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Original article

Prevalence and clinical value of IgA and hidden rheumatoid factors in juvenile rheumatoid arthritis.

Background: *Juvenile rheumatoid arthritis (JRA) is so difficult to be diagnosed early and a small subgroup of patients has positive IgM rheumatoid factor (RF) detected by the standard agglutination techniques.*
Objective: ***T****o investigate the prevalence of IgA and hidden RFs among patients with JRA, to evaluate their diagnostic value in comparison to classic RF and to outline their relation to disease activity, severity as well as to therapy.*
Methods: *The study included 46 patients with JRA (31 females and 15 males). Thirty patients had polyarticular JRA, 9 had oligoarticular JRA and 7 had systemic-onset JRA. Twelve patients had active disease. Thirteen systemic lupus erythematosus patients and 32 healthy subjects were studied as controls. Evaluation was carried out clinically and radiologically (using modified Larsen scoring). Laboratory investigations included CBC, ESR, classic IgM RF (latex agglutination), ANA (indirect immunofluorescence), IgA-RF (ELISA) and hidden RF seropositivity (ion exchange chromatography).*
Results: *All JRA patients had significantly higher IgA-RF (121.5 ± 195.4 mg/dL) and positivity of hidden RF (56.5%) than lupus (14 ± 6.6 mg/dL and 7.7% respectively) and healthy controls (13.7 ± 6.2 mg/dL and 0% respectively). Classic IgM RF had only 10.9% sensitivity in diagnosing JRA. IgA and hidden RFs had higher sensitivities (50% and 56.5% respectively). Specificity, positive and negative predictive values of IgA-RF were 97.7%, 95.8% and 65.7% and for hidden RF, they were 95.5%, 92.9% and 68.3%. Interestingly, combined positivity of IgA and hidden RFs had 100% specificity and positive predictive value for JRA. Classic RF did not correlate with disease activity and severity in terms of ESR, activity score and Larsen Index. In contrast, patients with active disease had significantly higher value of IgA-RF and positivity of hidden RF than those with quiescent disease. Also, IgA-RF had significant positive correlation with ESR, activity score and Larsen index. Similarly, patients with positive hidden RF had significantly higher values of ESR, activity score and Larsen index than those with negative hidden RF. Steroid therapy was associated with significantly higher level of IgA-RF and positivity of hidden RF, perhaps related to disease severity.*
Conclusion: *IgA and hidden RFs are more sensitive tests in diagnosing JRA than classic IgM RF. Also, the combined positivity of IgA and hidden RFs can confirm the diagnosis of JRA in doubtful cases. The fact that IgA and hidden RFs gave positive results in the meantime that classic RF was negative, together with their significant relation to disease activity and severity highlights their clinical value as reliable laboratory markers of JRA.*

Key words: juvenile rheumatoid arthritis, rheumatoid factors, IgA-RF, hidden RF, SLE.

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INTRODUCTION

Juvenile rheumatoid arthritis (JRA) is associated with certain generalized abnormalities of the immune system. A number of circulating autoantibodies including rheumatoid factors (RFs) are found in the serum of most patients with JRA [1].

Rheumatoid factors are a group of antibodies that react with the Fc portion of immunoglobulin G (IgG). Rheumatoid factors detected by standard agglutination techniques such as latex agglutination or the sheep cell agglutination tests are IgM. A small subgroup of patients with JRA has positive RFs.

Other RFs of IgG, IgA and IgE classes can also be identified by methods other than agglutination tests [2].

To date, the diagnosis of JRA has been based on the American College of Rheumatology (ACR) criteria and RFs are the only autoantibodies included among the classification criteria [1]. Raised titres of IgA-RF were detected in patients with rheumatoid arthritis and it was significantly associated with disease activity and worse functional capacity [3]. Similarly, Hidden RF was detected in the IgM containing fraction after separation of the sera of patients with JRA at an acid pH [4,5].

This work was conducted to determine the prevalence of IgA and hidden rheumatoid factors among patients with JRA and to evaluate their overall performance as diagnostic tests in this disease. The study also aimed at outlining their relation to disease activity, severity and mode of therapy.

