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Diagnostic Sensitivity of Anti-cyclic Citrullinated Peptide ACCP2 and Rheumatoid Factor Isotypes RFs in Sudanese Rheumatoid Arthritis RA Patients.

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ABSTRACT

The early diagnosis is essential, as it has been observed that progression occurs within 2 years of disease onset.. An aggressive intervention with new and effective biological treatments can alter the course of the disease, prevent joint destruction, lengthen life, and improve function . RA is known to be associated with broad range or the presence of a large number of autoantibodies The present study was designed as a case-control study, carried out in the National Ribat University Hospital, in Khartoum State, Sudan. The aim of this study was to assess the diagnostic value of RA serological markers. The study group composed of 88 patients with RA; all patients had fulfilled the criteria of the American College of Rheumatology (ACR) (5), whereas control group composed of 53 healthy individuals. Blood samples were collected from the study population in order to determine RF-isotypes namely (RF-IgM, RF-IgA), and ACCP2. RF-isotypes and ACCP2 were measured quantitatively using a Sandwich Enzyme–Linked Immunosorbant Assay ELISA (Euroimmune, Germany). displayed that 56.8% of the study group had positive RF-IgM, where it was 22.6% in control group. RF-IgA showed positive reaction in 47.7% and 30.2% of study group and control group, respectively Both RF-isotypes were found to be positive in 33(37.5%) and 6(11.3%) of study group and control group respectively. The ACCP2 was found positive in 52(59.1%) of study group and in 3(5.7%) of control group (table 2). The mean value of ACCP2 in RA patients was statistically significant than that of control group (*p*.value =0.000). Sensitivity for RA was highest for ACCP2 59%, followed by RF-IgM 56.82%. Utility of two combined RF-isotypes autoantibodies sensitivity for RA was 69(78.4%) and will be 90.9% when ACCP2 was enrolled with the 2 RF-isotypes. In this study 11(12.5%) patients were RF-isotypes negative and positive for ACCP2. Correlation between ACCP2 & RF-IgM was 0.386 and was 0.255 between ACCP2 and RF-IgA and there was no such correlations with the controls. This study showing that the ACCP2 had slightly better sensitivity than RFs among RA patients, and the diagnostic sensitivity of RA increased when ACCP2 was added in the panel along with RF-isotypes.

Keywords: Rheumatoid arthritis, Auto-antibodies of ACCP2 and RF-IgM plus RF-IgA, Sudan.

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INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease affecting about 1% of the general population worldwide (1). It is a chronic and progressive disease that leads to gradual destruction and functional damage of joints, and uncontrolled proliferation of synovial membrane. The inflammation spreads symmetrically from small to large joints, Knee, elbow, and ankle. The initial symptoms include painful swelling of the proximal interphalangeal and metacarpophalangeal joints, with morning stiffness (2, 3). Like many autoimmune diseases, RA occurs more frequently in females than in males (3:1 ratio), suggesting a role for sex hormones. Extra articular manifestations such as vasculitis and rheumatoid nodules may occur in patients with long-standing RA (4). Onset of RA usually occurs between 30 and 50 years of age. Diagnosis of RA is difficult in most of the times as it is based on clinical manifestations as indicated by the revised American College of Rheumatology (ACR) criteria (1987) (5). Those criteria may not be developed in the early stages of the disease, and the patients do not always show typical symptoms and signs to fulfill the (ACR) classification criteria The early diagnosis is essential, as it has been observed that progression occurs within 2 years of disease onset (6). An aggressive intervention with new and effective biological treatments can alter the course of the disease, prevent joint destruction, lengthen life, and improve function (7). RA is known to be associated with broad range or the presence of a large number of autoantibodies (8,9).Rheumatoid factor (RF) is a family of autoantibodies that recognize the crystalizable fraction (Fc) part of IgG molecules and exists as IgM, IgA and other isotypes. Serological testing for rheumatoid factor is complicated by moderate sensitivity and specificity and high rates of positivity in other auto-immune and infectious diseases such as Sjogren's syndrome and chronic viral hepatitis (10). RF is taken as non-specific marker because it is present in normal and elderly individuals (11,12).The most specific auto-antibody system for RA, anti-cyclic citrullinated peptide ACCP bind to antigenic determinant containing the unusual amino acid citrulline, formed by post-translational modification of arginine (13), and mediated by enzymes peptidyl-arginine deaminases (PADs) and may occur during inflammatory conditions (14). This test for anti-CCP antibodies is currently known as the anti-CCP1, and the second generation known as the anti-CCP2 assay, and has slightly better performance characteristics than anti-CCP1 (15). Also, it has been described as highly specific and useful for RA diagnosis (11,16,17,18). ACCP has high specificity (91-98%) but wide variability in diagnostic sensitivity (41-68%)(19). Anti-CCP2 is currently the most widely used anti-citrullinated peptide assay (20).

