Biomarker significance of interleukins, IL-37 and IL-38 in patients with juvenile idiopathic arthritis

Inas K. Sharquie, PhD

Department of Microbiology & Immunology, College of Medicine, University of Baghdad, Baghdad, Iraq

ABSTRACT

Introduction: Juvenile idiopathic arthritis (JIA) is the most common rheumatic condition that develops during child age and adolescence. Unbalanced production of pro-inflammatory cytokines is suggested to participate in the etio-pathogenesis of JIA, so the objective of this study is to evaluate the role of interleukins (IL), IL-37 and IL-38, in patients with JIA.

Materials and Methods: Sixty patients with JIA (19 males, 41 females) and 90 healthy controls (35 males, 55 females) were included in this study. Participants were assessed using the juvenile arthritis disease activity score-27 and underwent laboratory tests, including measurements for C-reactive protein, rheumatoid factor, and IL-37 and IL-38.

Results: Mean ages of JIA patients and controls were 10.37±4.21 years and 11.13±3.84 years, respectively. Compared to controls, serum IL-37 levels were increased in patients with JIA (117.98±209.282ng/ml vs. 37.87±24.496ng/ml; p<0.01), whereas serum IL-38 titres were diminished in individuals with JIA (106.2±95.781ng/ml vs. 182.24±108.428 ng/ml; p<0.01).

Conclusion: This study provides a further layer of evidence for the role played by IL-37 in JIA and creates new questions about the potential role of IL-38 in this condition.

KEYWORDS:
Juvenile idiopathic arthritis, IL-37, IL-38

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is the most prevalent rheumatic condition arising during childhood and adolescence.[1] JIA comprises a highly heterogeneous group of disorders, including systemic JIA (SJIA), which is characterised by a quotidian pattern of spiking fever, transient rash, as well as arthritis.[2] Patients with SJIA initially present with symptoms and signs seemingly non-specific to JIA, most likely owing to the syndrome's tendency to alternate between inactive and active (flare) periods.[1,2] SJIA was recently re-evaluated as an autoinflammatory syndrome rather than an autoimmune disease in keeping with the majority of rheumatic conditions.[3,4] Thus, in view of its heterogeneity, the diagnosis of JIA can be a challenge for professionals. Although it is impossible to draw conclusions relating to all JIA categories, owing to its elusive nature, SJIA is perhaps the best subgroup to study as it hypothetically allows for the identification of biomarkers that are specifically responsible for associated symptoms. Additionally, its re-classification as an autoinflammatory disease provides further excellent targets for future research strategies, most notably including the use of inflammatory biomarkers for the diagnosis and management of JIA.[1]

Some evidence, such as that arising from gene expression studies, has already demonstrated an existing link between the pathogenesis of SJIA and the balance of pro- and anti-inflammatory cytokines.[5] This is likely to reflect their involvement in systemic inflammation and joint damage, which are amongst the symptoms present in SJIA. Of these cytokines, the interleukins (IL), IL-6 and IL-17, as well as tumour necrosis factor-α are members of the group of pro-inflammatory cytokines associated with SJIA pathogenesis as well as being significantly elevated in the serum of patients with SJIA.[2,6,7] Furthermore, therapeutic targeting of these cytokines has been shown to cause less severe symptoms.[8] As such, these cytokines are useful when looking to diagnose and manage SJIA in active periods. However, since SJIA can often be sporadic in nature, the identification of specific cytokines that can be used as biomarkers for the purpose of managing the syndrome is also necessary. Additionally, the pro-inflammatory cytokines are often related to other diseases presenting with inflamed tissue; identifying biomarkers specific to SJIA is necessary.

One of the most potentially relevant cytokines in SJIA is IL-37,[9] which belongs to the IL-1 cytokine family.10 IL-37 is an anti-inflammatory cytokine that acts to reduce pro-inflammatory cytokine expression in several inflammatory diseases, including ankylosing spondylitis (AS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and adult-onset Still's disease (AOSD).[10,11] Although in healthy subjects the levels of IL-37 and IL-37 mRNA are both low, their expression in inflamed tissues and cells is markedly raised.[11,12] This occurs owing to the presence of increased levels of pro-inflammatory cytokines that stimulate IL-37 production. Moreover, IL-37 mRNA is significantly more stable in patients with inflammatory diseases, suggesting that IL-37 participates in the suppression of an excessive immune response. In pathologies such as JIA, it would be expected that IL-37 remains elevated and could therefore function as a reliable biomarker. There is some in vitro evidence to demonstrate that IL-37 inhibits the production of pro-inflammatory cytokines in peripheral blood monocytes

