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Original article

Expression and diagnostic utility of anti-cyclic citrullinated peptide antibodies in juvenile idiopathic arthritis

Background: *Juvenile idiopathic arthritis (JIA) is a clinically heterogeneous group of arthritis occurring in children. Anti-cyclic citrullinated peptide (anti-CCP) antibodies have been recently included in the revised diagnostic criteria for adult onset rheumatoid arthritis. Its diagnostic value in JIA is still debatable.* ***Objective:*** *The study is aimed to investigate the expression and diagnostic utility of anti-CCP antibodies in pediatric JIA in relationship to its various clinical phenotypes.* ***Methods:*** *Forty children and adolescents (13 males, 27 females) with JIA as well as 35 healthy children were enrolled in this cross-sectional study. Serum anti-CCP antibodies were determined by enzymatic immunoassay and its expression was statistically correlated to clinical, laboratory, and radiological data of the patients.* ***Results:*** *Anti-CCP antibodies were positive in 8 (20%) patients while not expressed in the control group. Seven out of the 8 positive cases (87.5%) had polyarticular JIA and only one patient (12.5%) had the oligoarticular onset variety. A significant positive correlation was elicited between the anti-CCP antibody levels and the number of tender joints (r= 0.39), swollen joints (0.68) and disease duration (r = 0.59). Radiographic erosive arthritis was found in 8 patients with positive anti-CCP antibodies; 7 of whom (87.5%) suffered the polyarticular subtype and only one patient (12.5%) had the oligoarticular subtype. All the rheumatoid factor (RF) seropositive patients had positive anti-CCP antibody as well as radiographic erosive arthritis. The overall anti-CCP antibody diagnostic value in our series showed a sensitivity and specificity of 20% and 100% respectively and the positive and negative predictive values were 100%, and 52.2%, respectively.* ***Conclusion:*** *Anti-CCP antibodies have a low sensitivity but high specificity in patients with JIA with a significant relationship to clinical and radiologic severity especially in RF seropositive cases. It may thus have a diagnostic and/or prognostic utility in severe polyarticular onset disease.*

Keywords: Anti-cyclic citrullinated peptide antibodies; Juvenile idiopathic arthritis; children.

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INTRODUCTION

Juvenile idiopathic arthritis (JIA) is a clinically heterogeneous autoimmune disease of unknown cause, characterized by chronic, inflammatory changes of the joints, as for rheumatoid arthritis (RA) in adults.[1] The diagnosis of JIA resembles RA because it is mostly dependent on clinical features of the disease after excluding infection and other inflammatory diseases.[2] Moreover, it is very difficult to establish the diagnosis of JIA, especially at the early stage of the disease, since the clinical symptoms are often not characteristic.[3, 4]

The clinical diagnosis of RA can be supported only by limited serological markers with confirmed serological value; one of them is IgM class rheumatoid factor (IgM-RF). However, contrary to RA, in JIA especially at the onset of the disease IgM-RF is found only in low numbers.[3-5]

Recent studies have demonstrated anti-citrullinated protein/peptide antibodies (ACPA) which include anti-CCP antibodies, have high specificity for RA, and are now included in the revised diagnostic criteria for RA.[6] Although anti-CCP antibodies have only been recently discovered to have a diagnostic value for RA, with a specificity of 96-100%[7] and a sensitivity of 40-85%[8] However, their diagnostic value for JIA is still debatable with a relatively few studies have shown the diagnostic efficacy of anti-CCP in JIA.[9]

This study sought to investigate the prevalence and diagnostic utility of anti-CCP antibodies in

children with JIA and its relationship to clinical, laboratory and radiologic parameters.

METHODS

Study groups

This cross-sectional controlled study was carried out in the Departments of Pediatrics and Rheumatology and Rehabilitation of El-Minia University Hospital. A sample of forty children and adolescents (13 males, 27 females) with JIA was recruited in the study. Their ages ranged from 3.5 to 18 years with a mean ± SD of 12.3 ± 4.4. They were already diagnosed cases of JIA fulfilling the 1997 International League Against Rheumatism (ILAR) classification criteria.[5] The patients were consecutively enrolled in the study during their regular visits to the outpatient clinic. They comprised 10 oligoarticular, 26 polyarticular, and four systemic onset JIA cases. Patients diagnosed with other autoimmune diseases, chronic diseases, or malignancies were excluded.

