Research Article

Synovial Anti Cyclic Citrullinated Peptide Antibodies in Comparison with Serum Antibodies in Early and Late Rheumatoid Arthritis

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Abstract

Aim: The study objective was to evaluate the diagnostic value of synovial fluid anti cyclic citrullinated peptide (sf-anti CCP) in comparison to serum anti CCP (s-anti CCP) in early and late rheumatoid arthritis and to clarify its correlation with disease activity.

Methods: In this cross-sectional study a total of 45 patients aged between 18 and 70 years with rheumatoid arthritis (RA) according to the ACR criteria were enrolled. Twenty six patients had a disease duration of more than 2 years (late RA) whereas 19 cases were referred to as early RA (<2 yrs). Baseline characteristics and laboratory studies including complete blood count (CBC), erythrocyte sedimentation rate (ESR) in addition to anti CCP and rheumatoid factor (RF) levels in the serum and synovial fluid were recorded. Disease activity was assessed according to disease activity score of 28 joints - ESR (DAS28 - ESR).

Results: Serum and synovial fluid anti CCP correlated significantly (p<0.001, r=0.6). Sf-anti CCP also significantly correlated with sf-RF (p=0.02, r=0.34). No correlation was found between sf-anti CCP and disease activity according to DAS28-ESR (p=0.38, r=0.13). There also was no statistically significant difference between early and late RA in terms of sf-anti CCP (p=0.9).

Conclusion: The diagnostic value of sf-anti CCP for RA is similar to s-anti CCP. Therefore, sf-anti CCP can be useful if RA is suspected. The current study did not show any difference in sf-anti CCP between early and late RA and revealed no correlation with disease activity.

Keywords: Rheumatoid arthritis (RA); Rheumatoid factor (RF); Anti-cyclic citrullinated peptide antibodies (anti CCP); Synovial fluid

1. Introduction

Diagnosis of rheumatoid arthritis (RA) is based on clinical manifestations; albeit, today serologic and immunologic studies are becoming more prominent in this respect. The most common serologic markers are RF and anti CCP, however in seronegative cases, RA is not ruled out [1, 2]. RF has some limitations in RA diagnosis such as low sensitivity and specificity [2]. On the other hand, anti CCP with a specificity of 95-98% can be used as a predictive and prognostic marker in RA [3]. Anti CCP antibodies may manifest years earlier than RA symptoms. Therefore, they are considered to have a high positive predictive value for RA development [4, 5]. One of the most important diagnostic challenges in RA is early inflammatory monoarthritis, in which, serum autoantibodies may still be negative. As the production of anti CCP occurs prior to the onset of arthritis [4, 6], the diagnostic value of sf-anti CCP prior to serum one is expected to be important. Citrullinated proteins originate from the synovium, and anti CCP is produced locally at the site of joints inflammation by local plasma cells in the first stages of RA [7]. However, recent studies have reported occasional local detection of anti CCP in other arthritis such as OA and reactive arthritis [8]. Limited studies have focused on the diagnostic utility of sf- anti CCP for RA and this issue has not been fully investigated yet [9-12]. Therefore, in the present study we aimed to investigate the diagnostic efficiency of sf-anti CCP in comparison to s-anti CCP; besides its potential benefits in prediction of disease activity and early RA diagnosis.

2. Material and Methods

In this cross-sectional study a total of 45 RA patients who were referred to Imam Reza Hospital, Mashhad, Iran between Jan 2008 and Mar 2012 enrolled. They recruited based on these inclusion criteria, (1) aged between 18 to 70 years, (2) patients with RA according to the ACR criteria, (3) at least one knee with effusion, (4) needing therapeutic arthrocentesis and Exclusion criteria consisted mainly of (1) active infection, (2) other connective tissue disorders, (3) any type of coagulopathy, (4) recent (<6 weeks) injection of corticosteroids into the knees, (5) diabetes. Twenty six patients had disease duration of more than 2 years and were referred to as "late RA", whereas 19 cases with a less than 2-year history from disease onset were referred to as "early RA". Disease duration was defined as the time from the onset of first RA symptoms up to inclusion in the study. Patients in the early and late RA groups were matched for age and sex. Disease activity was assessed according to DAS28-ESR. Patients with overlap syndromes, contraindication for joint aspiration were excluded from the study. At study entrance a designed questionnaire was completed for each patient including the following data: full medical history, physical examination, RA disease duration, disease activity according to DAS28-ESR, and anti CCP and RF levels in both serum and the synovial fluid. On the same day of the clinical evaluation, a 3cc venous blood sample and a 3cc synovial fluid sample (by standardized arthrosynthesis) were obtained to quantify the titer of anti CCP and RF antibodies. These samples were centrifuged and the serum was stored at -20ºC until tested. The serum and synovial fluid anti CCP levels were measured by the ELISA method (Stat Fast, Awareness Company) with the anti CCP kit