The aim of this study was to assess the diagnostic values of the RA serological markers, ACCP2, RF-IgM and RF-IgA, and to study the correlation between these markers in Sudanese RA patients. There are no published data on this issue from Sudan.

MATERIALS AND METHOD

The present Study was designed as a case-control study, carried out in the National Ribat University Hospital, in Khartoum State, Sudan. The study group composed of 88 patients with rheumatoid arthritis; all patients had fulfilled the criteria of the American College of Rheumatology (ACR) (5), whereas control group composed of 53 healthy individuals. Blood samples were collected from the study population in order to determine RF-isotypes namely (RF-IgM, RF-IgA), and ACCP2. Serum was separated from the blood samples and stored at -70oC until tested. RF-isotypes and ACCP2 were measured quantitatively using a Sandwich Enzyme–Linked Immunosorbant Assay ELISA (Euroimmune, Germany).

The assay is based on the use of specific plate coated with highly purified Fc fragment of human IgG that used to capture the relevant autoantibody in patient serum. The complex of the coated antibody-autoantibody reacted with the third antibody that specific to certain antibody isotype. This complex was detected by measuring the labeled enzyme that previously attached to the third antibody. Results were expressed in relative unit per milliliter RU/ml. Cutoff value ≥ 5.0 RU/ml was taken as positive for anti-CCP2-Ab, and ≥15 RU/ml was taken as positive for both RF-isotypes as recommended in leaflet. The present study was approved by the National Ribat University and the participants were informed about the purpose of study before samples collection.

Statistical analysis

All the data were computed using SPSS, version 18. Statistical analysis was done using ANOVA for continuous variables, and Chi square test was used for proportion. Correlation between quantitative variables was assessed by Pearson's correlation coefficient. *P. value* <0.05 was considered to be statistically significant

RESULTS AND DISCUSSION

The study population (control and study groups) was screened for the present of RF isoptypes and ACCP2. The mean age of the study group was 41.7 years and 38.8 years for control group. The result displayed that 56.8% of the study group had positive RF-IgM, where it was 22.6% in control group. RF-IgA showed positive reaction in 47.7% and 30.2% of study group and control group respectively (table 1). Both RF-isotypes were found to be positive in 33(37.5%) and 6(11.3%) of study group and control group respectively (table 1).

The ACCP2 was found positive in 52(59.1%) of study group and in 3(5.7%) of control group (table 2). The mean value of ACCP2 in RA patients was (46.26±7.23Ru/ml) and it was

(3.19±1.64Ru/ml) in control group which statistically significant different as indicated by (*p*.value =0.000). Sensitivity for RA was highest for ACCP2 59% followed by RF-IgM 56.82% and RF-IgA 47.7%. Utility of two combined RF-isotypes autoantibodies sensitivity for RA was 69(78.4%) and will be 90.9% when ACCP2 was enrolled with the 2 RF-isotypes. In this study 11(12.5%) patients were RF-isotypes negative and positive for ACCP2. Correlation between ACCP2 & RF-IgM was 0.386 (figure 1) and 0.255 between ACCP2 and RF-IgA (Figure 2) and there was no such correlations with the controls.