Onset and duration of JIA disease and demographic variables were recorded. Clinical examination of the patients included the status of arthritis and systemic manifestations including fever, rash, and visceral involvement. Active disease was defined by the presence of joint swelling or limitation of movement with either pain on movement or tenderness. Non-active disease (remission) was defined by the absence of joint swelling or limitation of movement with no pain on movement or tenderness.[10]

The control group comprised 35 clinically healthy children and adolescents (15 males and 20 females) who were consecutively recruited at their visits to Children's University Hospital Clinics for minor traumatic causes. Their age ranged from 6 to 18 years with a mean of 12.1±3.6.

An informed consent was obtained from the parents or care givers according to the medical ethical regulations and the protocol was approved by the Ethics' Committee of the Department of Pediatrics, Children's Hospital Minia University.

Methods

- The following laboratory tests were done for both patients and controls:
  i. Erythrocyte sedimentation rate (ESR), measured by the Westergren method.
  ii. Serum IgM rheumatoid factor (RF), measured by latex agglutination test.
  iii. Serum anti-cyclic citrullinated peptide (Anti-CCP) antibodies:

A blood sample (2-3 ml) was collected, centrifuged and sera were stored at -20˚C until assayed. Samples were tested without knowing the clinical details of patients and were done using the Quanta lite ™CCP IgG ELISA according to the manufacturer's instructions, a semi-quantitative enzyme-linked immunosorbent assay for the detection of IgG Anti-CCP IgG antibodies in patient sera (the antigen). According to the manufacturer, anti-CCP antibodies considered positive when its serum level is 20 units/ml or more.

- Radiographic assessment: a postero-anterior plain X-ray of the hands and wrists were obtained to detect radiological joint damage (defined as joint narrowing and/or erosions). The radiological data were collected from their files, nearly from the same time of blood collection from the patients.

The control subjects underwent serum Anti-CCP antibody and RF measurement and their samples were assayed in the same setting as the patients. They were also subjected to ESR assessment. Children with family history of autoimmune illness were excluded from this group.

Statistical analysis

Data entry and analysis were performed using the SPSS (Statistical Package for Social Science) for windows version 11.5. Categorical data were presented as numbers and percentage (%) and were compared using the Chi Square and Fisher's exact tests as appropriate. Numerical data were presented by mean and standard deviation (mean ± SD) and were compared using the t-student test. Correlations between anti-CCP antibody and clinical parameters were attempted by the Spearman's rank correlation coefficient test. The diagnostic value of anti-CCP antibodies was determined by calculation of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). P values less than 0.05 were considered significant.

RESULTS

The female gender was more represented in the studied sample with female: male ratio of 2.1:1, ($p<0.05$). Age was compatible between the patients and controls. The ESR was significantly higher in the JIA patients than control group ($p <0.0001$). The RF positivity was 7.5% (3 cases) in the patients' group versus 0/35 in control group; however the difference did not reach statistical significance ($p=0.24$). The number of positive anti-CCP patients was 8/40 (20%) compared to 0/35 (0.0%) in the control group ($p=0.01$); Table 1.

Among the 27 patients with polyarticular JIA, 8 patients had positive anti-CCP antibodies of whom 7 patients (87.5%) suffered the polyarticular subtype and only one patient (12.5%) had

oligoarticular JIA. Similarly, radiographic erosive arthritis was detected in 8 patients, 7 (87.5%) had polyarticular and only one patient (12.5%) had oligoarticular JIA. Three patients in the polyarticular JIA subtype were identified with both positive RF and Anti-CCP antibody; all of whom had radiographic erosive arthritis (Table 2).

Significant positive correlations could be elicited between the anti-CCP antibodies and disease duration (r=0.59), number of tender joints (r=0.68), and number of swollen joints (r=0.39); (Table 3).

Table 4 reveals a significant difference in age according to anti-CCP positivity in the JIA patients. The disease duration and number of tender and swollen joints showed a similar variation. RF positivity was also significantly higher in anti-CCP positive cases.

