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Original article

Diagnostic and prognostic values of antikeratin antibodies (AKA) and collagen III propeptides (PIIINP) in juvenile rheumatoid arthritis.

Background: *The need for specific and sensitive diagnostic as well as prognostic markers for juvenile rheumatoid arthritis (JRA) has urged scientific research toward this field.*

Objective: *We sought to assess the diagnostic and prognostic values of serum and synovial fluid collagen III propeptides (PIIINP) and antikeratin antibodies(AKA) in JRA.*

Methods :*Thirty-one JRA patients with a mean age of 10.6±4.2 years were enrolled in the study. They were compared to 10 SLE patients with arthritis and 15 age and sex matched healthy children as control groups. All patients were subjected at enrollment to laboratory evaluation by CBC, ESR, CRP, serum PIIINP and AKA. Patients with knee effusion were subjected to AKA and PIIINP analysis in their synovial fluid samples. Clinical evaluation was done by examination of joint swelling, tenderness and limitation of movement scores. Also, radiological evaluation by plain X-ray films for hands, knees and feet using modified Larsen score (MLS) was performed. Laboratory, clinical and radiological evaluation methods were re-performed after 3 months of proper treatment. A third radiological evaluation was done one year after the first evaluation.*

Results: *Serum PIIINP was significantly elevated in JRA patients (12.8±8.7) μg/L in comparison to healthy controls (7.0±1.9 μg/L, $p<0.05$). However, no significant difference was observed when compared to SLE patients. Reduction of serum PIIINP was observed after 3 months of treatment with no significant difference between JRA patients (10.5±8.8 μg/L) and the healthy controls. Synovial fluid PIIINP (56.4±6.6 μg/L) was significantly higher than that of the serum ($p<0.05$). Neither serum nor synovial fluid PIIINP correlated with any of the laboratory, clinical or radiological parameters. The serum AKA positivity rate was significantly higher in JRA patients (77% at the study onset and 90% after three months) as compared to the control group (7%, $p<0.001$). Serum AKA values recorded a sensitivity, specificity and diagnostic efficiency in JRA patients of 77%, 93% and 83% at the study onset. These values increased after 3 months to 90%, 93% and 91% respectively. Synovial AKA levels displayed no significant difference in comparison to that of the serum.*

Conclusion :*AKA is a specific and sensitive diagnostic marker for JRA. Meanwhile, PIIINP was significantly elevated during JRA disease activity particularly in the synovial fluid. Neither AKA nor PIIINP proved to have prognostic values in JRA.*

Key words: juvenile rheumatoid, arthritis, antikeratin antibodies, collagen III propeptides, SLE.

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INTRODUCTION

JRA is one of the most common rheumatic diseases of children. It is characterized by an idiopathic synovitis of the joints, associated with soft tissue swelling and effusion [1].

Advances in exploring joint tissue composition and pathophysiology have defined a number of biological markers that can serve as good diagnostic and prognostic predictors [2]. Antikeratin antibodies (AKA) are detectable at the time of initial diagnosis of adult RA and its positivity may have prognostic significance in the disease course [3,4]. Furthermore, it is considered now as one of the specific serological markers of RA [5]. PIIINP is a marker of type III collagen synthesis as well as an indicator of inflammation and healing processes in the joints [6]. PIIINP was reported to reflect

disease activity and to correlate with the grade of joint destruction in adult RA [7].

This study is aimed at evaluation of AKA and PIIIPN as diagnostic and prognostic markers in JRA.

METHODS

The study comprised 31 patients who fulfilled the American Association Criteria Update for Diagnosis of JRA [1,8]. They were enrolled from the Pediatric Allergy and Immunology Unit, Ain Shams University. They were 15 males and 16 females. Their age ranged from 4 to 17 years with a mean age of 10.6±4.2 years and a mean duration of illness of 53.7±42.4 months. Out of the 31 patients, eleven had knee effusion.

Any patient with evidence of hepatic or renal affection was excluded from the study to avoid extra-articular sources of changed PIIINP metabolism.

The patients were subgrouped into:

Group I : Comprised 20 patients with poly-articular JRA.

Group II : Comprised 5 children with pauci-articular JRA.

Group III : Comprised 6 children with systemic onset JRA.

The patients were compared to two control groups consisting of 25 age and sex matched children. The first group comprised 15 healthy children with a mean age of 9.8±4.7 years and the second comprised 10 systemic lupus erythematosus patients who were having arthritis with a mean age of 11.4±5.1 years.

All patients were subjected to :

I. Clinical history taking : to verify the diagnosis, duration of illness and the disease course.

II. Joints clinical evaluation : three clinical indices were used :

A. Joint swelling: graded from 0 to 3.

B. Pain or joint tenderness : graded from 0 to 3.

C. Limitation of movement (LOM): graded from 0 to 4.

Activity score was calculated according to Giannini et al., 1992 and VanRossum et al., 1998 [9,10].

