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Serum Neopterin Level in Children with Juvenile Idiopathic Arthritis

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ABSTRACT

Background: Juvenile idiopathic arthritis (JIA) is generally considered a clinical syndrome involving several disease subsets, with a number of inflammatory flows, leading to an eventual common pathway in which persistent synovial inflammation and associated damage to articular cartilage and underlying bone are present. Neoptrin is a reliable marker in the assessment of the rate of IFN-γ production. Levels of neoptrin increase in direct proportion with the level of interferon. Measurement of neopterin level is useful because of its relative stability also it is a prognostic indicator for cell-mediated immunity.

Aims: This study aims to assess serum level of neopterin in patients with Juvenile Idiopathic Arthritis (JIA) in relation to the disease activity, severity and response to conventional and biological therapy.

Methodology: The study was conducted on 30 patients (Group A) previously diagnosed as SoJIA, they were divided into two subgroups according to their therapy into Group AI on biological therapy (15 patients) and Group AII on conventional therapy (15 patients). These in addition to 20 healthy controls (Group B).

Results: Basic clinical evaluation and laboratory investigations were done. We found that JIA patients had significantly higher levels of serum neopterin than healthy controls. We also found a highly significant difference between neopterin levels in the activity and remission states among all patients (Group AI and Group AII).

Conclusion: We concluded that serum neopterin is a useful marker for cellular immune activation and also indicative of the activity of JIA. Our findings are supported by positive correlations between serum neopterin levels and other markers of activity as TLC, PLT counts, ESR, and CRP. We also concluded that serum neopterin is a sensitive and accurate predictor of disease activity where sensitivity of that test was 93.3% and accuracy was 72.5%.

Recommendations: Investigating the serum neopterin measurement in other autoimmune collagen diseases. Assessment the influence of biological therapy on neopterin levels in relation to disease progression.

Keywords: Serum Neopterin, Juvenile Idiopathic Arthritis.

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is generally considered a clinical syndrome involving several disease subsets, with a number of inflammatory flows, leading to an eventual common pathway in which persistent synovial inflammation and associated damage to articular cartilage and underlying bone are present[(1)].

Many biological therapies are now available for patients with rheumatoid arthritis (RA) who have an inadequate response to synthetic disease modifying anti-rheumatic drugs (DMARDs) especially methotrexate[(2)].

The 2015 American College of Rheumatology (ACR) treatment guideline addresses the use of DMARDs, biologics, tofacitinib, and glucocorticoids in early and established RA. It is also recommended to use various treatment approaches in frequently encountered clinical scenarios, including treat-to-target, switching between therapies, tapering of therapy, the use of biologics and DMARDs in high-risk RA patients[(3).]

Neopterin is a pyrazino-pyrimidine compound that is synthesized by monocytes and macrophages in response to interferon (IFN) which is produced by activated T-cells. It is a marker of cellular immune response, and levels are elevated in conditions of T-cell or macrophage activation, including autoimmune diseases such as systemic lupus erythematosus and JIA[(1)].

Neopterin is found at increased levels in biological fluids from individuals with inflammatory disorders. Due to its capacity to increase hemeoxygenase-1 content, it has been proposed as a protective agent during cellular stress[(4)].

Neopterin is a biologically stable metabolite, which gives an advantage of its detection in assessing the activity of the immune response[(5)]. An association between CRP and ESR levels as well as neopterin concentrations

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has been shown already earlier in patients with many diseases such as cardiovascular disease and malignancies as well as in patients with RA[6].

Neopterin is accepted as an immunologic marker and an indicator of activation of the immune system[7]. Neopterin levels in patients with active RA are noted to be accentuated[8].

AIM OF THE WORK

This study aims to assess serum level of neopterin in patients with Juvenile Idiopathic Arthritis (JIA) in relation to the disease activity, severity and response to conventional and biological therapy.

SUBJECTS AND METHODS

Study Design

This study was a pilot prospective study, conducted in the Pediatric Rheumatology, Allergy and Immunology Unit, Children's Hospital, Ain Shams University.

Sample

The sample was recruited between October 2016 and October 2017, on 30 patients with Systemic onset Juvenile Idiopathic Arthritis diagnosed according to American College of Rheumatology (ACR) for the diagnosis of SoJIA**,** as well as for International league of Association for Rheumatology (ILAR) classification of Juvenile idiopathic arthritis[9]. As shown in the following table:

Table (1): ILAR criteria for diagnosis of SJIA.[9]

Arthritis in any number of joints, together with fever of at least 2 weeks duration (quotidian in nature); daily for at least 3 days and is accompanied by one or more of the following:
• Evanescent erythematous rash.
• Generalized lymphadenopathy.
• Enlargement of the liver or spleen.
• Serositis.

A consent was obtained from each patients' legal guardian before enrollment in the study.

Study population

Thirty patients previously diagnosed as cases of JIA. They were studied over one year in relation to the clinical and laboratory indices of the disease activity and severity, and the relation to the response to conventional as well as biological therapy.

The study was approved by the Ethics Board of Ain Shams University.

20 control kids nearly of the same age and sex as the JIA patients, with neither history of rheumatologic illness nor family history of it. Those controls were collected from the outpatient clinic, Children's Hospital, Ain Shams University.

Children involved in the study were divided into the following groups:

Group A: 30 pediatric patients with systemic onset juvenile idiopathic arthritis, who were further subdivided into:

- ***Group AI*:** 15 patients with SoJIA on biological therapy (6 males and 9 females).
- ***Group AII:*** 15 patients with SoJIA on disease-modifying anti-rheumatic drugs (DMARDs) (10 males and 5 females).

Group B: 20 age and sex related healthy children serving as controls (11 males and 9 females).