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Corresponding Author: Associated Professor Dr. Inas K. Sharquie
Email: iksharquie@yahoo.com, inasksharquie@comed.uobaghdad.edu.iq

from individuals with SLE, RA, AS, and AOSD. The initial data are promising, as levels of IL-37 are correlated with the titres of pro-inflammatory cytokines in the serum of SJIA patients. It is also clear that IL-37 levels are higher in SJIA patients compared to those in healthy individuals.[9,13]

IL-38 is another lesser-known member of the IL-1 family, which has been recently discovered and characterised.[14,15] Owing to its novelty, little is known about this cytokine. Although it is essentially an anti-inflammatory mediator, in a similar manner to many cytokines, it may also function to upregulate other cytokines, such as IL-6. Currently, there is no link between IL-38 and SJIA. However, such a connection could be hypothesised as there is evidence suggesting an association with the adult-onset version of JIA, AOSD, indirectly through IL-36R,[15] which is one of the binding partners of IL-38.[10] Furthermore, in SLE, as with IL-37, higher levels of IL-38 are also detected in the blood of patients compared to healthy subjects.[10]

When this evidence is taken into account, a notable gap of knowledge in this field is the role of IL-38 in JIA. Thus, by attempting to replicate evidence surrounding the use of IL-37 as an inflammatory biomarker, this study aims to evaluate and consider the consistency of the roles of both IL-37 and IL-38 in children with JIA. Whilst confirming previous findings across JIA subgroups, a further objective is to provide novel evidence surrounding the part played by IL-38 in JIA.

MATERIALS AND METHODS

Sixty JIA patients fulfilling the International League of Associations for Rheumatology[16] classification criteria for JIA, who were referred to the rheumatology clinic at Baghdad Teaching Hospital and diagnosed by consultant rheumatologists, were recruited into this study. Additionally, 90 healthy age- and sex-matched control subjects were conscripted from healthcare units in Baghdad; their health status was ascertained based on a clinical evaluation by physicians.

All patients were assessed using the juvenile arthritis disease activity score-27.[17] Laboratory tests, including C-reactive protein (CRP) and rheumatoid factor (RF), were carried out for all subjects. Slide agglutination tests were used for the qualitative assessments of CRP (CRP-Latex, Spinreact Spain; Ref. ID: 1200305) and RF (RF-Immuno-Latex, La Wama Diagnostica, Brazil; Ref. ID: 28100-L).

IL-37 and IL-38 serum levels were measured by enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer instructions (MyBioSource, USA; Catalogue Numbers: RDEEH1120 (IL-37) and RDEEH14717 (IL-38)).

This research study has been approved by the Scientific Ethical Committee of the College of Medicine, University of Baghdad. All subjects' guardians gave informed consent according to the 2008 Declaration of Helsinki.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (version 21; SPSS, IBM). An independent samples' Student's *t*-test was employed for comparisons of quantitative variables between the studied groups, i.e., age/year and serum IL-37 and IL-38 levels. Data in a normal distribution were expressed as mean ± standard deviation. A Pearson chi-square test (χ^2) was used for comparisons of the qualitative variables between studied groups, i.e., age groups/year and gender. A binomial Z test was applied for RF and CRP assays. Data were given as (number) percentage. Finally, the validity of the ELISA test was estimated using the following parameters: ROC curve; cut-off value; area under curve (AUC); sensitivity (true positive) (%); specificity (true negative) (%); positive predictive value (PPV) (%); negative predictive value (NPV) (%); and accuracy (%). A *p*-value <0.05 was deemed statistically significant.

RESULTS

Sixty patients with JIA were recruited in the current study, i.e., 19 (31.7%) males and 41 (68.3%) females. Their ages ranged from 1 to 17 years, with a mean of 10.37±4.21 years. Additionally, 90 healthy persons were included as controls, i.e., 35 (38.9%) males and 55 (61.1%) females, with ages ranging from 3 to 19 years; mean age was 11.13±3.84 years. Evaluation of the groups' demographics showed that there were no differences between the subjects with JIA and the control group. The demographic data for the two cohorts are presented in Table I.

In patients with JIA, 42 (70%) were seronegative for RF and 18 (30%) were seropositive ($p<0.01$). The number of patients with negative and positive CRP levels were equivalent, i.e., 26 (45.33%) and 34 (56.67%), respectively (Table II).