Activity score =

$$\frac{\text{Sum of the 3 clinical indices for the examined joints}}{\text{Number of the affected joints}}$$

III. Laboratory investigations :

1. Routine investigations :

- ESR (Westergren method).
- C-reactive protein (latex agglutination test kit, Biotec Laboratories Ltd, UK).
- Complete blood picture (Coulter MicroDiff 18,USA).
- Serum rheumatoid factor (RF) by latex agglutination (Avitex-RF;Omega Diagnostics,UK).
- Antinuclear antibodies by indirect immuno-fluorescent microscopy (Sanofi Diagnostics-Pasteur,USA).

2. Measurement of PIIINP in serum and synovial fluid (PIIINP radioimmunoassay diagnostic kit, Orion Diagnostica, Finland) [6].

3. Detection of AKA in serum and synovial fluid (AKA immunofluorescence diagnostic kit, IMMCO Diagnostics, USA) [11].

Sampling for CBC, ESR, PIIINP and AKA were repeated for all patients after 3 months of the study onset.

IV. Radiological Assessment :

Knee joints, hands and feet were examined on 3 occasions : at the study onset, after 3 months and after 12 months.

Radiograph assessment was carried out by :

1. Rau and Herborn[12] method (Modified Larsen Scoring Method): scoring soft tissue swelling, joint space-narrowing, erosions.
2. Fuchs et al [13] method for scoring malalignment.

Statistical Methods

Statistical analysis of the results was done using a standard computer program (Statview 40, Abacus Concepts, Inc, Berkeley, CA,USA) employing the student t test as well as Mann-Whitney test for non-parametric data. The correlation coefficient (r) test was used to inter-relate the numerical data .

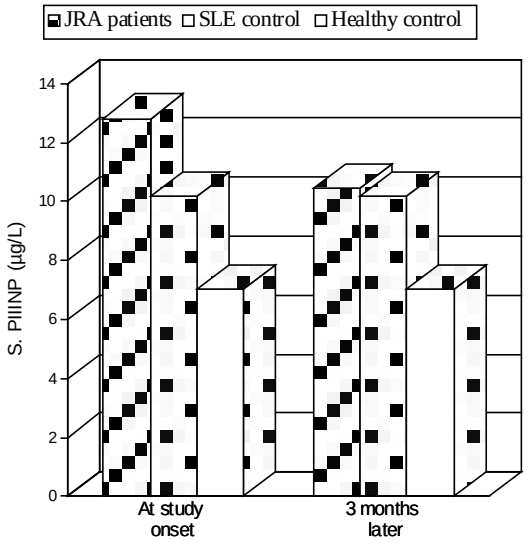
RESULTS

Serum PIIINP showed significant elevation at the study onset in JRA patients (12.8±8.7 μg/L) as compared to the healthy controls (7.0±1.9 μg/L, $p<0.05$). Meanwhile, comparing JRA patients to SLE patients with arthritis (10.2±3.1 μg/L) revealed non significant difference ($p>0.05$). After 3 months of proper treatment, no significant difference was observed between JRA patients (10.5±8.8 μg/L) and healthy children (7.03±1.95 μg/L, $p>0.05$) (Fig. 1).

On comparing serum PIIINP to SF PIIINP (56.4±6.6 μg/L); PIIINP was significantly higher in synovial fluid.

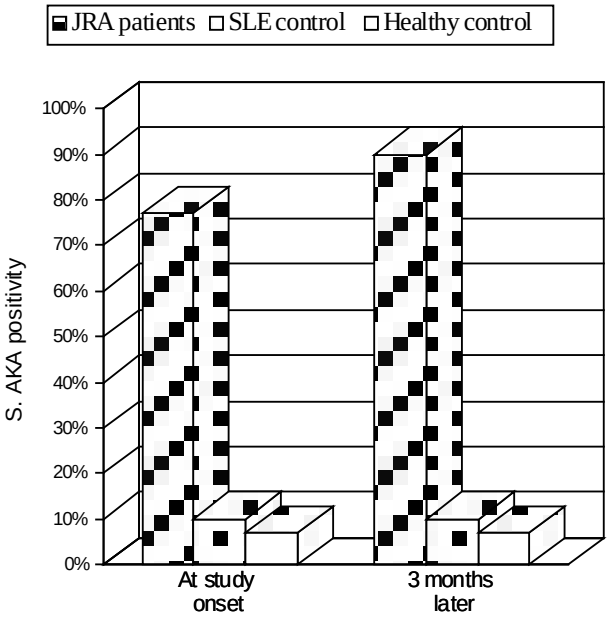
Serum AKA positivity was significantly higher in JRA patients at the study onset (77%) as compared to both healthy controls (7%, $p<0.001$) and SLE patients (10%, $p<0.001$). Three months later while still under treatment, serum AKA was significantly higher in JRA patients (90%) in comparison to both groups ($p<0.001$). Serum AKA showed increasing positivity with time despite treatment from 77% to 90% (Fig. 2).