Study measurements

All patients in the study were subjected to the following:

1- **Full medical history laying stress on:**
   - Demographic data; name, age, sex and consanguinity.
   - Symptoms and signs of disease activity.
   - Detailed drug history laying stress on the use of steroids, NSAIDs, DMARDs or biological drugs e.g.: anti-TNF monoclonal antibody or IL-1 receptor antagonist, and the duration and response to each of the previously mentioned medications.
   - Co-morbid illness as hypertension.

2- **Thorough clinical examination laying stress on** joint examination; arthritis, deformity, range of motion.

JIA disease activity assessment was done according to the Simplified Disease Activity Index (SDAI) depending on the recommendations of ACR, 2012.

Table (2): SDAI score for assessment of JIA activity [10]

Elements	SDAI	SDAI score interpretation
Number of swollen joints	Simple count (0-28)	0.0– 3.3 = Remission 3.4 – 11.0 = Low activity 11.1 – 26.0 = Moderate activity 26.1 – 86.0 = High activity
Number of tender joints	Simple count (0-28)	
Acute phase reactants	CRP in mg/dL	
Total index	simple calculation possible (0.1-86.0)	

3- Laboratory investigations:

- Complete blood count (CBC).
- Erythrocyte sedimentation rate (ESR).
- Serum level of C-reactive protein (CRP).
- Antinuclear autoantibodies (ANA).
- Serum neopterin assay using Enzyme-Linked Immunosorbent Assay (ELISA) technique to be withdrawn from all Children with JIA. Two samples were withdrawn from each patient, one sample during activity and the other one during remission both clinically and biochemically.

Blood collection and processing:

- Aseptic withdrawal of the blood specimens into sterile disposable syringes from every patient and control.
- 3 ml blood collected, in the plane red-top venipuncture tube without additives or anticoagulants were clotted.
- Centrifuge the specimen to separate the serum from cells, then stored at -80ºC, till the time of assay to avoid erroneous results from repeated freeze-thaw cycles.
- Thereafter, the frozen serum was calibrated at room temperature using ELISA.
- The yielding serum was used to measure serum neopterin level.

Assay of serum neopterin (nmol/L)

Serum neopterin levels were examined using an ELISA kit (Neopterin ELISA, IBL, Hamburg, Germany). The assay is based on the basic principle of competition between a peroxide-conjugated and non-conjugated antigen for a fixed number of antibody binding sites. The peroxidase conjugated antigen-antibody complexes bind to the wells of the micro filter strips, which are coated with a good anti-rabbit antibody. Unbounded antigen is then removed by washing. After the substrate reaction, the optical density is measured at 450 nm. A standard curve is plotted and neopterin concentration in the sample is determined by interpolation from the standard curve.

Data were analyzed using SPSS© Statistics:

Categorical variables were presented as numbers and percentage or ratio, and numerical data as mean and SD, range, and percentiles.

Normality of numerical data distributed was examined using the Shapiro-Wilk test. Non-normally distributed numerical variables were presented as median and interquartile range and intergroup differences were compared using Mann-Whitney test (for two-group comparison) or the Jonckheere-Terpstra trend test (for comparison of multiple ranked groups). Correlations were tested using the Spearman rank correlation.

As almost all laboratory data studied in different groups were unequally distributed, normality of numerical data distribution was examined using the Shapiro-Wilk test. Non-normally distributed numerical variables were presented as median and interquartile range and intergroup differences were compared using Mann-Whitney test (for two-group comparison) or Jonckeere-Terpstra trend test (for comparison of multiple ranked groups).

Basically, we compared the main 3 groups with each other (Group AI on biological therapy, Group AII on conventional therapy, and normal controls); patients were further divided into 2 subgroups (in activity and in remission).

RESULTS

I- Descriptive data

This study was conducted on 30 patients with SoJIA (Group AI: 15 patients with active SoJIA on biological therapy, Group AII: 15

patients with active SoJIA on DMARDs), and group B were healthy controls.

Patients' mean age was 8.4 ± 4 in group AI, 7.5 ± 3 in group AII, and 6 ± 3.5 in group B. In group AI, 6 (40%) patients were males and 9 (60%) were females. In group AII, 10 (66%) were males and 5 (33%) were females. Whereas, in group B 11 (55%) were males and 9 (45%) were females. Some personal data is summarized in table (3).

Table (3): Characteristics of the 3 studied groups

Variable		Value
Age (Years)	**Group AI**	8.4 ± 4 (range = 2 - 14)
	Group AII	7.5 ± 3 (range = 2.5 - 13)
	Group B	6 ± 3.5 (range = 1.5 - 12)
Sex (M/F)	**Group AI**	6/9
	Group AII	10/5
	Group B	11/9
Consanguinity	**Group AI**	4 (26.67%)
	Group AII	3 (20%)
	Group B	7 (23.33%)

Table (4) showed hypertension as a comorbidity in SoJIA where only 5 (33%) patients were hypertensive in Group AI, while all patients included in Group AII were not hypertensive.

Table (4): Hypertension as an associated comorbidity

HTN	Groups						Chi-Square	
	Group AI		Group AII		Total			
	N	%	N	%	N	%	X^2	P-value
Negative	10	66.67	15	100.00	25	83.33	6.000	0.014*
Positive	5	33.33	0	0.00	5	16.67		
Total	15	100.00	15	100.00	30	100.00		

There was a statistically significant difference between the two groups as regards hypertension (P value = 0.014).

ANA marker assay among patients is shown in table 5, where 14 (93%) patients were negative for ANA in Group AI, and 14 (93%) were negative for ANA in Group AII. While, only 1 (6%) patient was positive for ANA in either group.

Table (5): ANA marker assay

ANA	Groups						Chi-Square	
	Group AI		Group AII		Total			
	N	%	N	%	N	%	X^2	P-value
Negative	14	93.33	14	93.33	28	93.33	0.000	1.000
Positive	1	6.67	1	6.67	2	6.67		
Total	15	100.00	15	100.00	30	100.00		

Table 6 showed modalities of treatment the patients received, we had 12 (80%) patients on Actemra + corticosteroids + Methotrexate in Group AI, while 3 (20%) were on anakinra in the same group.