(Euroimmun, Medizinische Labordiag Nostike AG). Positive anti CCP was defined as a serum concentration ≥5 IU/mL according to the manufacturers' instructions. The study protocol was reviewed and approved by the local Ethics Committee of Mashhad University of Medical Sciences and an informed consent was obtained from each participant prior to study entrance. The collected data were analyzed using SPSS v16. In order to study the correlation between the different variables Pearson's test was used for normally distributed data whereas Spearman's test was applied in case of abnormal data distribution. The significance level was set at p<0.05.

3. Results

3.1 Demographics

45 RA patients were studied consisting of 36 females and 9 males. The patients mean age was 43.6 ± 13.73 yrs. Their mean disease duration was 69.05 ± 77.9 months. Mean DAS28 and VAS scores were 6.03 ± 1.3 and 57.81 ± 24.6 respectively, whereas the mean serum ESR level was 43.11 ± 27.53mm/h at baseline. In total, 19 (42.2%) cases had early RA whereas 26 (57.8%) had late RA. Baseline demographic data of two groups of early and late RA are presented in (Table 1) and other demographic and laboratory data in all patients are showed in (Table 2).

Variable	Early RA (n=19)	Late RA (n=26)	p-value
Sex (female%/male %)	83.3% / 16.7%	76.9% / 23.1%	0.7
Serum RF	111.67 ± 101.03	149.35 ± 100.84	0.282
Synovial fluid RF	116.41 ± 96.65	138.30 ± 101.37	0.481
Serum anti-CCP	103.73 ± 104.927	81.27 ± 93.401	0.482
Synovial fluid anti-CCP	$100.54 \pm 105\ 48$	99.98 ± 104.76	0.986

RA-Rheumatoid Arthritis; RF-Rheumatoid factor; CCP-Cyclic Citrullinated Peptide

Table 1: Demographic data of the early and late RA groups at baseline.

Variable	Serum RF	Synovial fluid RF	Serum anti CCP	Synovial fluid anti CCP
Age	P=0.46, r=0.13	P=0.84, r=0.03	P=0.73, r=0.05	P=0.29, r=0.17
Duration	P=0.17, r=0.4	P=0.1, r=0.26	P= 0.44,r=0.12	P=0.23, r=0.18
Disease activity	P=0.03,r=0.36	P=0.17, r=0.21	P=0.64, r=0.07	P=0.38, r=0.13
S-anti CCP	P=0.04, r=0.35	P=0.02, r=0.37	-	P=0, r=0.64
Sf-anti CCP	P=0.25, r=0.19	P=0.02, r=0.34	-	-
S-RF	-	P=0, r=0.68	-	-

Sf-synovial fluid; S-Serum

Table 2: Correlations between serum and synovial fluid RF and anti CCP with different variables.

3.2 Important correlations between RF, anti CCP and disease markers

3.2.1 RF: In total, 36 (80%) patients had positive serum and 31 (73.8%) had positive synovial fluid RF. The

mean serum and synovial fluid RF levels were 133.2 ± 101.21 U and 128.4 ± 98.68 U, respectively. T-test showed no meaningful difference between mean serum and synovial fluid RF levels in early (p=0.2, t=-1.09)

and late (p=0.48, t=-0.7) RA groups. Taken together, 88% of positive serum RF patients also had positive serum anti-CCP. Moreover, 80% of positive synovial fluid RF patients had positive synovial fluid anti CCP.

3.2.2 Anti CCP: In total, 30 (66.6%) patients had positive serum and 32 (71.1%) patients had positive sf-anti CCP. The mean level of s-anti CCP was 89.49 ± 97.08 IU/mL and sf-anti CCP was 100.21 ± 103.86 IU/mL, respectively. According to t-test analysis, no statistically significant difference was observed regarding the serum (p=0.48, t=0.7) and sf-anti CCP levels (p=0.98, t=0.01) in the early and late RA groups. Sensitivity of sf-anti CCP was calculated as 86.6% and its specificity was 72.7%. Positive predictive value for sf-anti CCP was 89.6% and its negative predictive value was 42.8%. Fisher's Exact test did not show any statistically significant difference in distribution of serum (P=0.98, OR= 1.01; 0.24<CI<4.25) and sf-anti CCP levels (P=0.74, OR=1.24; 0.33<CI<4.64) between the early and late groups. (Table 2) shows the correlation between serum and sf-anti CCP and different variables. A meaningful correlation was found between s-anti CCP and sf- anti CCP (p<0.000, r=0.64), s-anti CCP and s-RF (p=0.045, r=0.35), s-anti CCP and sf-RF (p=0.021, r=0.37). Moreover, sf-anti CCP showed a significant relationship with both sf-RF (p=0.026) and number of tender joints (p=0.038).