When the distribution of positive and negative assays for CRP and RF, respectively, were compared within the varying demographic groups, no differences were seen (Tables III and IV).

The only exception was in those patients who were seronegative for RF, where a higher proportion of females compared to males was noted, i.e., 34 (78.05%) versus 9 (21.95%) ($p<0.05$) (Table IV). An equivalent distribution of RF seropositivity was noted between the sexes.

Compared to controls, serum IL-37 levels were increased in patients with JIA (117.98±209.282ng/ml vs. 37.87±24.496ng/ml; $p<0.001$), whereas serum IL-38 titres were diminished in individuals with JIA (106.2±95.781 ng/ml vs. 182.24±108.428 ng/ml) (Table V).

A positive correlation between serum IL-37 and IL-38 levels was demonstrated (r=0.633, *p*-value <0.001). In contrast, no correlation was identified between patients' age/year and either serum IL-37 levels (r=0.099, *p*-value =0.454) or IL-38 concentrations (r=0.021, *p*-value =0.88).

Validity of tests

The validity of the tests for serum IL-37 and IL-38 titres relating to their potential value for the diagnosis of JIA was assessed. For serum IL-37, the following parameters were obtained: cut-off value, 31.2 ng/ml; AUC, 0.549; sensitivity,

Table I: Demographic data for study groups: patients with juvenile idiopathic arthritis versus controls

Demographics		Control, n=90	Patient, n=60	*p*-value
Age / Year, Mean±SD	11.13±3.84	10.37±4.21	0.251	
Age groups / Year, n (%)	1–5	16 (17.8%)	8 (13.3%)	0.389
	6–10	43 (47.8%)	24 (40%)	
	11–15	22 (24.4%)	17 (28.3%)	
	16–20	9 (10%)	11 (18.3%)	
Gender, n (%)	Male	35 (38.9%)	19 (31.7%)	0.367
	Female	55 (61.1%)	41 (68.3%)	

Table II: Distribution of rheumatoid factor (RF) and C-reactive protein (CRP) amongst patients with juvenile idiopathic arthritis

		Patient, n=60		
Assay		N	%	*p*-value
CRP	Negative	26	43.33	0.366
	Positive	34	56.67	
RF	Negative	42	70	0.003
	Positive	18	30	

Table III: Distribution of C-reactive protein (CRP) amongst patients (n=60) with juvenile idiopathic arthritis: comparison of demographic data

Demographics		CRP		*p*-value
		Negative, n=42	Positive, n=18	
Age (Year), Mean±SD		10.50±4.052	10.26±4.385	0.832
Age groups (Year), n (%)	1–5	2 (7.7%)	6 (17.6%)	0.664
	6–10	12 (46.2%)	12 (35.3%)	
	11–15	7 (26.9%)	10 (29.4%)	
	16–20	5 (19.2%)	6 (17.6%)	
Gender, n (%)	Male	9 (34.6%)	10 (29.4%)	0.668
	Female	17 (65.4%)	24 (70.6%)	

Table IV: Distribution of rheumatoid factor (RF) amongst patients (n=60) with juvenile idiopathic arthritis: comparison of demographic data

Demographics		RF assay		*p*-value
		Negative, n=42	Positive, n=18	
Age (Year), mean±SD		10.57±4.162	9.89±4.404	0.569
Age groups (Year), n (%)	1–5	5 (11.9%)	3 (16.7%)	0.869
	6–10	16 (38.1%)	8 (44.4%)	
	11–15	13 (31%)	4 (22.2%)	
	16–20	8 (19%)	3 (16.7%)	
Gender, n (%)	Male	9 (21.95%)	9 (50%)	0.046
	Female	32 (78.05%)	9 (50%)	

Table V: Mean distribution of serum IL-37 and IL-38 levels amongst patients with juvenile idiopathic arthritis and controls and rheumatoid factor (RF) and C-reactive protein (CRP) assay results

Parameters		n	IL-37 (ng/ml)		IL-38 (ng/ml)	
			Mean±SD	*p*-value	Mean±SD	*p*-value
Study groups	Control	90	37.87±24.496	<0.001	182.24±108.428	<0.001
	Patient	60	117.98±209.282		106.20±95.781	
CRP assay	Negative	26	129.67±209.388	0.709	96.49±82.120	0.497
	Positive	34	109.04±211.903		113.61±105.651	
RF assay	Negative	42	98.20±158.040	0.267	112.08±103.094	0.472
	Positive	18	164.15±297.567		92.47±76.949	

55 %; specificity, 47.8%; PPV, 41.2%; NPV, 61.4%; and accuracy, 50.67%. These values failed to reach significance, (p=0.31).