Table 1: RF-isotypes positivity among RA patients and controls

	Patient (n=88)) positive	**Control (no 53)**	**Chi-square**	***p*.value**
RF-IgM	50 (56.8%)	12 (22.6%)	17.72	0.000
RF-IgA	42 (47.7%)	16 (30.18%)	4.2	0.052
nixed positivity of IgM and IgA	33 (37.5%)	6 (11.3%)	11.33	0.001

Table 2: **ACCP2 antibodies positivity among RA patients and healthy controls**

Group of patients and controls	**ACCP2 positive**	**ACCP2 negative**	**Mean ± SD**	**Chi square**	***P.* value**
Patients (no 88)	52(59%)	36(40.9%)	46.26 ±7.23	39.7	0.001
Controls (no 53)	3(5.7%)	50(94.3%)	3.19 ± 1.64		

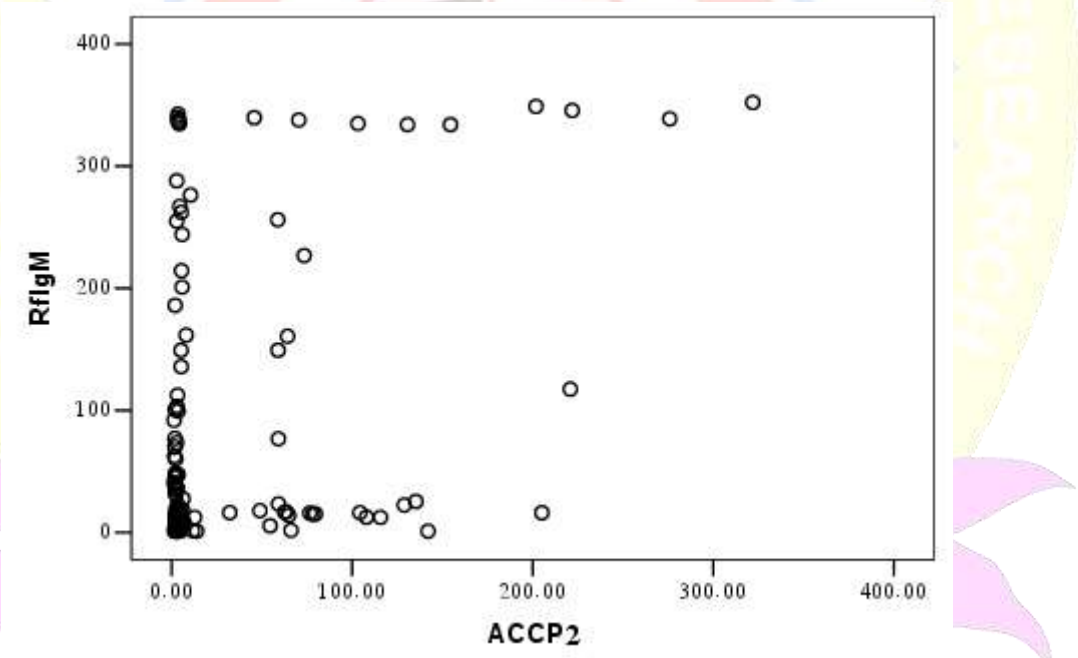

Figure 1: Correlation between ACCP2 and RF-IgM in RA patients (r=0.386)