METHODS

This study was carried out in the Pediatric Allergy and Immunology Unit, Children's Hospital, Ain Shams University. It included 46 patients fulfilling the American College of Rheumatology criteria for diagnosis of JRA [6]. They were 31 females and 15 males. Their ages ranged from 6 – 17 years with a mean of 12.4 ± 2.97 years and their disease duration ranged from 1 to 12 years with a mean of 5.26 ± 2.83 years. They were grouped according to the type of disease onset into:

*** *Group I:*** Comprised 30 children with polyarticular JRA. They were 20 females and 10 males. Their ages ranged from 7-16 years (mean 12.58 ± 2.9 years). Twenty-five patients were RF negative and only 5 were RF positive. Ten children were studied during disease activity [based on clinical ground and ESR according to ***Pinals et al., (1981)***] [7]. The remaining 20 children were studied during disease remission. All patients were receiving NSAIDs. In addition, 15 of them were on low dose oral prednisone, 4 were on methotrexate (MTX) and 6 were receiving both MTX and oral prednisone.

*** *Group II:*** Included 9 children with pauciarticular JRA. They were 6 females and 3 males. Their ages ranged from 7 – 16 years with a mean of 12 ± 3.3 years. Eight out of the 9 patients were in disease remission at the time of the study, while only one was studied during disease activity. NSAIDs were the only line of therapy in 7 patients, whereas one patient was also receiving oral prednisone and one was receiving MTX.

*** *Group III:*** Included 7 children with systemic-onset JRA (5 females and 2 males). Their ages ranged from 8-16 years (mean 12.14 ± 3.23 years). One patient only was studied during disease activity. All patients were receiving NSAIDs. In addition, 4 of them were on oral prednisone and 3 were on MTX therapy.

The range of doses of the received drugs was:

- NSAIDs (ibuprofen): 10 – 40 mg/kg/day.
- Oral prednisone: 0.25 – 1.5 mg/kg/day.
- Methotrexate (MTX): 7 - 10 mg/m^2/week either oral or IM.

Two groups of children were studied as control subjects.

Control group A: Included 13 patients with SLE attending the Pediatric Allergy and Immunology Clinic and diagnosed according to the (1982) revised criteria for the diagnosis of SLE [8]. All had evidence of arthritis at the time of the study. They were 9 females and 4 males and their ages ranged from 7-16 years (mean 12.3 ± 3.66 years).

Control group B: Comprised 32 healthy age and sex-matched children. They were 21 females and 11 males and their ages ranged from 7–16 years (mean 12.56 ± 2.83 years).

Methods

Patients were subjected to:

I) Clinical evaluation: with special emphasis on joint examination, systemic manifestations and current medications used.

Three clinical indices of articular inflammation were used to evaluate each joint according to the American Rheumatism Medical Information System (ARAMIS, 1984) [9]. They include joint swelling grades (0 = none, 1+ = mild synovial swelling or effusion with visible bony landmarks, 2+ = moderate swelling with definite obscuring of bony landmarks, 3+ = severe swelling with no obvious landmarks), pain on motion and / or joint tenderness grades (0 = none, 1+ = mild pain with no subjective reaction, 2+ = moderate pain, 3+ = marked pain), and limitation of motion grades (0 = full range of motion, 1+ = 25% limitation, 2+ = 50% limitation, 3+ = 75% limitation, 4+ = no motion possible).

The mean for each of the 3 clinical indices of all joints was calculated. Joints examined were right and left metacarpophalangeal, proximal interphalangeal, distal interphalangeal, wrist, elbow, shoulder, temporo-mandibular, knee, ankle, small joints of the foot with exclusion of the sacroiliac and hip girdle joints.

In addition to these indices, the total articular activity for each patient was then calculated considering the **affected joints only** as follows:

Activity score [10,11] =

1. Sum of the 3 clinical indices for each joint = X
2. $$\frac{\text{Sum of X of all examined joints}}{\text{Number of affected joints}}$$

II) Laboratory investigations: (for patients and controls)

Complete blood count (CBC) by Coulter Counter (Coulter Instruments, Model T660, Fullerton, California, USA), ESR (mm/1st hr) by Westergren method, CRP by latex agglutination test, ANA by indirect immunofluorescent microscopy.

