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Research Article

Comparative study of anti-mutated citrullinated vimentin, anti-cyclic citrullinated peptides, and rheumatoid factor predictability in the diagnosis of rheumatoid arthritis

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ABSTRACT

Background: Rheumatoid arthritis (RA) is characterized by synovial joint inflammation, which often leads to progressive joint destruction and disability. Several other auto-antibodies specific to RA have been found .Among them, antibodies against cyclic citrullinated peptides (CCP) are useful for diagnosing RA. Antibodies to mutated citrullinated vimentin (MCV) were described recently in RA. The aim of this study was to determine the diagnostic values of ACCP compared to anti-MCV and Rheumatoid Factor in rheumatoid arthritis patients.
Methods: This study included 92 patients with Rheumatoid arthritis (RA) and 35 matching healthy controls. Blood samples were obtained from patients and controls for Erythrocyte Sedimentation Rate (ESR), C Reactive Protein (CRP), Rheumatoid factor (RF). Anti-CCP2 and anti-MCV were determined using ELISA technique.
Results: RA group was significantly higher than control group as regard ESR, CRP, RF, Anti-CCP, and Anti- MCV.
Conclusion: It was concluded, compared to ACCP, anti-MCV has approximately the same accuracy for the diagnosis of rheumatoid arthritis but higher than Rheumatoid Factor.
Level of Evidence: Level II, prospective study, as per guidelines for authors.

Keywords: Anti-cyclic citrullinated peptide (anti-CCP2), Anticitrullinated vimentin antibody (anti-CMV), Rheumatoid arthritis (RA)

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune, systemic disease, which primarily involves the joints, leading to inflammation, swelling, pain, stiffness, with inevitable progressive functional deterioration.[1] The global prevalence of RA ranges between 0.5-1%, mostly in young women and elderly people.[2] Considering the aggressive nature of the disease process with the significant residual disability, the International Societies of Rheumatology potentially recommended early aggressive treatment for tight control of the inflammatory process aiming to prevent joint destruction and preserve function. Rheumatoid factors (RFs) were the first biological markers discovered for RA and remain the only laboratory criterion included in the American College of Rheumatology criteria for RA classification. The currently laboratory diagnostics of RA particularly early RA, is based on a highly specific marker of the disease such as antibodies against citrullinated proteins. The positive test for anti-cyclic citrullinated protein (ACCP) antibody is now used as a classification criterion of RA.[3]

ACCP positivity predisposes individuals to more advanced course of the disease, with extensive bony erosions, accelerated atherothrombotic disease and worse overall prognosis.[4,5] From the different types of ACCP antibodies, ACCP2 is found to be the most sensitive and specific diagnostic marker.

Anti-mutated citrullinated vimentin (Anti-MCV) is another anti-citrullinated antibody reacting with mutated citrullinated vimentin.[6] Vimentin is an intermediate filament that is widely expressed by mesenchymal cells and macrophages and easy to detect in the synovium. Modification of the protein occurs in macrophages undergoing apoptosis, and antibodies to citrullinated vimentin may emerge if the apoptotic material is inadequately cleared. Recently, citrullinated vimentin, a protein highly released in synovial microenvironment, has been identified as potential autoantigen in the pathophysiology of RA and an enzyme-linked immunosorbent assay (ELISA) for the detection of Antibodies directed against a mutated citrullinated vimentin (anti-MCV) was developed.

The objective of this work was to investigate the impact of seropositivity to antibodies against modified citrullinated vimentin antibodies (anti-MCV) in comparison with anti-CCP2 and Rheumatoid Factor in terms of their respective sensitivity and specificity in rheumatoid arthritis (RA).

METHODS

This case control study was conducted on RA patients attending the outpatient Orthopaedics clinic, in the period between Jan 2012 to November 2013. Ninety two patients (66 females and 26 males) fulfilling the 1987 American College of Rheumatology (ACR) criteria for a diagnosis of RA were studied, thirty five healthy age and sex matched control subjects (23females, 12 males) were included for comparative assessment of the investigated serological disease markers.

At enrollment caretakers provided informed consents and the following data were collected: age, gender, duration of RA, treatment and hospital admission. The RA group Comprised 92 patients of age ranged from 18-60 years. They were 66 females and 26 males. Their disease duration ranged from 6 months to 22 years. Diagnosis of RA was based and confirmed according to (ACR)/ (EULAR) 2010 criteria.

Six mL of peripheral venous blood were withdrawn aseptically from each patient and from each control subject. Two mL blood were left to clot for 15 minutes then centrifuged and sera were put into aliquots and stored at -20°C until assayed for anti-MCV and anti CCP2 antibodies for both patients and controls. The remaining 3 ml were used for other investigations done to patients:

a) Complete blood picture CBC was performed on The CELL-DYN 3700 automated hematology analyzer.
b) Renal and liver function tests were performed on Auto-analyzer Bechman Synchron cx5 system.
c) Measurement of ESR by the Westergren method.
d) Serum CRP concentrations were determined by immuno-nephelometry methods on a Turbox nephelometer (Orion Diagnostica, Finland). The titer of 6 mg/l were considered positive for CRP.
e) Rheumatoid factor IgM isotype was analyzed using the ELISA kit for RF IgM quantitation (Orgentec Diagnostika GmbH, Germany) according to the manufacturer's instructions. The titer of 20 IU/ml was regarded as positive.[7]

Anti MCV and Anti CCP testing was performed in an investigator blinded fashion. Anti CCP antibody reactivity was tested using a commercially available automated ELISA on automated analyzer according to manufacturer recommendation. Value of 25.0 U/ml or greater were considered to be positive.

