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ARAŞTIRMA RESEARCH ARTICLE

Diagnostic performances of anti-cyclic citrullinated peptide (anti-CCP) assay in patients with rheumatoid arthritis, asthma, systemic lupus erythematosus

Romatoid artrit, astım, sistemik lupus eritematozuslu hastalarda "anti-cyclic citrullinated peptide (anti-CCP)"in tanısal değeri

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ABSTRACT

Objective: Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown etiology with chronic joint inflammation. The major autoantibody detected in RA patients is rheumatoid factor (RF). RF has demonstrated lower sensitivity for the diagnosis of RA. The aims of this study were to evaluate the diagnostic performance of anti-cyclic citrullinated peptide (anti-CCP) by ELISA method and compared those with the diagnostic performance of RF test in patients with RA, and to determine how frequently anti-CCP antibodies can be found in patients with asthma, systemic lupus erythematosus, and in normal blood donors and control groups.

Materials and Methods: Diagnostic performance of anti-CCP assay by enzyme linked immunosorbent assay (ELISA) was compared with that of RF by nephelometry test. RF and anti-CCP tests were per-

ÖZET

Giriş: Romatoid artrit (RA) kronik eklem inflamasyonlu etyolojisi bilinmeyen bir sistemik otoimmün hastalıktır. Romatoid faktör (RF) RA'nın tanısı için düşük duyarlılığa sahiptir. Çalışmada, RA'lı ve astımlı, sistemik lupus eritematozuslu, hastaların, sağlıklı ve kan donörlerinin serumlarında enzim immunsorbent assay (ELISA) kullanarak "anti-cyclic citrullinated peptide (CCP)"in tanısal performasyonu çalışıldı.

Materyal ve Metod: Anti-CCP'nin tanısal değeri nefelometrik çalışılan RF ile karşılaştırıldı. RA'lı 23, sağlıklı kontrol grup 23, astımlı çocuk 20, sistemik lupus eritematozuslu 7 ve 15'i kan donöründen elde edilen serumlarda anti-CCP ve RF testleri çalışıldı.

Bulgular: Hastaların %95.7'sinde ve sağlıklı kontrol grup %4.3'ünde anti-CCP pozitif bulunmasına rağmen, astımlı, sistemik lupus eritematozuslu has-

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formed in 23 RA patient, 23 healthy control groups, 20 asthma, 7 systemic lupus erythematosus, and 15 normal blood donors.

Results: However, anti-CCP antibody showed positivity in 95.7% of RA patient and 4.3% of healthy control groups, this autoantibody has demonstrated negativity for asthma, systemic lupus erythematosus, and normal blood donors. In the RA groups, sensitivity and specificity were 95.7% and 95.7% for anti-CCP, and 100% and 78.3% for RF antibody, respectively.

Conclusion: It was considered that anti-CCP could be very useful serological assay for the diagnosis of RA, because anti-CCP revealed higher diagnostic specificity than RF.

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Key words: Anti-CCP, asthma, rheumatoid arthritis, systemic lupus erythematosus

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taların ve kan donörlerinin serumlarında negatif bulundu. Anti-CCP ve RF testlerinin sırasıyla; duyarlılığı %95.7 ve %100 ve özgüllüğü %95.7 ve %78.3 olarak belirlendi.

Sonuç: Anti-CCP testi RA'nın tanısında özgüllüğü RF ye göre daha yüksek olduğundan dolayı tanıda faydalı serolojik test olarak düşünülebilir.

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disorder that affects 1% of the population worldwide[1]. There is growing evidence that therapeutic intervention early in the disease course of RA leads to earlier disease control and less joint damage. Since treatment with disease-modifying antirheumatic drugs is only justified when the risk: benefit or cost effectiveness ratios are favourable, it is mandatory to be able to differentiate between RA and other forms of arthritis early after symptom development[2]. RA is diagnosed primarily according to clinical disease manifestations, and serologic support is primarily restricted to the determination of rheumatoid factor (RF). Increased levels of RF can be detected in 50-80% of RA sera but are also encountered in the sera of patients with other connective tissue diseases, patients with infectious diseases, and elderly healthy individuals. This incomplete sensitivity and specificity of RF tests for RA limits their diagnostic usefulness[3]. Schellekens et al. discovered that antibodies specific for RA in to antigenic determinants that contain the unusual amino acid citrulline, and could be detected in over 80% of RA sera with a high disease specificity using several synthetic peptides containing citrulline[4]. These new serological tests have advantage of easy performances by convenient enzyme linked immunosorbent assay (ELISA) method[5].

We studied the diagnostic performance of anti-cyclic citrullinated peptide (anti-CCP) by ELISA method and compared those with the diagnostic performance of RF in patients with RA, asthma, systemic lupus erythematosus, normal blood donors and control groups.