Assay for rheumatoid factors:

- Classic IgM RF by latex agglutination method[5].
- IgA-RF by enzyme linked immunosorbent assay (ELISA) (Sigma Diagnostics, St. Louis, MO, USA)[12,13].
- Hidden RF by ion exchange chromatography (Sigma Chemical Co., St. Louis, MO, USA) [4,5].

Three ml whole blood were aspirated and were allowed to clot at 37°C for ½ an hour and then centrifuged at 3000 rpm for 10 min. Serum was collected into a clean Ependorf's tube and preserved at -20°C until assayed. Detection of hidden RF in the IgM fraction of the serum was done after separation of the serum at an acid pH. Results were expressed as positive or negative for hidden RF.

Quantitative measurement of IgA-rheumatoid factor was assayed by ELISA. The microplate was coated with Fc fragment of highly purified human immunoglobulin G. A cut off value was taken at the 95th percentile of healthy controls (23 mg/dL). Patients who had IgA-RF level above this value were considered to be positive for IgA-RF.

III) Radiographic evaluation (of knees, hands and feet)***:***

X-ray films were taken at the time of sampling. Evaluation was done after the method of Rau and Herborn [14] for scoring soft tissue swelling, joint space narrowing, osteoporosis and erosions:

0 Normal
1 Soft tissue swelling and/or joint space narrowing/subchondral osteoporosis.
2 Erosions with destruction of the joint surface (DJS) < 25%.
3 DJS (26 – 50%).
4 DJS (51 – 75%).
5 DJS (> 75%).

Joints examined radiologically were those of hands, feet and knees. A mean value of the score of all joints (Larsen index) was obtained for each patient.

Statistical Methods

The results were analyzed by commercially available software package (StatView, Abacus Concepts, Inc, Berkley, CA, USA). The data are presented as mean and standard deviation (SD). Student's "t" test was used to compared between two groups as regards parametric data, whereas Mann Whitney test was used for non-parametric data. Pearson "r" correlation coefficient was used to determine the relationship between different quantitative variables. Chi-square test was used for contingency tables. For all tests, a probability (p) of less than 0.05 was considered significant.

RESULTS

All patients with JRA had significantly higher mean value of IgA-RF and positivity of hidden RF than both lupus and healthy controls. The results of lupus and healthy controls were comparable. On the other hand, patients with polyarticular JRA had significantly higher value of IgA-RF and positivity of hidden RF than patients with oligoarticular and systemic-onset disease. The results of patients with oligoarticular and systemic-onset disease were comparable (Table 1, Fig. 1 and 2).

Classic IgM RF had only 10.9% sensitivity in diagnosing JRA. In contrast, IgA and hidden RFs had much higher sensitivities. The sensitivity of IgA-RF was 50% (76.7% for polyarticular JRA and 0% for both oligoarticular and systemic-onset disease), while the sensitivity of hidden RF was 56.5% (66.7%, 33.3% and 42.9% for polyarticular, oligoarticular and systemic-onset disease respectively). Specificity, positive and negative predictive values of IgA and hidden RFs were (97.7%, 95.8% & 65.7%) for the former and (95.5%, 92.9% & 68.3%) for the latter. Combined positivity of both IgA and hidden RFs had 100% specificity and positive predictive value for JRA, which were much higher than their sensitivity (43.5%) and negative predictive value (63.4%).

Table (1): Comparison of mean IgA-RF and hidden RF positivity in all groups.

	Mean IgA-RF		Hidden RF positivity	
	z	p	Chi-square	p
All patients versus healthy controls	4.49	< **0.001**	21.64	< **0.001**
All patients versus lupus controls	3.4	< **0.001**	5.32	< **0.05**
Lupus controls versus healthy controls	0.15	> 0.05	2.16	> 0.05
Polyarticular versus oligoarticular	2.9	< **0.05**	5.64	< **0.05**
Polyarticular versus systemic-onset	2.7	< **0.05**	5.03	< **0.05**
Oligoarticular versus systemic-onset	0.47	> 0.05	0.15	> 0.05