4 (26.67%) patients were on corticosteroids only in Group AII, 11 (73.33%) were on corticosteroids + Methotrexate in the same group.

Table (6): Treatment modalities in Group AI and Group AII

Treatment	Groups			
	Group AI		Group AII	
	N	%	N	%
Actemra + CS + MTX	12	80.00	0	0.00
Anakinra + CS	3	20.00	0	0.00
CS	0	0.00	4	26.67
CS + MTX	0	0.00	11	73.33
Total	15	100.00	15	100.00

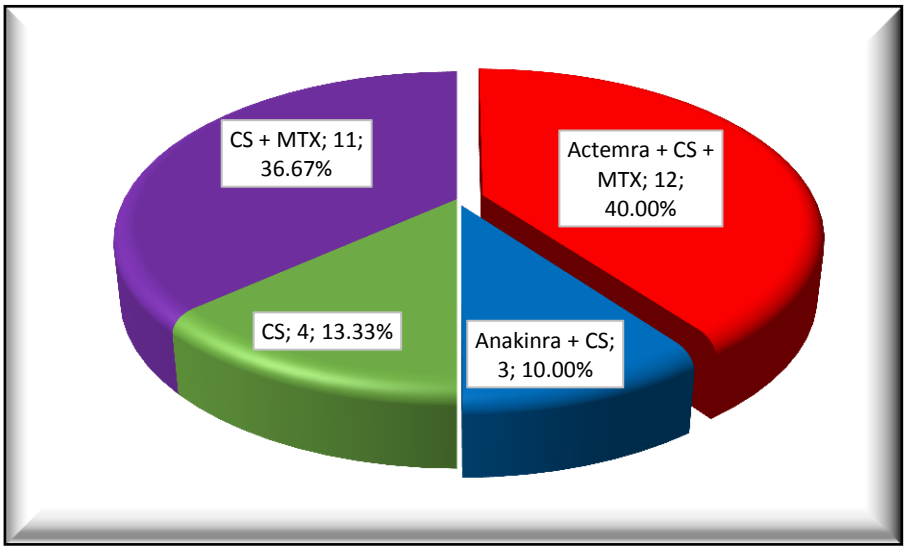

Figure (3): Treatment modalities in Group AI and Group AII.
(CS, corticosteroids; MTX, methotrexate)

II- Comparative data

Tables 7-11 display the comparison between total leukocytic count (TLC), platelet count (PTL), ESR, and CRP levels respectively in activity and remission states among patients.

In table 7 total leucocytic count (TLC) levels in patients in the activity ranged from 11 to 30 x10³/mm³ with a mean 18.123 x10³/mm³, while levels in same groups in the remission state ranged from 3.8 to 14.3 x10³/mm³ with a mean 9.300 x10³/mm³.There was a highly significant difference between TLC levels in both activity and remission states among patients (Group AI and Group AII) where $P < 0.001$.

Table (7): Total leukocytic count in the activity and remission states in Group AI and Group AII

Time	Total leucocytic count (× 10^3/mm^3)			Paired Differences		Paired Samples Test	
	Range	Mean ± SD		Mean	SD	T	P-value
Activity	11 - 30	18.123 ± 4.545		8.823	3.979	12.146	<0.001*
Remission	3.8 - 14.3	9.300 ± 3.309					

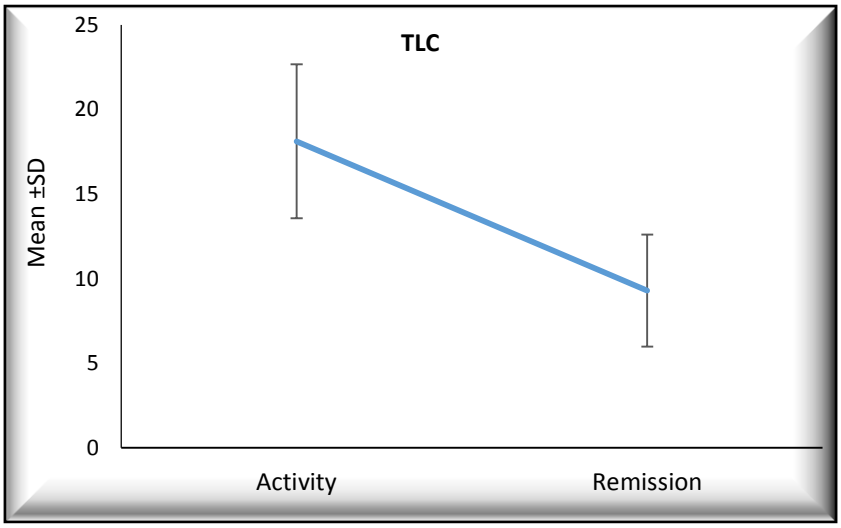

Figure (4): Total leukocytic count in both activity and remission states among patients (Group AI and Group AII).

Table (7) and figure (4) showed a highly significant difference between TLC levels within the two groups Group AI and Group AII in the activity and remission states (P < 0.001).

Table 8 shows platelet count(PLT) in the activity and remission states in patients, where PLT levels in patients in the activity states ranged from 201 to 940 x10³/mm³ (mean = 414.567x10³/mm³), while levels in the same groups in the remission state ranged from to 105 to 450 x10³/mm³ (mean 291.600x10³/mm³).