For serum IL-38, the following parameters were obtained: cut-off value, 95.56 ng/ml; AUC, 0.749; sensitivity, 61.7 %; specificity, 67.8%; PPV, 56.1%; NPV, 72.6%; and accuracy, 65.34% (p-value <0.001).

DISCUSSION

Since JIA is an autoinflammatory condition, the roles of inflammatory and anti-inflammatory cytokines in this pathology are undisputed.[18] Amongst the key anti-inflammatory cytokines involved in the downregulation of inflammation in JIA is IL-37, whilst the function of its close and recently discovered family member, IL-38, remains unclear. Thus, the aim of this study was to confirm previous findings that described the role of IL-37 in patients with JIA as well as to investigate the role of IL-38 in this condition.

Demographic analysis of the study participants showed no significant differences in either age or gender between control and patient groups, thus indicating a high degree of subject matching. Analysis of CRP and RF status amongst the 60 patients with JIA revealed an equal distribution of patients between the CRP-positive and the CRP-negative groups. However, an uneven distribution of patients amongst the RF-positive and RF-negative groups was observed, with 70% of JIA patients exhibiting a negative RF status. Such results are consistent with the literature, which shows that most subtypes of JIA are RF-negative.19 Polyarticular JIA is the exception and can be either RF-positive or RF-negative.[19]

Demographic analysis of the JIA patient group according to RF status demonstrated that almost 80% of RF-negative patients were females, compared to the RF-positive group, in which the gender distribution was equal. This is in contrast to previous studies, which have shown that RF positivity is more common amongst females with JIA and, particularly, amongst those with polyarticular JIA.[19,20]

In line with previous evidence describing a key role of IL-37 in JIA, the current analysis revealed significantly higher levels of IL-37 in the JIA patient group compared to the controls, thus confirming previous findings.[13] More specifically, IL-37 has been shown to have an immunosuppressive function, mediated by the inhibition of inflammatory cytokine production.[9]

Although the levels of IL-37 expression were in line with the present hypothesis, results from this study demonstrated that the titres of IL-38, a recently discovered cytokine belonging to the IL-1-family, were significantly higher in the control group. This result is unexpected as previous study has shown that titres of IL-38 were increased in patients with autoinflammatory diseases, such as RA[21] and SLE.[22] Given the similar biological mechanisms underlying RA and JIA, the hypothesis that IL-38 may differ seems unlikely. However, there is some evidence that cytokine expression profiles differ between adult RA and JIA.[23] Thus, future study should aim to repeat the current experiment to ensure that no experimental shortcomings have interfered with the results. Furthermore, given the high levels of IL-38 in the control cohort, special attention should be paid to the possibility of higher-than-average levels of IL-38 amongst these patients, which could be owing to potentially undiagnosed conditions or the presence of outlier values. No significant differences were observed between the levels of IL-37 or IL-38, or between the CRP-negative and CRP-positive groups, or between the RF-negative and RF-positive cohorts, which may suggest the absence of a relationship between these factors.

In the patients with JIA, a strong positive correlation was revealed between the levels of IL-37 and IL-38. Although further study would have to be carried out to confirm this hypothesis, this finding could point towards a biological relationship between both interleukins in JIA. However, given the observation that IL-38 levels were significantly higher than IL-37 titres in control patients, it is likely that the positive correlation between IL-37 and IL-38 is only true in the context of JIA.

Finally, the suitability of IL-37 and IL-38 measurements to aid in the diagnosis of patients with JIA was analysed. A low specificity and sensitivity for IL-37 was obtained, together with an AUC of 0.549, indicating poor discrimination. Although the results for IL-38 revealed a good specificity and sensitivity, with an AUC of 0.749, the lower-than-expected levels of IL-38 in patients with JIA compared to healthy controls suggest the need for future validation of the role of IL-38 in JIA before its diagnostic utility can be determined.

CONCLUSION

This study provides another layer of evidence relating to the role played by IL-37 in JIA and creates new questions regarding the potential role of IL-38 in this condition.

CONFLICTS OF INTEREST

The author declares no conflicts of interest.

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