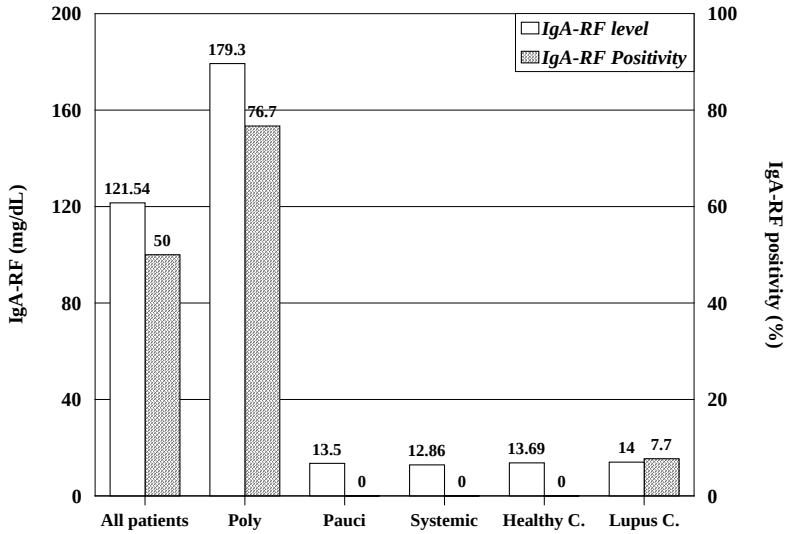


Fig. (1): Mean level and percent positivity of IgA-RF in different studied groups

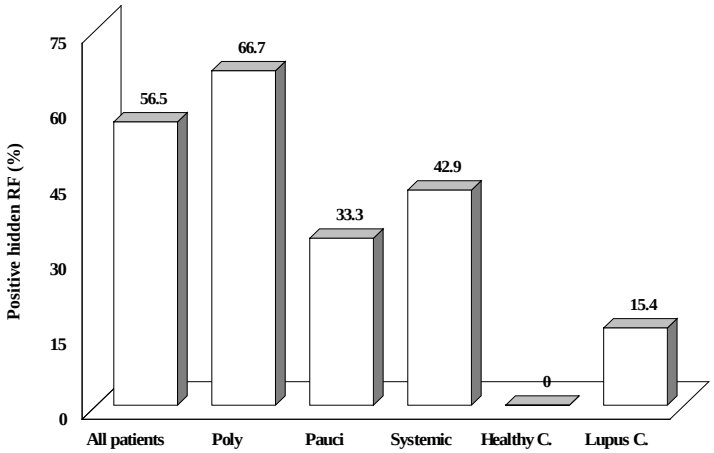


Fig. (2): Percentage of positivity of hidden RF in different studied groups

Classic IgM RF had no relation to disease activity and severity as evidenced by non-significant difference between values of activity score, ESR and Larsen index of patients with positive (5.5 ± 3.3, 22.7 ± 21.8 mm/1st hr and 1.9 ± 1.1) and those with negative classic RF (6.2 ± 0.4, 21 ± 2.2 mm/1st hr and 2.2 ± 0.4) ($p>0.05$). In contrast, patients with active disease had significantly higher value of IgA-RF and positivity of hidden RF than patients with quiescent disease. In addition, IgA-RF correlated positively with activity score, ESR and Larsen index and correlated negatively with Hb. Also, patients with positive hidden RF had significantly higher mean values of activity score and Larsen index than those with negative hidden RF (Figures 3, 4 and 5).

Steroid-treated JRA patients had significantly higher value of IgA-RF and positivity of hidden RF than non-steroid-treated patients (Fig. 6).

There was significant positive association between IgA-RF and hidden RFs as 20 out of the 23 IgA-RF positive patients had also positive hidden RF and 17 out of the 23 IgA-RF negative patients had negative hidden RF as well (Fig. 7). In addition, IgA-RF level was significantly higher in patients with positive hidden RF than those with negative hidden RF (202.2 ± 23.2 vs 16.7 ± 15.8 mg/dL) ($z = 3.58$, $p < 0.001$).