MATERIALS and METHODS

Patients and Control Groups

Serum samples were obtained from 23 patients diagnosed as RA according to the criteria from American College of Rheumatology (ACR) criteria from Department of Rheumatology and from 27 outpatients with systemic lupus erythematosus (SLE) (n= 7) and asthma (n= 20) from Department of Rheumatology and Allergy[6]. Two groups of healthy individuals (n= 23) and normal blood donors (n= 15) were considered as

control groups and all the samples were screened by comprehensive medical testing from the Family Medicine and Blood Center at Erciyes University Hospital. Consent and assent procedures for serum samples were obtained from patients diagnosed as asthma in different study were reviewed and approved by Ethical Committee of Erciyes University, Medical Faculty. Other serum samples were screened as routine. The study was carried out with 23 RA patients (13 female, 10 male, mean age 40.21), 7 SLE patients (5 female, 2 male, mean age 38.42), 20 asthma patients (14 female, 6 male, mean age 7.5), 23 control groups (15 female, 8 male, mean age 7.8) and 15 healthyblood donors (10 female, 5 male mean age 36.6): 88 serum samples in total.

Methods

Anti-CCP assay was determined by ELISA method (Euroimmun Labor diagnostika AG, Germany) according to the manuals. RF was measured by nepholometric methods (Dade Behring BN II, Germany). The cut off value of anti-CCP and RF was 5 IU/mL, and 9 IU/mL, respectively.

Statistical Methods

These results were analyzed by SPSS (version 11.0) and Anova test and ROC curves. Comparisons of date between groups were made by chi-square test and Mann Whitney U test. p-value less than 0.05 was regarded as significant.

RESULTS

The range of anti-CCP and RF tests results in RA patients was 1.4-> 100 IU/mL and 14-1070 IU /mL, respectively. Distribution of anti-CCP and RF tests results in 88 sera of patients with RA, asthma, systemic lupus erythematosus, blood donors, and healthy control groups were showed in Table 1. These results showed significant difference in anti-CCP and RF tests results between RA patients and asthma, systemic lupus erythematosus patients, and normal blood donors and healthy control groups ($p < 0.05$).

Anti-CCP was found positive in 22 of the 23 RA patients (95.7%) and in one of the 23 control groups (4.3%). None of the asthma, systemic lupus erythematosus patients, and normal blood donors had positive anti-CCP values. RF tests results was positive in all of RA patients (100%) and in 5 of the 23 controls groups (21.7%) (Table 2.).

Anti-CCP showed 95.7% of sensitivity, 95.7 % of specificity, 95.8% of positive predictive value and 95.8% negative predictive value. Anti-CCP positively was 95.7% among RA patients. False positively of anti-CCP and RF assay in healthy control groups were 4.5% and 21.7%, respectively. False positively of anti-CCP in patients with asthma and SLE was not found. RF showed 100% of sensitivity, 78.3% of specificity, 82.1% of positive predictive value and 100% negative predictive value. The anti-CCP

Table 1. Distribution of anti- CCP and RF tests results in 88 sera of patients with RA, asthma, systemic lupus erythematosus, blood donors, and healthy control groups

	Anti-CCP (IU/mL)		RF (IU/mL)	
Characteristics of patients (number)	Mean± SD	Range	Mean ± SD	Range
RA (n= 23)	89.0 ± 29.4	1.4-100	224.1 ± 256.2	14-1070
Control (n= 23)	6.0 ± 20.6	0.5-100	24.1 ± 63.7	1-305
Asthma (n= 20)	0.4 ± 0.4	0-1.4	3.8 ± 1.9	1-7
Blood donors (n= 15)	0.7 ± 1.2	0.2-2	4.6 ± 2.0	1-8
SLE (n= 7)	1.5 ± 1.0	0.6-3.4	4.6 ± 1.5	3-7

Anti-CCP: Anti-cyclic citrullinated peptide, RF: Rheumatoid factor, RA: Rheumatoid arthritis, SLE: Systemic lupus erythematosus, SD: Standard deviation.

Table 2. Tests results for anti-CCP and RF in patient with RA and without RA, and normal blood donors and healthy control groups

	Anti-CCP		RF	
Characteristics of patients (number)	**Positive**	**Negative**	**Positive**	**Negative**
RA (n= 23)	22	1	23	-
Control (n= 23)	1	22	5	18
Asthma (n= 20)	-	20	-	20
Blood donors (n= 15)	-	15	-	15
SLE (n= 7)	-	7	-	7

Anti-CCP: Anti-cyclic citrullinated peptide, RF: Rheumatoid factor, RA: Rheumatoid arthritis, SLE: Systemic lupus erythematosus.

