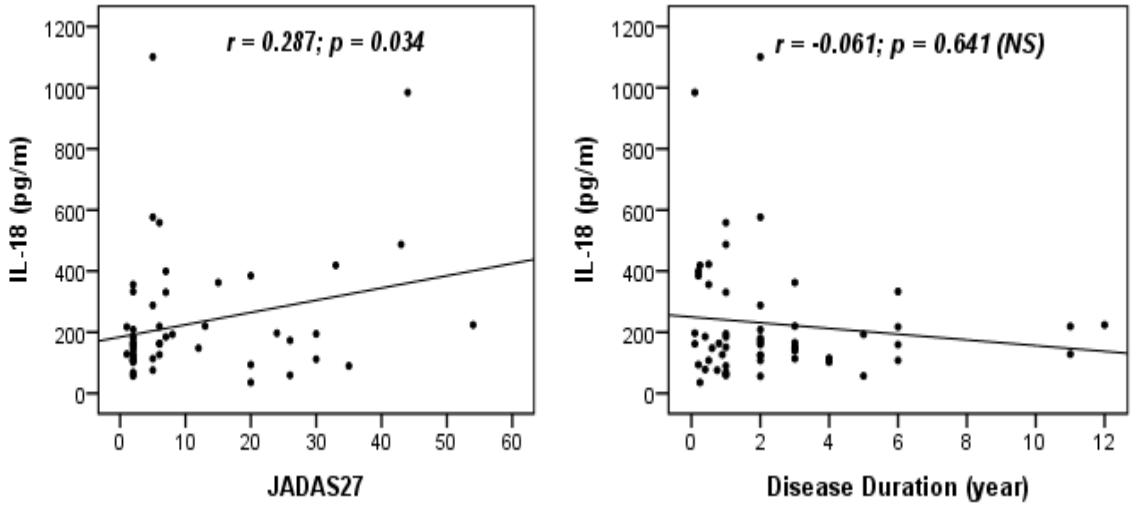


Figure 2-Spearman's correlation coefficient (*r*) between IL-18 serum level, JADAS27, and disease duration in JIA patients. *p*: Probability, NS: Not significant ($p > 0.05$).

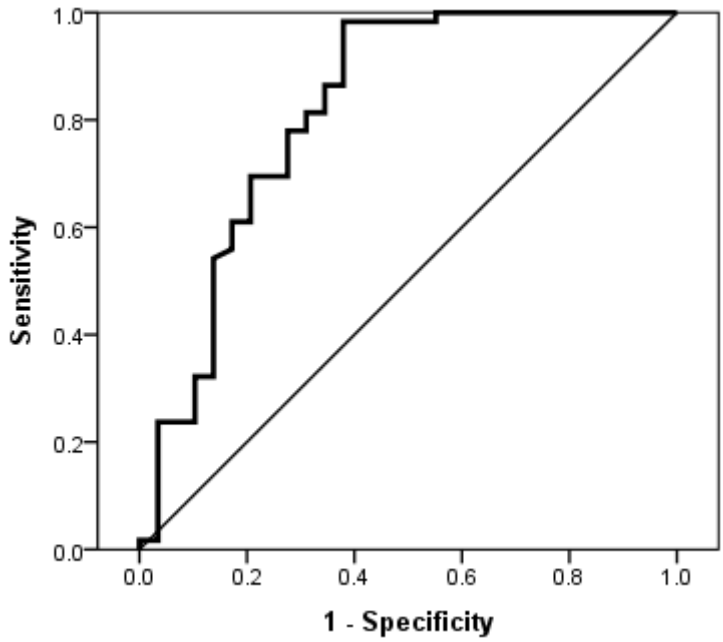


Figure 3-Receiver operating characteristic analysis of IL-18 serum level in JIA patients (Area under curve = 0.819; 95% Confidence interval: 0.739-0.898; $p < 0.001$; Sensitivity = 78%; Specificity = 73%; Cut-off value = 107.6 pg/ml).