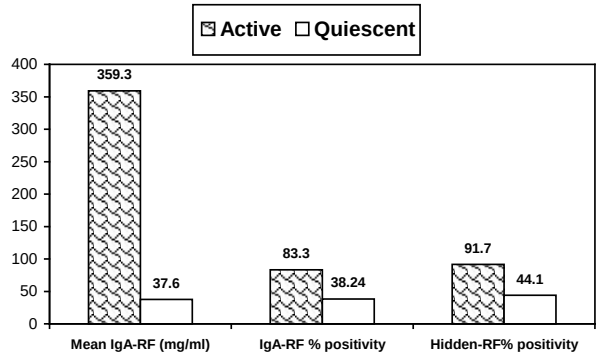


Fig. (3): IgA and hidden-RF in relation to disease status

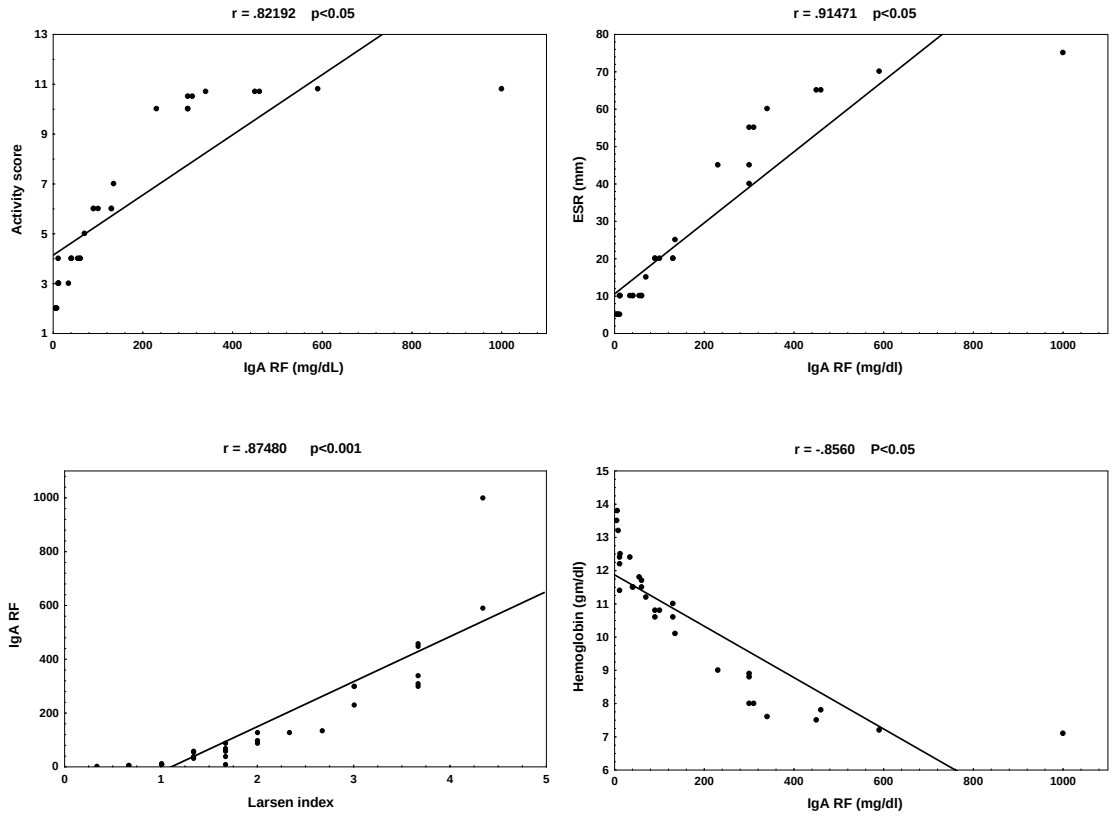
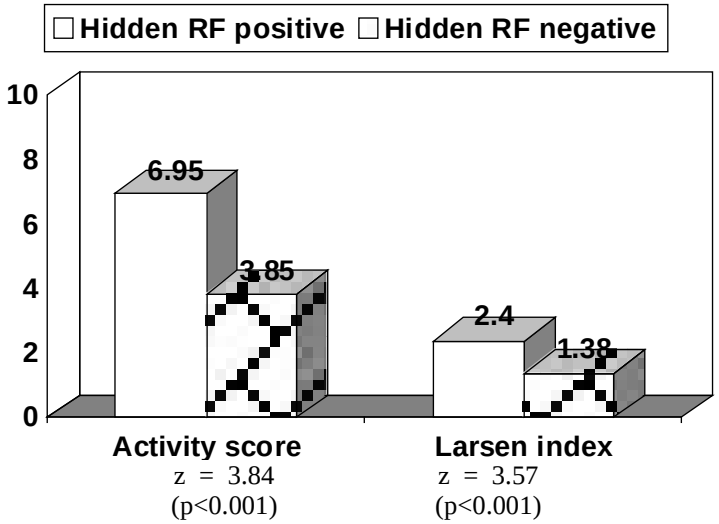