Calculation of the diagnostic performance of anti-CCP antibodies in JIA showed a sensitivity of 20% and specificity of 100%; the positive predictive (PPV) and negative predictive (NPV) values NPV were100%, and 52.2%, respectively.

Table 1. Demographic and laboratory data of the studied sample.

Variables	JIA patients (n = 40)	Control (n = 35)	P
Age (yr) mean ± SD	12.3 ± 4.4	12.1 ± 3.6	0.86
Female /male	27/13	23/12	0.02
ESR (mm) mean ± SD	40.2 ± 27.4	10.6 ± 5.1	0.0001
Positive RF (%)	3/40 (7.5%)	0/35 (0%)	0.24
Positive Anti-CCP (n)	8/40 (20%)	0/35	0.01*

* significant; CCP: cyclic citrullinated peptide; SD: standard deviation; yr: years

Table 2. Variation of the disease duration, RF positivity, and radiographic joint affection according to JIA subtypes.

Variables	Oligoarticular JIA (n=10)	Polyarticular JIA (n=26)	Systemic Onset JIA (n=4)
Disease duration (mo)	22.6 ± 19.8	36.5 ± 35	16.2 ± 3.5
Positive RF (%)	0 (0%)	3 (11.5%)	0 (0%)
Radiographic joint erosion	1 (10%)	7 (26.9%)	0 (0%)

mo: months; n: number; RF: rheumatoid factor

Table 3. Correlations between anti-CCP antibodies and clinical data.

Variables	r	p
Disease duration (mo)	0.59	0.0001
Tender joints (number)	0.68	0.001
Swollen joints (number)	0.39	0.01

CCP: cyclic citrullinated peptide; r: correlation coefficient

Table 4. Variation of clinical data and RF results according to anti-CCP status.

Variables	Anti-CCP positive (n=8)	Anti-CCP negative (n=32)	P
Disease duration (mo): mean ± SD	62.1±44.1	23.7±20.9	0.001
Tender joints: median (range)	10 (3-14)	2 (0-5)	0.002
Swollen joints: median (range)	4 (1-6)	2 (0-4)	0.01
Positive RF	3	0	0.01

CCP: cyclic citrullinated peptide; n: number; RF: rheumatoid factor

DISCUSSION

Juvenile idiopathic arthritis has clinical features which greatly differ from those of RA.[1,11] Since a highly specific and sensitive laboratory marker of JIA has not yet been found, the diagnosis of JIA is mainly dependent on the clinical manifestations. Although RF has a high sensitivity in the diagnosis of RA (but with a low specificity), it has a low sensitivity in the diagnosis of JIA[8]. Anti-CCP are auto-antibodies which are being increasingly used to aid in early diagnosis of RA as they have been found to be highly specific (96-98%) in the diagnosis of RA.[12]

Among our series, anti-CCP was detected in 8/40 (20%). A limited number of studies with controversial results are available as far as the diagnostic value of anti-CCP antibodies in JIA is concerned. Van Rossum et al.[13] reported 10 cases (14%) with positive anti-CCP among 71 children with JIA. Similarly, prevalence rates of 20.8% and 23.1% of anti-CCP antibodies in JIA were reported by Habib et al[14] and Gupta et al[15] respectively. A more recent study by Tebo[16] et al has also detected prevalence of 14.3% in children with JIA (OR 8.2, $p < 0.01$).

On the other hand, several studies have not shown usefulness of anti CCP antibodies either in diagnosis or prognosis of JIA. Avcin et al[17] and Kasapcopur et al[18] reported that anti-CCP antibodies were rare in patients with JIA (1.8% out of 109 and 2% out of 122 respectively). Similarly, Machado et al[19], found that only one out of 45 JIA patients was positive for anti-CCP**.** Avcin et al[17] speculated that the low rate of positivity may be due to the fact that their study was a multicenter one, with no consistent inclusion criteria other than clinical diagnosis. In addition, the time of serum collection in correlation with state of disease activity might have influenced the anti-CCP antibody expression. The divergence also of the results in various researches could be due to the use of different ELISA kits in the assay. The validity of our results is definitely limited by the sample size.