Table (8): Platelet count in the activity and remission states in Group AI and Group AII

Time	Platelets x 10³/mm³			Paired Differences		Paired Samples Test	
	Range	Mean ± SD		Mean	SD	T	P-value
Activity	201 - 940	414.567 ± 150.450		122.967	164.508	4.094	<0.001*
Remission	105 - 450	291.600 ± 93.473					

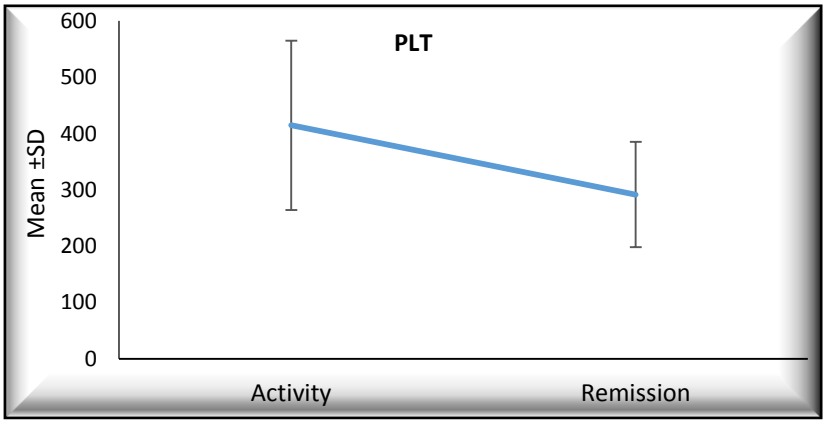

Figure (5): Platelet count in the activity and remission statesinGroup AI and Group AII.

Table (8) and figure (5) showed a highly significant difference between platelet count within the two groups Group AI and Group AII in the activity and remission states (P < 0.001).

Erythrocyte sedimentation rate (ESR) levels in the activity and remission statesin patients, table 9 shows that ESR levels in patients in the activity states ranged from 25 to 120 mm/hr (mean = 91.067mm/hr), while levels in same groups in the remission states ranged from 5 to 16 mm/hr (mean = 10.967 mm/hr).

Table (9): ESR levels in the activity and remission statesin Group AI and Group AII

Time	Erythrocyte sedimentation rate (mm/hr)		Paired Differences		Paired Samples Test	
	Range	Mean ± SD	Mean	SD	T	P-value
Activity	25 - 120	91.067 ± 21.641	80.100	21.392	20.509	<0.001*
Remission	5 - 16	10.967 ± 3.146				

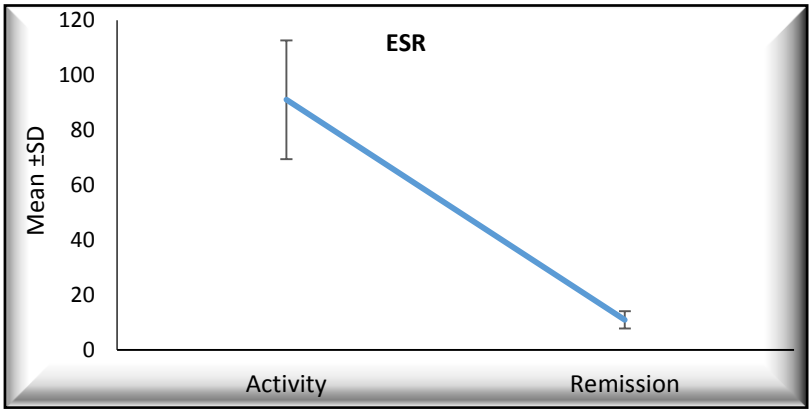

Figure (6): ESR levels in the activity and remission states in Group AI and Group AII.

Table (9) and figure (6) showed a highly significant difference between ESR levels in both the activity and remission states in Group AI and Group AII ($P < 0.001$).

C-reactive protein (CRP) level is shown in table 10, where CRP levels in patients in activity ranged from 6 to 171 (mean = 71.433), while levels in same groups in the remission was with a mean 6.

Table (10): CRP levels in the activity and remission statesin Group AI and Group AII

Time	C-reactive protein (mg/dl)		Paired Differences		Paired Samples Test	
	Range	Mean ± SD	Mean	SD	t	P-value
Activity	6 - 171	71.433 ± 42.126	65.433	42.126	8.508	<0.001*
Remission	6 - 6	6.000 ± 0.000				

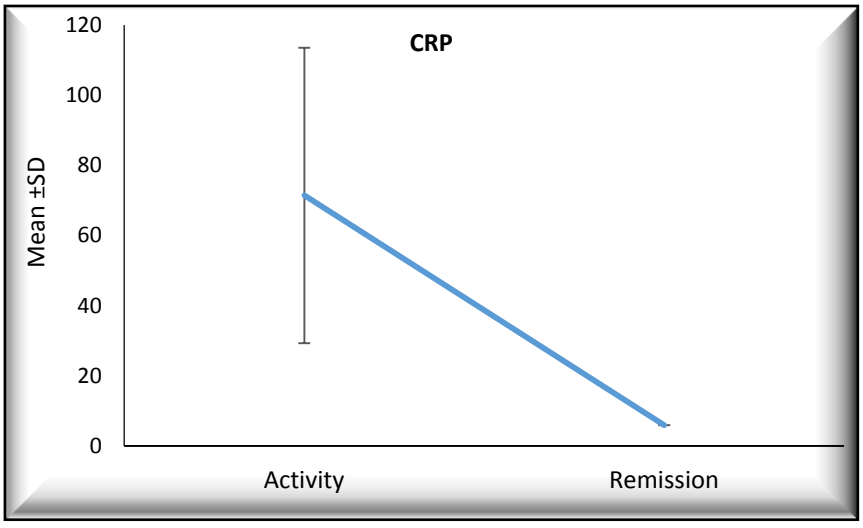

Figure (7): CRP levels in the activity and remission states inGroup AI and Group AII.

Table (10) and figure (7) showed a highly significant difference between CRP levels within the two groups (Group AI and Group AII) in the activity and remission states (P < 0.001).