Anti MCV antibodies were measured using a commercially available ELISA according to manufacturer instruction. Value of 20.0U/ml or greater considered as positive.

Serum diluted 1: 1000 for anti-MCV and 1:50 for anti-CCP. Than incubated on coated plates with kit standard and control at room temperature for 30 min than washed and added horseradish peroxidase labelled anti human IgG for 15 min. the reaction were revealed by the addition of TBM (3,3;5,5-tetramethylbenzidine) substrate and the colour intensity was measured at 450/620 nm.

RESULTS

Laboratory and serological assessment showed a mean serum Anti-CCP2 in patients with RA which was significantly higher than controls. The anti-MCV levels and Rheumatoid Factor were also significantly higher in RA patients compared to healthy control subjects.

Of 92 patients with RA, 72 patients were positive for anti-MCV antibodies (78.26%), 70 patients were positive for anti-CCP antibodies (76.08 %), 54 patients were positive for RF (58.60%), any of them were positive in 89 patients (96.73%) and all of them positive in 34 patients (37%). By contrast, of 35 healthy controls, 1 person was positive for anti-MCV antibodies (97.14%), 2 persons were positive for anti-CCP antibodies (94.28%), 4 persons were positive for RF (77.15 %) any of them were positive in 11 subjects (68.57%)and all of them positive in no subjects (0%).

DISCUSSION

The main focus of our study was to investigate the usefulness of anti-MCV for diagnosing and assessing severity of RA in comparison to anti-CCP and Rheumatoid Factor. In recent years, many studies have evaluated the presence of anti-MCV, anti-CCP antibodies, and RF in RA patients. In our study, at the cutoff values recommended by the manufacturer, the sensitivity and specificity of RF, anti-CCP, and anti-MCV in diagnosing RA compared.

In most of the published works that we studied, the sensitivity of anti-MCV was somehow higher than ACCP but ACCP was more specific.[6,8-11] The same results have been mentioned in some other studies that Ernest Wagner et al. referred to. They found that in RF negative patients, the sensitivity of anti-MCV is higher (43.8% versus 30%).[10]

The study showed that the levels of anti-CCP2 were significantly increased in the sera of patients with RA in comparison with the controls, which agrees with what has been reported in late studies by Zhu and Feng, 2013 in chinese patients with RA, Sariyildiz et al. 2013 in Turkish patients amongst other multi-ethenic studies.[7,12-14] In confirmation to what has been previously reported the study revealed a significantly higher serum anti-MCV antibody level in Egyptian RA patients when compared to healthy controls , supporting the hypothesis that citrullinated vimentin plays an integral role in triggering the inflammatory immune response in RA.[7,15] This antigenic self-protein activates T lymphocytes by binding on HLA-DR4 on the surface of antigen presenting cells and may contribute to certain pathways in the pathogenesis of RA. Several late studies have demonstrated significant elevation in serum anti-MCV in RA patients versus controls which correlated with severity of inflammatory process as evidenced by the associated increase in the inflammatory biomarkers, and evidences of association of anti-MCV with higher incidence of radiographic progression in these patients,[7,12-17] in contrast to this finding, Morbach et al.,[17] found no significant difference.

Our study did not show the significant differences between sensitivity and specificity of ACCP and anti-MCV (sensitivity 85%, 81%, specificity 96% and 95%, respectively) but significant difference than Rheumatoid Factor (sensitivity 58.60%; specificity 88.57%).

Liu et al,[16] and Al-Shukaili et al.,2012 showed that the sensitivities of anti-MCV antibodies was the highest in comparison to anti-CCP antibodies and RF were (78.2% and 72%), (61.8% and 52%), and 72.4% and 57%), respectively. While a contradictory to Maraina et al., 2010, who stated that the sensitivity of RF was higher than the sensitivity of anti-CCP or anti-MCV antibodies. Also, contradictory to Bartoloni et al., 2012, who stated that, anti-MCV demonstrated lower sensitivity than anti-CCP.

Roland et al., 2008, and Damjanovska et al 2009, showed that, the specificity of anti-MCV antibodies was the highest in comparison to anti-CCP antibodies.

While a contradictory to, Soos et al., 2007, Sghiri et al.[8] and Al-Shukaili et al., 2012, who stated that; the specificity of anti-CCP antibodies was higher than that of anti-MCV antibodies or RF.

Positive anti MCV was also reported in SLE, Sjögren syndrome, psoriatic arthritis.

CONCLUSION

Our study suggests that ACCP is an informative diagnostic test for RA. Anti-MCV does not have additional value. This statement is based on the somehow more sensitivity and specificity and the results of kappa, indicating positivity of ACCP in patients positive for anti-MCV and vice versa.

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