Studying serum AKA sensitivity, specificity and diagnostic efficiency in JRA revealed percentage values of 77%, 93% and 83% respectively at the study onset, that increased to 90%, 93%, 91% respectively after 3 months (Table 1).



JRA = Juvenile rheumatoid arthritis; SLE = Systemic lupus erythematosus; S. PIIINP = Serum aminoterminal propeptide of type III procollagen

Fig. (1): Comparison of mean S. PNIIIP in all studied patients versus the control groups at study onset and 3 months later.



S. AKA = Serum antikeratin antibodies; JRA = Juvenile rheumatoid arthritis; SLE = Systemic lupus erythematosus

Fig. (2): Comparison of mean S. AKA positivity in all studied patients versus the control groups at study onset and 3 months later.

Synovial fluid AKA levels showed no significant difference as compared to those of serum AKA. The 3 subgroups of JRA were statistically comparable to each other in terms of serum PIIINP and serum AKA. Also, groups on different modalities of treatment were quite comparable to each other in serum PIIINP and AKA concentrations.

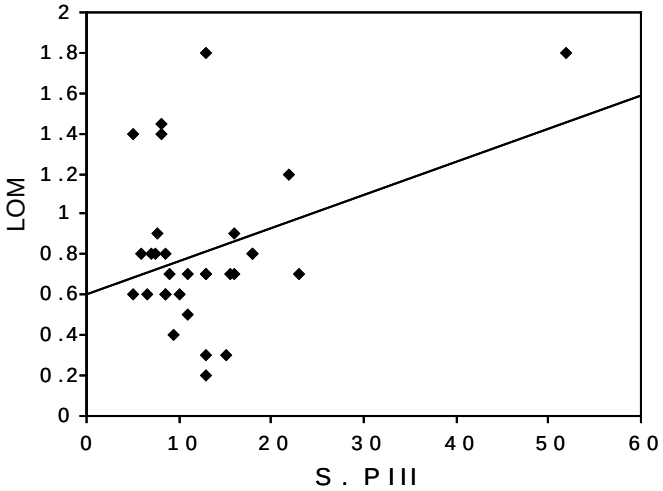
Serum PIIINP and SF/S PIIINP had no significant correlation with other studied laboratory indices including serum AKA, total clinical activity score or radiological score. The only exception was that serum PIIINP correlated positively to one clinical index which is the limitation of movement LOM ($r = 0.36$, $P<0.05$) (Fig.3).

Table (1): S. AKA sensitivity, specificity and diagnostic efficiency for JRA at study onset and 3 months later.

	S. AKA sensitivity	S. AKA specificity	S. AKA diagnostic efficiency
Month 0	77%	93%	83%
Month 3	90%	93%	91%

S. AKA = Serum antikeratin antibodies; JRA = Juvenile rheumatoid arthritis

Although the clinical activity score and laboratory indices showed significant improvement after 3 months of treatment ($p>0.05$) (Table 2), the radiological score did not show any significant change over 3 months or after 12 months ($p>0.05$). Moreover, 8 patients were reported to have some radiological changes after 12 months. On comparing their serum PIIINP levels to those who had no radiological changes, no statistically significant difference could be elicited ($p>0.05$) (Table 3).



r = 0.36; p<0.05 (Sig)

Fig. (3):Positive correlation between serum aminoterminal propeptide of type III procollagen (S. PIIINP) and limitation of movement (LOM).

Table (2): Comparison of the clinical scores of JRA patients at month 0 versus those at month 3.

	Joint swelling score	Tender-ness score	Limitation of movement score	Activity score
n	30	30	30	28
Month 0				
Mean	1.50	1.31	0.82	8.34
± SD	± 0.37	± 0.44	± 0.40	± 1.78
Month 3				
Mean	1.09	0.88	0.59	5.28
± SD	± 0.29	± 0.32	± 0.23	± 2.18
Z	4.35	4.26	3.40	3.92
P	<0.001 (Sig)	<0.001 (Sig)	<0.001 (Sig)	<0.001 (Sig)

Table (3): Comparison of the mean S. PIIINP in patients with radiographic changes versus those without, after one year of follow up.