The results of neopterin level assay is displayed in table 11 which compares between neopterin levels in the activity states in Group AI and Group AII, and in Group B, where it was shown that neopterin levels in Group AI patients in the activity state ranged from 4.9 to 12 nmol/L (mean = 7.23 nmol/L), and levels in Group AII patients in the activity state ranged from 5.3 to 10 nmol/L (mean = 7.32 nmol/L). While neopterin levels in Group B of control ranged from 2.4 to 8.8 nmol/L (mean = 5.53 nmol/L).

Table (11): Neopterin levels in the activity state in Group AI, Group AII, and Group B

Groups	Neopterin in Activity (nmol/L)				ANOVA	
	Range	Mean	±	SD	F	P-value
Group AI	4.9 - 12	7.233	±	2.096	5.177	0.009*
Group AII	5.3 - 10	7.320	±	1.286		
Group B	2.4 - 8.8	5.535	±	2.062		

TUKEY'S Test		
AI&AII	AI&B	AII&B
0.991	0.029*	0.021*

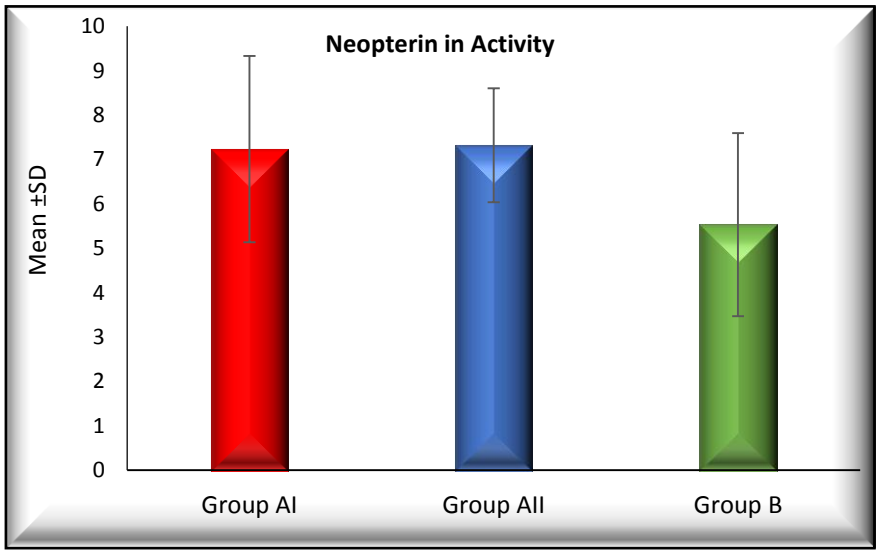

Figure (8): Neopterin levels in the 3 studied groups.

Table (11) and figure (8) showed neopterin levels in patients and in control, it was revealed that there was a highly significant difference in neopterin levels between AI and B groups, as well as between AII and B (p=0.029 & p=0.021) respectively, while there were no significant difference in neopterin level between AI and AII groups (p=0.991).

Neopterin levels in the activity and remission states among patients were compared and displayed in table 12, where it was revealed that neopterin levels in activity ranged from 4.9 to 12 nmol/L (mean = 7.277 nmol/L), while levels in the same groups in remission ranged from to 2 to 6.8 nmol/L (mean = 3.507 nmol/L).

Table (12): Neopterin levels in the activity and remission states among all patients (Group AI and Group AII)

Time	Neoptrin (nmol/L)			Paired Differences		Paired Samples Test	
	Range	Mean ± SD		Mean	SD	t	P-value
In Activity	4.9 - 12	7.277 ± 1.709		3.770	1.241	16.635	<0.001*
In Remission	2 - 6.8	3.507 ± 1.092					

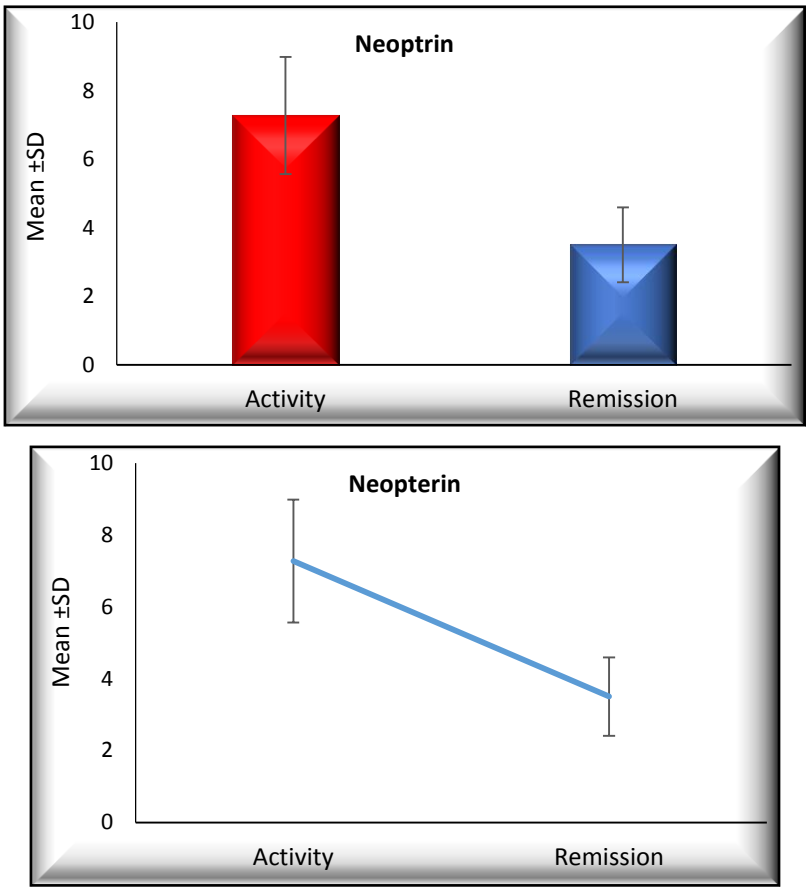

Figure (9): Neopterin levels in the activity and remission states among all patients (Group AI and Group AII).