In the current study, as anticipated, most cases positive for anti-CCP [7 of 8 (87.5%)] had polyarticular JIA and demonstrated radiographic erosive arthritis. Only one patient (12.5%) with oligoarticular JIA developed serum anti-CCP antibodies. Moreover, all RF-positive polyarticular JIA children in the present study had anti-CCP antibody positivity. Our results are in line with those reported by van Rossum et al[13] who reported anti-CCP antibody positivity in 73% of RF positive polyarticular JIA and radiological damage was observed on the radiographs of 8 out of 10 (80%) anti-CCP positive patients. A relevant study also reported that 7 of 8 (87.5%) patients with RF IgM positive polyarticular JIA had anti-CCP antibodies in their sera. Also, 11 out of 19 (58%) patients with joints erosions had significant levels of anti-CCP antibodies compared to only 12% of patients without erosions.[15]

Over again, a group of investigators from China[20] reported anti-CCP antibody positive rates of 31.25%, 14.8% and 0%, in polyarticular, oligoarticular, and systemic-onset JIA respectively. Anti-CCP antibodies seem to play an important role in the pathogenesis of RA inflammation, because RA patients with anti-CCP antibodies have a more aggressive disease course with joint erosion and damage. Citrullinated proteins might be targets of the local immune response in patients with RA and may perpetuate a persistent state of synovitis leading to joint destruction.[21]

Another notable finding in our series is the positive correlation between anti-CCP antibodies and disease duration, and number of joints showing signs of arthritis. This observation was supported by a significant difference between anti-CCP antibody positive and negative JIA patients in terms of the number of swollen and tender joints. These findings might indicate that anti-CCP antibodies can be good markers of disease activity. This is in accordance with the findings of Lipinska J et al[12] who reported that serum anti-CCP concentrations were significantly higher in children with higher activity of the rheumatoid process ($p=0.014$; $R=0.25$).

Recently, a relevant study by Omar and coworkers[22] has also concluded that Anti-CCP antibodies were prevalent among a group of Egyptian children and adolescents JIA patients with polyarticular patterns compared to other disease patterns ($F=4.845$, $P=0.012$) with significant correlation with radiological damage as assessed by Sharp/Van der Heijde Score ($r=0.457$, $P<0.001$).

It is suggested that JIA with both RF and anti-CCP positive antibodies could be an indicator of more aggressive and severe disease that leads to joint damage and disability. Thus their simultaneous presence help in putting more aggressive immunosuppressive treatment plans such as the use of biological therapy.[23] Bacos et at[24] has recently shown that pediatric JIA patients with high anti-CCP antibodies and older onset of disease frequently can have continuous inflammatory activity during adult life and may need further care in the adult rheumatology clinic. Another recently published study by Gilliam et al[25] has also reported JIA patients with joint damage were positive for

various combinations of anti-citrullinated antibodies, with IgG anti-CCP and anti-citrullinated fibrinogen antibody positivity demonstrated in 7/20 (35%) patients with joint damage

The overall diagnostic performance of anti-CCP antibodies in JIA in the present study has shown low sensitivity (20%), high specificity (100%), high PPV (100%), and moderate NPV (54.2%). This comes in concordance with a couple of previous studies.[13,20] The high specificity, was mostly demonstrated in RF-positive polyarthritis, may be explained by the fact that this particular form of the disease phenotypically resembles rheumatoid arthritis (RA) in adults, and might actually represent the childhood onset of RA.[1]

The present study has the following limitations including the limited size of the studied sample and being a cross sectional study; it does not explore the relationship between the levels of anti-CCP antibodies and treatment and/or long term outcome. Also, the time of sampling for anti-CCP assay was not studied in relation to an objective activity scoring methodology.

In conclusion, anti-CCP antibodies can be detected with low rates but high specificity in patients with JIA being more frequently detected in the polyarticular subtype. The anti-CCP positivity was positively correlated to RF positivity, disease severity and radiographic joint erosion. It could therefore be a useful serologic marker of severity and may guide optimum treatment decision-making in order to reduce joint damage and disability.

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