4. Discussion

Rheumatoid arthritis is primarily diagnosed based on clinical manifestations, supported in many cases by serologic findings. Due to the progressive and disabling nature of RA, early diagnosis of RA especially in atypical presentations like large joint monoarthritis is important to provide better opportunity for treatment and saving joints [13, 14]. This research revealed that sf-anti CCP and RF increase in parallel with serum values. In addition, serum and sf-anti CCP and RF values did not show any significant difference between

early and late RA. In our study serum and sf-anti CCP correlated significantly. Moreover, serum and sf-anti CCP showed no correlation with disease activity. Sensitivity of sf-anti CCP was calculated as 86.6% and its specificity was 72.7%. Positive predictive value for sf-anti CCP was 89.6% while it had a negative predictive value of 42.8%. Therefore, sf-anti CCP may be a valuable marker for diagnosis of early-undifferentiated arthritis or monoarticlular RA. Other studies have also evaluated the role of sf-anti CCP in RA in different populations; here we summarize some study findings: In the study by Caspi et al. [7], on 29, 20 and 19 patients with RA, psoriatic arthritis (PsA) and osteoarthritis, the anti CCP level was measured in their synovial fluid. Significantly increased levels of anti CCP and IgA-RF were observed in the serum and synovial fluid of patients with RA in comparison with other joint effusion conditions. Moreover, they showed a positive relationship between serum and sf-anti CCP [14, 15]. Heidari et al. [6], also reported the same correlation between serum and sf-anti CCP. Our results were in line with the aforementioned studies. Spadaro et al. [16] found lower levels of IgG anti CCP antibodies in synovial fluid and serum in PSA respect to RA patients without difference with patients with osteoarthritis (OA). They also showed a higher sf/serum ratio for anti CCP in RA compared to sf/serum ratio for total IgG in psoriatic arthritis.

Olivares et al. [9] found a positive correlation between sf-anti CCP and s-anti CCP in their patients. They also reported a great variability of citrullinated proteins in the synovial tissue of RA patients, which their level is associated with that of s-anti CCP antibody concentration. Guo et al. [10] searched synovial tissue and sf-anti CCP in comparison with osteoarthritis and reported synovial tissue plasma cells as an important source of anti CCP production. They reported the level of sf-anti CCP as high as the serum level. Mrabet et al.

[11] measured synovial fluid and serum anti CCP in RA, osteoarthritis and seronegative spondyloarthropathy patients. They found a positive correlation between those two markers in RA and seronegative spondyloarthropathy. Both serum and synovial fluid anti CCP showed a cut-off value for determining RA from non RA arthritis. However, sf-anti CCP values discriminated RA from non-RA at a higher cut-off value.

Snir et al. [12] also reported similar results to our study. Other studies have proposed that anti CCP positive RA patients share differences in synovial tissue and fluid characteristics with anti CCP negative patients [17, 18]. Guo et al. showed that anti-CCP levels in Synovial tissue were higher than in serum therefore synovium is a better place for detection of anticcp antibodies [19]. Regarding certain studies suggesting that due to the earlier appearance of antibody in synovial fluid compared to serum, lower threshold level for RA diagnosis, and more frequent sf-anti CCP positivity compared with s-anti CCP positivity in seronegative arthritis, Arthrosynthesis is recommended in any individual with recent onset arthritis [20].

Our study did not find any difference in sf-anti CCP of early and late RA seropositivity. Therefore we could not conclude that in early RA, synovial fluid anti-CCP could be beneficial in better diagnosis. Our study had some limitations such as the effect of drugs on anti CCP values. Moreover, although the study population was more than most previous studies, still larger studies specially focused on newly diagnosed individuals whom have received no type of treatment is highly recommended. The highlights of our study were investigating the difference in sf-anti CCP between early and late RA, its association with disease activity according to DAS28-ESR and serum and sf-RF.

5. Conclusion

It seems that the diagnostic ability of sf-anti CCP for RA is similar to s-anti CCP. Therefore, in monoarthritis cases with an unknown origin, sf-anti CCP may be more helpful if RA is suspected. Our study revealed no difference in sf-anti CCP between early and late RA and showed no correlation with disease activity.

Conflict of Interest

The authors confirm that this article content has no conflict of interest.

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