Table (12) and figure (9) showed a highly significant difference between neopterin levels within the two groups (Group AI and Group AII) in the activity and remission states (P < 0.001).

Basically, we compared neopterin levels in the activity and remission states among patients in each group, to assess its relation with remission. Table 13 displays this comparison. For Group AI, neopterin levels in patients in the activity states ranged from 4.9 to 12 nmol/l (mean = 7.233 nmol/l), while levels in same group in the remission states ranged from 2 to 5.8 nmol/l (mean = 3.780 nmol/l), and a meandecline value in serum neopterin 3.780 nmol/l.

For Group AII, neopterin levels in patients in the activity states ranged from 5.3 to 10 nmol/l (mean = 7.320), while levels in same group in the remission states ranged from 2.4 to 6.8 nmol/l (mean = 3.560), and a meandecline value in serum neopterin 3.760 nmol/l.

Table (13): Neopterin levels in both the activity and remission states and decline values among patients in each group

Neopterin		Groups						T-Test	
		Group AI			Group AII			t	P-value
In Activity	Range	4.9	-	12	5.3	-	10	-0.136	0.892
	Mean ±SD	7.233	±	2.096	7.320	±	1.286		
In Remission	Range	2	-	5.8	2.4	-	6.8	-0.263	0.794
	Mean ±SD	3.453	±	1.137	3.560	±	1.082		
Decline values	Mean ±SD	3.780	±	1.182	3.760	±	1.339		
Paired Test	P-value	<0.001*			<0.001*				

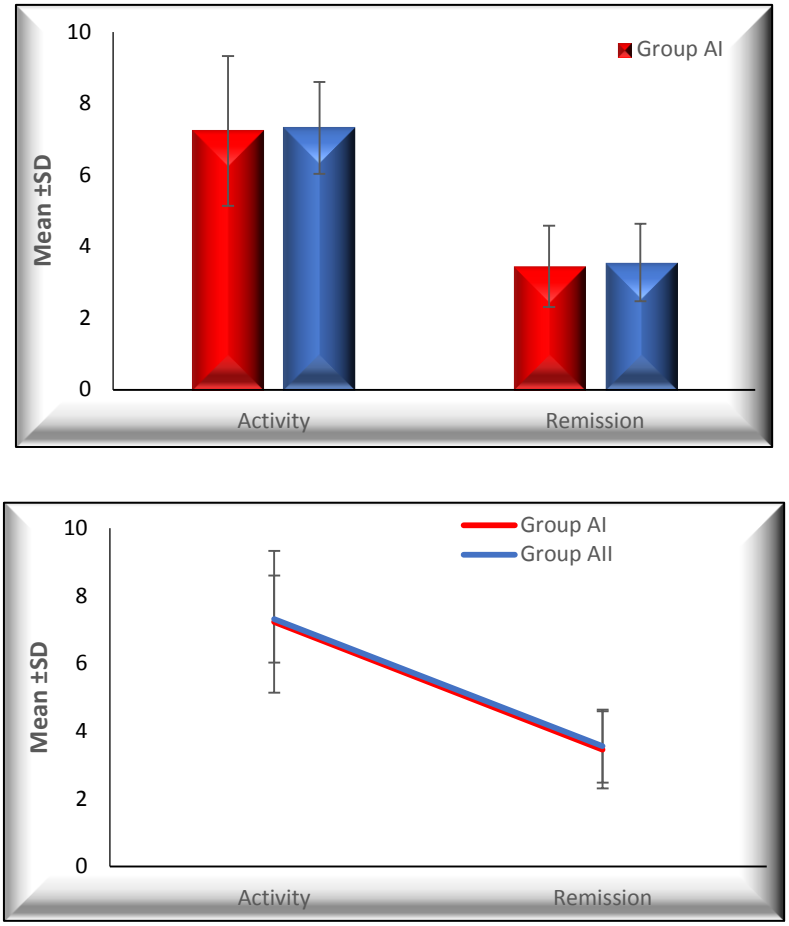

Figure (10): Neopterin levels in the activity and remission states among patients in each group.

Table (13) and figure (10), a highly significant difference between neopterin levels in the activity and remission states among patients in both Group AI and Group AII was revealed ($P < 0.001$).

III- Correlative data for serum neopterin and parameters listed in our study:

Table 14 displays the correlation between serum neopterin level in both activity and remission states with different modalities of treatment in Group A.

Table (14): Relation between neopterin level in the activity and remission states with different modalities of treatment

			N	Mean	±	SD	T or F	P-value
Neopterin in activity states	**Group AI**	**Actemra + CS + MTX**	12	7.017	±	1.912	0.521	0.672
		Anakinra + CS	3	8.100	±	3.041		
	Group AII	**CS**	4	6.725	±	0.885		
		CS + MTX	11	7.536	±	1.374		
Neoptrin in Remission states	**Group AI**	**Actemra + CS + MTX**	12	3.325	±	1.045	0.295	0.828
		Anakinra + CS	3	3.967	±	1.595		
	Group AII	**CS**	4	3.450	±	0.603		
		CS + MTX	11	3.600	±	1.235		

Mean neopterin levels in the activity state in the 12 patients receiving Actemra + CS + MTX and the 3 patients receiving Anakinra + CS in Group AI were 7.17 and 8.1 nmol/l respectively. Whereas, mean neopterin levels in the 4 patients receiving CS and the 11 patients receiving CS + MTX were 6.72 and 7.53 nmol/l respectively. In the remission states, mean neopterin levels in the 12 patients receiving Actemra + CS + MTX and the 3 patients receiving Anakinra + CS in Group AI were3.32 and 3.97 nmol/l respectively, and in the 4 patients receiving CS and the 11 patients receiving CS + MTX were3.45 and 3.60 nmol/l respectively.