Discussion

The obtained results constantly consider IL-18 as a cytokine of pivotal role in JIA pathogenesis. Such consideration was not affected by the gender or age of JIA patients. The extremely elevated levels of IL-18 in sera of male and female patients, as well as patients at the age groups < 12 and 12 – 16 years, were a consistent observation as shown in the results. However, IL-18 level was positively correlated with disease activity. Patients with medium or high JADAS27 showed increased levels compared to low JADAS27 patients. Most of medium-high JADAS27 patients were presented with a level higher than the median of IL-18 (76.7%). These results complement an existing body of

publications in JIA in general or specifically in sJIA, adult-onset Still's disease (AOSD) and macrophage activation syndrome (MAS) [11, 13–15]. Accordingly, the key role of IL-18 in the pathogenesis of JIA, as well as its biomarker significance in the disease, is augmented. Lotito and colleagues (2007) analyzed IL-18 level in sera of 50 JIA patients, and increased levels were recorded compared to control. The increase was much higher in patients with sJIA compared to other subtypes of disease. A correlation with disease activity was also reported [16]. In a recent investigation, it has been confirmed further that IL-18 is a potential biomarker to support the diagnosis of sJIA, especially in the active disease [14]. The present study shares similar findings, with the exception of those related to sJIA specification. IL-18 medians showed no significant variations between the clinical subtypes of JIA, but there was a tendency for IL-18 serum level to increase in RF+Poly JIA compared to other clinical subtypes of disease; however, the observation was based on a non-significant difference between the clinical subtypes of JIA.

Accordingly, it is strongly suggested that IL-18 is involved in pathogenesis of JIA. *In vivo*-based experimental evidence stated that collagen-induced arthritis was markedly reduced in IL-18-deficient mice compared to heterozygous or wild-type mice. Production of pro-inflammatory cytokines (IFN-γ, tumor necrosis factor-α, IL-6, and IL-12) by spleen and lymph node cells was also reduced in these animals. Further, treating the deficient mice with recombinant IL-18 completely reversed the disease to that of the wild-type mice. These findings insistently demonstrated the pivotal role of IL-18 in the development of inflammatory arthritis [17]. In a further investigation, IFN-γ-producing T helper (Th) cells and Th1/Th2 ratio in peripheral blood of AOSD patients were correlated significantly with clinical activity and serum level of IL-18 [18]. Thus, the overproduction of IL-18 may shift the immune response to the Th1 type, which is suggested to have a role in pathogenesis of arthritis [19]. Equally important, infectious microbial agents are suspected in the etiology of JIA. Different external pathogens have been suggested to drive multiple immune response pathways in JIA patients, including production of pro-inflammatory cytokines [5]. Among these cytokines is IL-18, which plays a prominent role in host defense against various infectious agents *via* its enhancement effects in the induction of IFN-γ, nitric oxide, and reactive oxygen species in phagocytes [20]. Therefore, it is suggested that pathogenic agents are involved in activating immune cells in JIA patients and promoting their production of IL-18.

With regard to medications, none of the four therapies (MPS, MTX, ETP and ADM) affected the level of IL-18. It remained elevated in sera of JIA patients irrespective of the administrated therapy. Such findings suggest that IL-18 is not a target for the four therapeutic protocols. However, it has been demonstrated that treating sJIA patients with canakinumab (anti-IL-1β monoclonal antibody) resulted in reduced expression of other inflammation-related genes (*IL6* and *IL18*), and consequently, IL-6 and IL-18 levels were declined [21]. It has also been demonstrated that IL-18 can be counterbalanced by the naturally occurring IL-18BP (IL-18 binding protein), which is an IL-18 endogenous antagonist with a high-affinity [22]. Several publications have addressed using exogenous IL-18BP as a novel therapeutic approach for inflammatory diseases; including sJIA, AOSD and MAS, and encouraging results have been gained [15, 23, 24].

In conclusion, IL-18 is an important biomarker for JIA that may have a role in the pathogenesis of disease. However, further investigations based on larger samples of patients and control are necessitated to validate these conclusions.

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