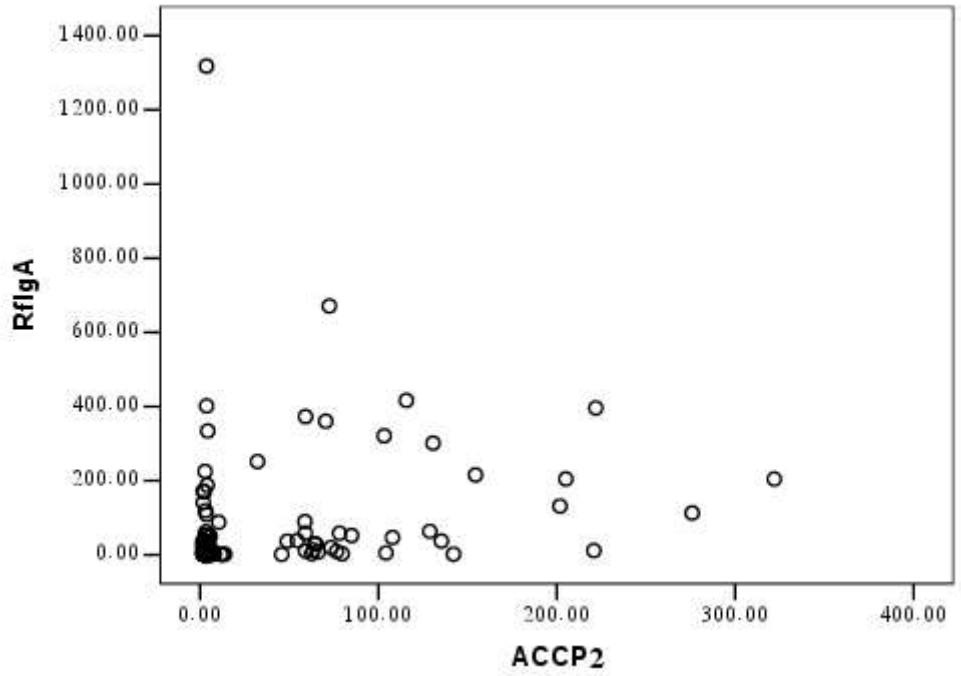

Figure 2: Correlation between ACCP2 and RF-IgA in RA patients (r=0.255)

DISCUSSION

Rheumatoid arthritis is a common autoimmune polyarthritis showing positivity for various autoantibodies (11). The aim of this study was to assess the diagnostic value of RA serological markers. The study showed that the female/male ratio was 2:1 which agreed with Gabriel 2001 (2). The frequency of positive RF among RA patients had a wide variability; in our study the positive rate of RF was found to be (78.4%) in patient group, this finding was similar to that reported by Lindqvist et al (21) (79%), and agreed with the range (50-80%) reported by Bas (19) and relatively consistent with 66.4% reported by Vallbracht et al (16).

Our results showed that the positive RF-IgM and RF-IgA were 56.8% and 47.7% in patients group respectively. However Usha(11) and Vallbracht(16) reported 47.89% and 66.4% positive RF-IgM respectively, whereas Sigita et al (22), Usha et al (11) and lindqvist et al (16) had reported 36.89%, 64%, and 78% positive RF-IgA respectively. The dissimilarity of these results might be due to variation of titration and cutoff levels (1) of each study, some patients were on continuous medication, or partly explained by different sample size. When the RF-isotypes combined with ACCP2, considerable sensitivity will be obtained 90.9%, because there were 11 of the patients had only ACCP2 positive. This study revealed that both autoantibodies of RF-isotypes could exist within the same patient, similar observation reported by Sigita et al, (22).

Our study showed that ACCP2 had slightly better sensitivity than RF in diagnosing RA patients. It was found positive in 52(59%) in the study group. Bas et al (19), found that the ACCP2 positive rate was within range of (41-68%) and concluded that ACCP2 had a wide

variability in diagnostic sensitivity. Contrary to these findings, several researchers found a higher positive rate for ACCP2 among RA patients (1, 16, 23, 24), while other authors reported a low positive rate of ACCP2 among RA patients (11). In this study the results exhibited that 3 (3.6%) of the control group were positive for ACCP2, this finding may reflect early phase of disease, however Usha et al (11) and van Venrooij et al (25) noted that 1% of healthy individual may also have positive ACCP2.

In our results we found that 11(21.2%) of patient group were positive for ACCP2 and negative for the two RF-isotypes, similar results were reported by different authors (11, 26, 27). Previously, there was evidence supporting the existence of correlation between ACCP and RF in RA patients (17). Our study indicated a positive correlation 0.386 between ACCP2 and RF-IgM, whereas correlation between ACCP2 and RF-IgA was 0.25. This result in alignment with Zaval-Cerna et al (8) result who suggesting that there were different pathways and times for the generation of both antibodies,

CONCLUSION

This study showing that the ACCP2 had slightly better sensitivity than RFs among RA patients, and the diagnostic sensitivity of RA will increased when ACCP2 was added in the panel along with RF-isotypes to 90.9%

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