Table 15 displays the correlation between different modalities of treatment and the decline value of serum neopterin in Group AI and AII (difference between levels in activity and remission states for each single line of treatment).

Table (15): Correlation between different modalities of treatment and the value of neopterin decline post-treatment

			Value of neopterin decline post-treatment				ANOVA	
			N	Mean	±	SD	F	P-value
Treatment	**Group AI**	**Actemra + CS + MTX**	12	3.692	±	1.164	0.354	0.787
		Anakinra + CS	3	4.133	±	1.447		
	Group AII	**CS**	4	3.275	±	0.299		
		CS + MTX	11	3.936	±	1.535		

There was no correlation between different lines of treatment and the value of neopterin decline post-treatment (p=0.787).

Studying the relation between neopterin levels and patients' sex and age was displayed in table 16 and 17.

Table (16): Relation between neopterin levels in the activity and remission states and patients' sex

	Neopterin in Activity	Neoptrin in Remission
	Mean	**Mean**
Male (16)	7.956	3.656
Female (14)	6.500	3.336
T-Test or ANOVA	**P-value**	**P-value**
	0.017*	0.432

Positive correlation was seen between serum neopterin level and male sex, as the mean in males was higher than that in females in the activity states only (p=0.017).

Table (17): Correlation between neopterin level in the activity and remission states and patients' age

Correlations				
	Neopterin in activity		Neopterin in Remission	
	r	P-value	R	P-value
Age	-0.153	0.419	-0.172	0.362

As per table 16 and 17, neopterin has a positive correlation with male sex and has no correlation with patients' age.

Correlation between serum neopterin level and other markers of inflammation:

In table 18 a positive correlation between TLC, PLT count, ESR levels, CRP levels and neopterin levels was shown in the activity state, with a P value <0.001 for TLC, PLT count, and CRP, while P value was 0.039 for ESR.

In table 19 a positive correlation between TLC and ESR, and neopterin levels in remission ($p < 0.001$).

Table (18): Correlation between neopterin andtotal leukocytic count (TLC), platelet count (PLT), ESR, and CRP in the activity state

Correlations		
	Neoptrin in activity	
	R	P-value
TLC in activity	0.797	<0.001*
PLT in activity	0.688	<0.001*
ESR in activity	0.379	0.039*
CRP in activity	0.839	<0.001*

Table (19): Correlation between neopterin andtotal leukpcytic count (TLC), platelet count (PLT) and ESR in the remission state

Correlations		
	Neoptrin in remission	
	r	P-value
TLC in remission	0.603	<0.001*
PLT in remission	0.303	0.104
ESR in remission	0.607	<0.001*

Neopterin level shows a positive correlation with other markers of inflammation in both activity and remission states with statistically significant P values.

IV – Predictive data:

At a cutoff value > 5.5, neopterin shows 93.3% sensitivity, 55% specificity in the 75.5 PPV and 84.6 NPV, and 72.5% accuracy.

Table (20): ROC curve between Group A in activity and Group B

ROC curve between Group A in activity and Group B					
Cutoff	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
>5.5	93.33	55.00	75.7	84.6	72.5%

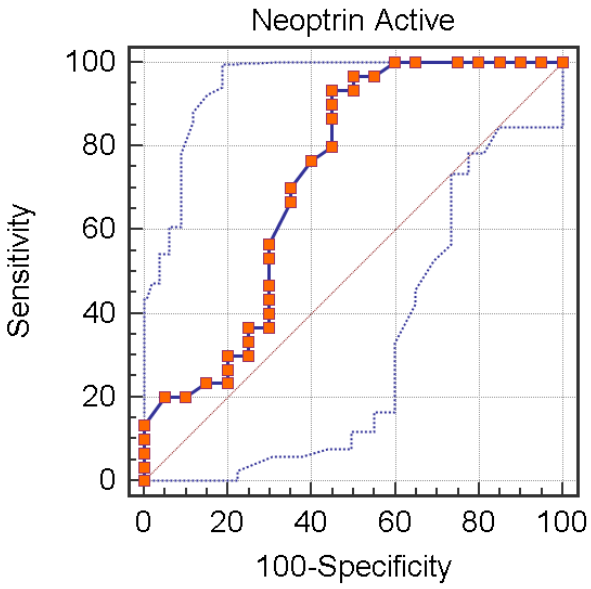

Figure (11): ROC curve for sensitivity of neopterin.

From the above table and figure, it was shown that neopterin is a sensitive and accurate predictor for disease activity in JIA.

DISCUSSION

Juvenile idiopathic arthritis (JIA) is the common term for all forms of arthritis that begin before age 16 years, persist for more than 6 weeks, and for which the etiology is unknown[(11)].

Patients with JIA often experience diminished health-related quality of life (HRQOL) with respect to both physical functioning and emotional state due to the pain, stiffness, fatigue and disability that can result from this inflammation.[(12)]

Early and sensitive markers are needed to evaluate the activation of the immune system and relate it to its pathogenesis as well as selecting the most suitable therapy for each patient[(13)].

Neopterin levels have been regarded as a biochemical marker of cell-mediated immune response and inflammation. Good correlation between neopterin concentrations and disease activity may facilitate assessing disease activity.[(13)]

Elevated neopterin concentrations in patients with early JIA indicate immune stimulation related with IFN-γ which is identified as a major participant in the inflammatory process[(13)].

Neopterin levels in chronic inflammatory disease may provide a better understanding of progression of the disease as neopterin pathway could be involved in the inflammation ongoing in the disease[(14)].