Fig. (4): Correlation between IgA-RF and ESR, Hb, activity score and Larsen index in patients with polyarticular JRA



z = 3.84 (p<0.001) z = 3.57 (p<0.001)

Fig. (5): Activity score and Larsen index in relation to hidden RF status

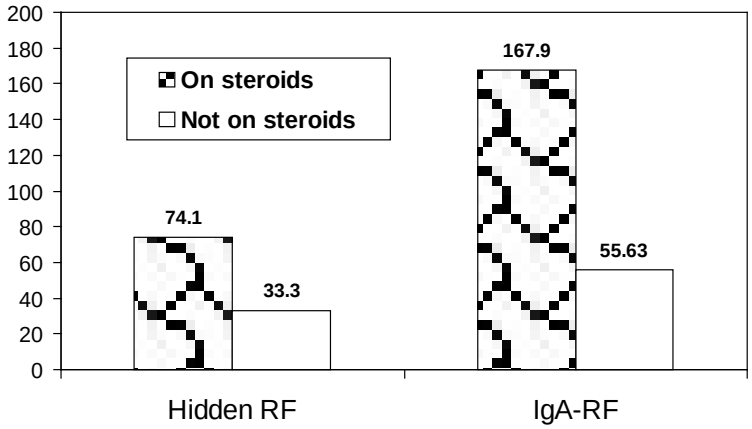
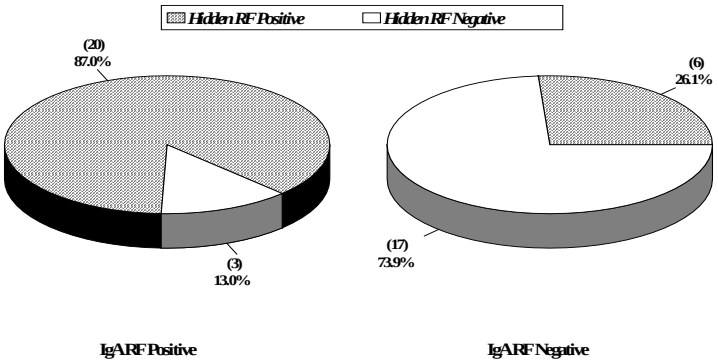


Fig. (6): IgA and hidden RFs in steroid versus non-steroid-treated JRA patients



$X^2 = 17.38$, $p < 0.001$

Fig. (7): Association between IgA-RF and hidden RF

DISCUSSION

Rheumatoid factor detected by standard agglutination techniques is IgM and it is detected in a small subgroup of patients with JRA. Hidden RFs are IgM-RFs detected in the IgM containing fraction after separation of the serum at an acid pH [4]. Other RFs of IgA, IgG and IgE classes can also be identified by methods other than agglutination tests [2].

In the present study, mean IgA-RF was significantly elevated in all JRA patients compared to the corresponding values of healthy and lupus controls. Similar findings were observed by Bharadwaj et al.[3], who also found a non-significant difference between serum IgA level of patients with positive and those with negative IgA-RF indicating that the elevated IgA-RF was not secondary to a rise in the serum IgA level. In addition, IgA-RF was polymeric suggesting its local production in the mucosa. The prevalence of IgA-RF seropositivity among our studied JRA patients was 50%. Previous researches reported a prevalence ranging from 22 – 58% in one study [15] and 40.8% in another one [3].

The prevalence of IgA-RF seropositivity was high in patients with polyarticular-onset disease (76.7%), whereas in patients with pauciarticular-onset disease and in those with systemic-onset disease the prevalence of IgA-RF was 100% negative. Therefore, it can be deduced that IgA-RF is specific for JRA patients of the polyarticular variety and that its presence in the serum is not a pre-requisite for joint or systemic manifestations of the inflammatory process in the other types of JRA. Walker et al. [16], reported IgA-RF positivity in 58% of patients with polyarticular-onset disease and in one only out of their 17 patients (5.9%) with pauciarticular JRA but it was negative in all systemic-onset disease patients.