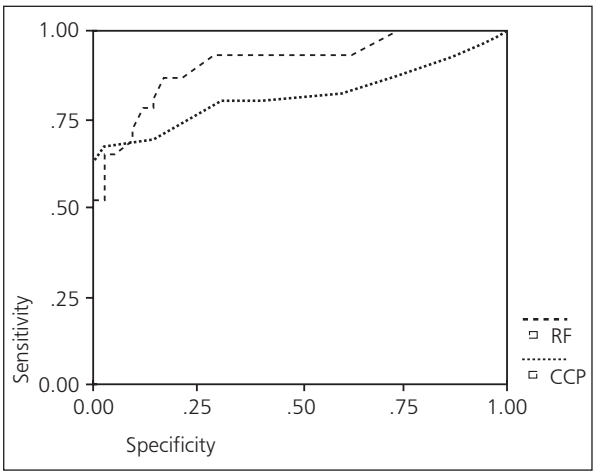

Figure 1. Receiver operator characteristics (ROC) curve of anti-CCP and RF with or without RA ROC curve (p= 0.00).

assay showed better specificity than RF test. Diagnostic accuracy among anti-CCP and RF assays by ROC curve was compared and the area under the curve of anti-CCP and RF were 0.911 and 0.820, respectively. Diagnostic accuracy between anti CCP and RF was significantly different (p= 0.00) (Figure 1).

DISCUSSION

The recently trend of RA treatment has been changed to start treatment as early as possible, based on the concept that early control of inflammation results in reduced joint damage. The RF assay has known to have sub optimal specificity for diagnosis of RA[5]. Although RF showed higher diagnostic sensitivity than anti-CCP in sera of our patients with RA, RF was lower specificity (78.3%) than anti-CCP (95%). Therefore, it is during the first period of the disease, when not all clinical parameters are manifest, that a specific serological test is needed[5]. Recently, anti-CCP by ELISA method was developed using the synthetic cyclic citrullinated peptide for diagnosis of RA, which was reported highly specific and significant[5-9]. Schellekens et al. reported that by ELISA methods using the cyclic citrullinated peptide had a diagnostic sensitivity of 68% for a specificity of 96%, 84% of positive predictive value and 81% negative predictive value[5]. Choi et al. reported that the diagnostic performances of anti-CCP by ELISA method as useful new serological tests for the diagnosis of RA[10]. The diagnostic performances of anti-CCP and RF in sera obtained from patients with RA had the sensitivity of 72.8%, 80.6% and the specificity of 92.0%, 78.5%, respectively. Altun et al. reported that RA patients were found to be anti-CCP positive 70%; specificity was 98.6 % and sensitivity 70%. In this study, the diagnostic sensitivity proposed for anti-CCP-ELISA showed better than that of other studies, and slightly low specificity than in the studies of Schellekens et al. and Altun et al.[5]. We concluded that anti-CCP showed high specificity for the diagnosis of RA. Our study showed that the false positive of anti-CCP and RF was not and following diseases were included asthma and SLE (Table 2). Choi et al. reported that the false positive of anti-CCP was 8.0% included Behcet's disease, fib-

romyalgia, gout, juvenile RA, osteoarthritis, reactive arthritis, spondyloarthritis, and SLE[10]. Dubucquoi et al. showed that percentages of false positives were low with most EIA methods (4% and 2% for the anti-CCP2 antibody kit and the home made EIA, respectively) included SLE and Sjögren's syndrome patients[8].

IgE-RF could focus an array of inflammatory mediators at a site containing basophils or mast cells and an appropriately high epitope density of IgG. Some patients with asthma have positive tests for RF that is detected in systemic hyper immunoreactive disease such as RA. Kobayashi et al. showed that RF levels were significantly higher than those in severe asthma, treated with high dose inhaled steroids, with history of systemic steroids use for the last one month[11]. These results suggested that RF levels reflect eosinophilia and asthma severity. Similarly, Zuraw et al. reported that present evidence of IgE-RF activity of sera from patients with RA and asthma[12]. But our study showed that positive of anti-CCP and RF was not founded in sera of our patients with asthma (Table 2). Pedersen et al. were reported that difference between risk associations with anti-CCP-positive and anti-CCP-negative RA were not statistically significant, whereas physician-verified asthma before age 45 years were appeared to be more strongly inversely associated with anti-CCP-negative[13].

Diagnostic accuracy among anti-CCP and RF assays by ROC curve was compared and the area under the curve of anti-CCP and RF were 0.911 and 0.820, respectively. Diagnostic accuracy between anti CCP and RF was significantly different (p= 0.00) (Figure 1). Choi et al. reported that sensitivity of among the anti-CCP and RF was compared, and the area under the curve of anti-CCP and RF was 0.837, 0.833, respectively[10]. Diagnostic accuracy between anti-CCP and RF was not significantly different (p= 0.857). Some studies had suggested that the discrepancies between the performances of tests, which may result from the composition of the series of patient's analyzed and from interlaboratory technical differences in performing the same tests and different anti-CCP antibodies kits were quite comparable[8,14].

In conclusion, anti-CCP ELISA is a new diagnostic test with extremely high specificity for RA. However, the test may therefore prove to be useful for diagnostic and therapeutic strategies in patients with recent-onset arthritis. There are needs to further studies for discrepancies between the performances with commercially kits of anti-CCP ELISA and in patients with other rheumatologic and autoimmune diseases.

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