The aim of our study was to explore serum levels of neopterin in children with JIA in relation to clinical and laboratory indices of disease activity and severity, in addition to its relation to the conventional as well as biological therapy.

Our prospective study included 30 patients with SoJIA, 15 of which were on biological therapy (Group AI: 12 patients on Actemra + corticosteroids + Methotrexate, while 3 patients were on Anakinra + corticosteroids), the other 15 patients were on conventional therapy, (Group AII: 4 patients were on corticosteroids only, while 11 patients were on corticosteroids + Methotrexate).

In our study, the mean neopterin level in the activitystate in Group AI patients (on biological therapy) was 7.23 nmol/l, and the mean of neoptrin levels in Group AII patients (on conventional therapy) was 7.32 nmol/l. Mean neopterin level in Group B (controls) was 5.53 nmol/l. We reported a highly significant difference between group AI and B groups, as well as between group AII and B regarding neopterin levels. This agrees with **Arshadi *et al.*[(8)]**who reported that mean neopterin level of 4.92 nmol/l was significantly higher in JIA patients, compared to that in the control group which was 4 nmol/l.

Our results revealed that sensitivity of neopterin was 93.3% and accuracy was 72.5% at a cutoff value >5.5 nmol/l, this displays the fact

that neopterin is a sensitive and accurate predictor for JIA activity.

This also comes in agreement with **Shady *et al.*** [1], whose study found high levels of neopterin in patients with JIA as compared to controls, and that neopterin is a significant predictor of disease activity.

D'agostino *et al.* [13]also agreed with our study results as the mean neopterin concentrations in early JIA patients (8.92nmol/l)were significantly higher than the mean neopterin concentrations in healthy volunteers (5.62 nmol/l).

This is also in agreement to **El-Lebedy *et al.*** [15] who found significantly higher levels of neopterin in JIA patients (11.46 ± 3.56 nmol/L) compared to healthy controls (4.74 ± 1.98 nmol/L).

In comparing neopterin levels during activity and remission, we observed a highly significant difference between neopterin levels in both states among all patients (Group AI and Group AII) with a mean 7.27 nmol/l during the activity state and 3.507 nmol/l during the remission state.

This agrees with the study done by **Arshadi *et al.*** [8]who stated that theneopterin levels were significantly higher in patients with active JIA compared to patients in remission group. Thus, indicating that neopterin is a sensitive marker for assaying background inflammation and disease activity in JIA patients and may be used as a marker for evaluation of therapy efficacy.

On the contrary **El-Lebedy *et al.*** [15] found no significant difference regarding neopterin levels in the different disease activity phases. The results indicated that neopterin is a marker of JIA but not a marker of disease activity in treated JIA patients.

In the current study, we found a positive significant correlation between TLC, PLT count, ESR levels, as inflammatory markers and neopterin levels in the activity state, with a P value <0.001 (my means were18, 414, and 91 respectively). This comes in agreement with **Shady *et al.*** [1]**.** In the present study, positive significant correlation was also found between CRP levels and neopterin in the activity states (p<0.001), while no detected significant correlations was found between serum neopterin level and CRP according to the study done by **Shady *et al.*** [1].

Our study also agrees with **Arshadi *et al.*** [8] who reported that neopterin levels were significantly correlated with inflammatory parameters including ESR and CRP, where CRP level had a higher correlation with neopterin level in JIA patients.

D'agostino *et al.* [13]also found a correlation between CRP and disease activity.

As for **Ozkan *et al.*** [14]their results also agree with our study as they reported significantly correlated neopterin levels with ESR in JIA patients.

However, in contrast to our study, **El-Lebedy *et al.*** [15] found no significant correlations between neopterin levels and any of the disease activity parameters.

Our current study revealed difference between neopterin levels in males and femalesand its level was significantly higher in males than femaleswith a mean 7.95 and 6.5 respectively. This is in agreement with **Arshadi *et al.*** [8]**,** wherethere was difference between neopterin level in males and females but its level was significantly higher in males (7.4) than females (4.8).

This agrees also with **El-Lebedy *et al.*** [15] where a significantly higher neopterin levels were observed among male patients [median 13.44 (12.65–16.21)] than female patients [median 11.86 (7.91–13.44)], P <0.0001.

Our study agrees with **El-Lebedy *et al.*** [15]where no significant correlations between neopterin and age were found. (p=0.419 in activity, p=0.362 in remission).

Although, in our study there was no correlation between age and neopterin levels in the activity and remission states, **Ozkan *et al.*** [14]reported that the ages were positively correlated with neopterin levels. That was the same as for **Arshadi *et al.*** [8] as there was a significant correlation between age and neopterin level in both JIA group and control group.

In our current study, to evaluate the effects of the different lines of treatment on measured parameters, JIA patients were divided into conventional treatment group and biological treatment group, and the neopterin decline values were measured in each group. None of the values of measured parameters differed significantly between treatment groups. The neopterin value of decline for Anakinra + CS comes in the first rank with a mean 4.133, followed by CS + MTX with a mean 3.936 in the second rank, followed by Actemra + CS + MTX with a mean3.692 in the third rank, while treatment with CS only

comes at the fourth and the last rank with a mean in neopterin value of decline3.275.

As for **Ozkan *et al.*[14]** there were no observed significant differences between treatment groups methotrexate treatment group (patients taking only methotrexate or methotrexate plus other drugs) and non-methotrexate treatment group (patients taking drugs without methotrexate).

CONCLUSION

From this prospective pilot study, we concluded that serum neopterin is a useful marker for cellular immune activation and also indicative of the activity of JIA. The serum neopterin levels showed significant increments during disease activity.

Our study results demonstrate that neopterin concentration measurement is a worthy immune activation marker for JIA.

RECOMMENDATIONS

It is recommended to assess the serum neopterin measurement in other autoimmune collagen diseases, and address the influence of biological therapy on neopterin levels in relation to disease progression.

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