In the present study, the seropositivity to hidden RF was detected in 56.5% of all JRA patients. Previous investigators reported a prevalence quite near to ours and a prevalence of 68% was reported in 2 of these earlier studies [5,17]. The positivity of hidden RF was highest in JRA patients with polyarticular-onset disease (66.7%) followed by patients with

systemic-onset disease (42.9%) and the least percentage was detected in patients with pauciarticular-onset disease (33.3%). Varbanova et al.[18], reported hidden RF positivity in 55% of patients with polyarticular-onset disease and 42% of patients with pauciarticular-onset disease.

Compared with the percentage of positivity of classic RF in the different types of JRA (16.67% in polyarticular-onset JRA and 0% in both the pauciarticular and systemic-onset types), hidden RF appears more reliable in the diagnosis. This is based on the fact that it was not only positive in those patients who were positive for classic RF, but it was also positive in 15/25 (60%) of those who were ranked as RF negative.

Concerning the effect of disease activity on IgA-RF, it was found that patients with active disease had significantly higher IgA-RF level than those with quiescent disease. Also, IgA-RF correlated positively with activity score, ESR and CRP. Similar findings were reported by previous two studies [1,3]. The 95th percentile of IgA level in JRA patients with quiescent disease was as high as 135 mg/dl. All patients with active disease had IgA-RF level greatly above this value. This ranks IgA-RF as one of the most accurate laboratory markers for diagnosis and follow up of disease activity in JRA.

A close relation between hidden RF positivity and disease activity was also observed as 91.7% of patients with active disease were seropositive for hidden RF compared to 44.1% only of patients with quiescent disease. Also, patients with positive hidden RF had significantly higher values of activity score and ESR when compared to the corresponding values of those with negative hidden RF. Similarly, Moore et al.[5] and Walker et al.[16], reported significant positive correlation between hidden RF and disease activity.

In contrast, classic RF had no correlation with disease activity as evidenced by the non-significant difference between levels of activity score and ESR of JRA patients with positive and those with negative classic RF. These results lend support to the study of Vasiliauskiene et al.[1].

Although steroid therapy reduce the levels of immunoglobulins including RFs, in this study, patients who were on steroid therapy had significantly higher value of IgA-RF and hidden RF positivity when compared to those who did not receive this therapeutic modality. This may be explained by the fact that patients who were on steroid therapy had more severe disease than patients who did not receive steroids and this may be an indirect evidence of the relation between IgA and hidden RFs and disease severity. The latter assumption was supported by the positive correlation between IgA-RF and the studied clinical and radiological scores. This was also the case with hidden RF as patients with positive hidden RF had significant elevation of the clinical and radiological scores when compared to those with negative hidden RF. Bharadwaj et al. [3] reported higher prevalence of IgA-RF in children in the poor functional class and significant association of deforming joint disease with the presence of hidden RF. Houssien et al. [12] reported that IgA-RF correlated positively with the subsequent onset of severe erosive disease.

Evaluation of the efficacy of each of IgA and hidden RFs in the diagnosis of JRA revealed comparable results for both tests. Broadly speaking, each test alone had a high specificity and positive predictive value and a low sensitivity and negative predictive value indicating that they are good positive but not good negative tests.

Combined positivity of IgA and hidden RFs was not found in the lupus control group. Furthermore, this combination was 100% specific for JRA patients, whereas the specificity of IgA and hidden RFs separately were 97.7% and 95.5% respectively. Similarly, Jonsson et al. [19] mentioned that combined positivity of IgA and hidden RFs was highly specific for RA. Therefore, the mutual evaluation of both types of RFs can help in settling the diagnosis of JRA in cases of doubt.

In conclusion, IgA-RF and hidden RFs can be considered specific markers in diagnosing JRA. The former was essentially positive only in polyarticular-onset JRA patients. Hidden RF was found in all the 3 types of JRA but its positivity was highest among those with polyarticular-onset disease. The mutual evaluation of both IgA and hidden RFs was considered to be more reliable given the better overall performance of both tests together. Both markers had also significant positive correlation with disease activity, and disease severity, so we recommend their addition to the currently used tests of JRA to facilitate the diagnosis especially in doubtful cases and as indicators of disease activity and severity.

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