S. PIIINP (µg/L)	JRA patients with radiographic changes	JRA patients without radiographic changes	Z	P
Month 0				
n	8	23		
Mean	9.71	13.03	0.81	>0.05
± SD	± 5.31	± 9.54		
Month 12				
n	7	22		
Mean	14.50	9.17	0.05	>0.05
± SD	± 16.6	± 4.07		

S. PIIINP = Serum aminoterminal propeptide of type III procollagen; JRA = Juvenile rheumatoid arthritis

DISCUSSION

JRA is characterized by the presence of numerous serum antibodies to various antigens [14]. However, most of these antibodies are poorly specific for JRA [15]. Rheumatoid factor is present in approximately 8% only of total JRA cases [1]. Moreover, the role of non specific markers of inflammation like ESR and CRP is limited in monitoring the disease course. Identification of reliable diagnostic and prognostic markers of the disease seems important[16].

In the current study two markers were investigated as potential diagnostic and prognostic markers in JRA namely collagen III propeptides (PIIINP) and antikeratin antibodies AKA.

In agreement with Hakala et al. [7] who reported significant elevation of serum PIIINP in adult RA, we found it significantly elevated in the studied JRA children. PIIINP is one of the main fibrillar collagens present in the synovium, and is primarily synthesized during inflammation and proliferation [17]. Therefore, it is expected that joint synovial tissue will be the main source of serum PIIINP in JRA patients. In the current study, a highly significant elevation was reported in synovial fluid taken from knee effusion in comparison to serum PIIINP level of the same patients. However, we could not record any superiority for SF/S PIIINP ratio over serum PIIINP in detecting disease activity, which may indicate that elevation of serum PIIINP might reflect its elevation in the synovial fluid.

In a trial to study the specificity of serum PIIINP to JRA disease activity, its mean level was compared to that of a group of SLE patients with arthritis. No significant difference could be detected indicating that it might be a product of the joint inflammatory process rather than a specific marker for JRA.

Follow up after 3 months of proper treatment revealed significant drop in the clinical activity score and other laboratory parameters. Meanwhile, serum PIIINP showed significant drop to near normal level. This indicates that serum PIIINP could be used to monitor the therapeutic response in JRA . There was no significant correlation between the total clinical activity score and serum PIIINP; however, serum PIIINP correlated positively with LOM, which is one component of the complicated clinical activity score. LOM index in JRA patients usually reflects disease inflammatory activity rather than joint destruction explaining why PIIINP correlated only with this component of the clinical activity score.

In the present study, there was no correlation between serum PIIINP and the radiological score. Also there was no significant difference in serum PIIINP between 8 cases who showed some radiological changes after 1 year and those who did not. The last 2 results indicated that a prognostic value for serum PIIINP in JRA patients could not be elicited in this study.

The prevalence of AKA positivity in the patients' sera was (77%) at study onset, which was relatively higher when compared to other studies. Gabay et al. [18] reported 27% AKA seropositivity in their study on JRA, while Hromadnikova et al.[19] reported 50% seropositivity. Paimela et al. [20] reported 40% positivity rate in adult RA patients. Ethnic variability and variations in disease duration might explain the difference.

A highly significant positivity of serum AKA was recorded in JRA patients (77%) in comparison to the healthy control group (7%) and also to SLE patients (10%) implying reasonable sensitivity and specificity of the test in JRA. The calculated AKA sensitivity, specificity and diagnostic efficiency in JRA patients at the study onset was 77%, 93% and 83% respectively. Meyer et al.[4] reported 63% sensitivity and 93% specificity for AKA in their study on adult RA patients, results that seemed comparable to ours.

AKA is considered one of the antifilaggrin autoantibodies (AFA). Filaggrin is thought to cross react with antibodies to a protein expressed by synoviocytes[21]. This may explain the diagnostic value of this marker. Another member of the (AFA) is the antiperinuclear factor (APF). El-Gamal et al.[22] reported APF specificity, sensitivity and diagnostic efficiency in JRA of 92%, 53% and 74% respectively. The lower sensitivity of APF reported in their study, in comparison to AKA in our study, could be because of the technical difficulties in the procedure of APF estimation.

After 3 months follow up, AKA seropositivity was significantly elevated from 77% to 90%. The calculated sensitivity, specificity and diagnostic efficiency increased to 90%, 93%, and 91% respectively. This means that diagnostic efficiency can improve with the advance in disease duration. In agreement, Paimela et al.[20] reported elevation in the seropositivity of AKA in adult RA patients from 26% to 40% over 2 years.

Serum or SF AKA had no correlation with clinical activity score, radiological score or other laboratory parameters. Gabay et al.[18] came to the same conclusion that there was no relation between AKA and disease severity or activity in JRA patients. On the contrary, strong relation to disease activity and severity in adult RA was reported by Girbal and associates[5].

In conclusion, serum PIIINP may reflect the inflammatory process of the joints and is a good marker for therapeutic control. Serum AKA positivity seems to be a good diagnostic marker for JRA; however, the study could not prove any prognostic value for either of the two markers.

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