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Original Article

Angiotensin-Converting Enzyme as Immune Inflammatory Biomarker in Rheumatoid Arthritis Patients

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ABSTRACT

Rheumatoid arthritis (RA) is one of the most common autoimmune diseases which mainly affects joints. The link between angiotensin-converting enzyme (ACE) and Rheumatoid Arthritis (RA) still needs more investigation, especially in the pro-inflammatory process and the production of inflammatory molecules. Blood specimens were collected from 71 patients and 24 healthy individuals, and all needed information was collected in a questionnaire. Laboratory investigations for CRP, anti-CCP, RF, and ACE were performed. Mean concentrations for the patients were significantly higher for anti-CCP, hs-CRP, RF, and ACE, respectively. The statistical analyses compared three pairs of variables: "ACE versus. Anti-CCP, ACE versus. CRP, and ACE versus RF, Which was based on Pearson correlation coefficients; showed there was no correlation between “ACE versus Anti-CCP" with a very weak negative correlation in both ACE versus CRP and ACE versus RF respectively. Current study concludes that there is a link between ACE concentration in participants' sera and numerous immuno-inflammatory markers in RA patients.

Keywords: Rheumatoid arthritis, Angiotensin-converting enzyme, Inflammatory biomarker, anti-CCP, RF.

1 Introduction

Rheumatoid arthritis (RA) is a chronic, systematic inflammatory autoimmune disease of unclear etiology 60% of the risk factors are genetic. Other environmental factors such as smoking, oral health, and diet can be considered the remaining percentage.[1-3]. RA manifests as a progressive and destructive arthritis associated with serological evidence of autoreactive antibodies and with systemic complications leading to progressive disability and premature death [1]. The risk of sudden cardiac death and ischemic heart disease (IHD) is significantly higher in patients with RA than in those without it, contributing significantly to RA-related mortality. [1]

The prevalence rate of RA disease in the world was recorded at about 0.3 - 1.2 %, cartilage destruction is one of the main properties of this disease [4]. In RA patients, the development of synovium angiogenesis (new blood vessels are growing with infiltration of Immunocytes) will be leading to the invasion of tissue and joint destruction [5], and these will stimulate inflammation associated with increased production of cytokine, chemokine, inflammatory reactants such as C-reactive protein (CRP) and overproduction of autoantibodies like an increase of immunoglobulin M (RF) and anti-cyclic citrullinated peptide (anti-CCP) in RA patients [3] Moreover, the CRP, RF, and anti-CCP represent immunological RA immune response and diagnostic blood biomarkers [6]. Many researchers approved the relationship between RA, Inflammatory cytokines production like TNF-α, acute-phase and immune complexes due to the role of TNF-α which increases expression other pro-inflammatory cytokines (i.e., interleukin-1 (IL-1), IL-6 and IL-8), local functional renin-angiotensin systems (RAS), as well as stimulate hepatocytes for acute phase proteins production all these will sustain systemic inflammation that leads to increase endothelial activation [7,8]. ACE is one component of RAS that is expressed in a wide range of tissues, like skin, vascular endothelium, immune cells and activated monocytes [9,10]. Cobankara and his team in 2005 concluded that the angiotensin-converting enzyme is strongly linked to joint destruction in rheumatoid arthritis [11]. The ACE is a membrane-bound, zinc metalloendopeptidase and it is known as peptidyl dipeptidase A or kininase II, encoded by

the ACE gene. It is mapped on chromosome 17q23 and plays a central role in several diseases, includes RA [12, 13]. This study aims to investigate the link between ACE concentration in participants sera and numerous immuno-inflammatory markers in RA patients and also to see the progress of the disease with age and the health state of individuals.

2 Methodology

2. 1 Rheumatoid arthritis patients and Control group

This is a case-control study was done from (September 2021 to January 2023). Ninety-five individuals were involved in the study, with an age range 23–74 years. They were divided into two groups:

The patients group consist of 71 individuals (6 males and 65 females), their ages ranging between (23 -74) years, who regularly attended to Rezgari Teaching Hospital in Erbil city**.** Verbal consent was taken from all participants during specimen collection. The patients subjected to clinical examination to confirm a diagnosis and answer the questions in a specially designed questionnaire form prepared according to the purpose of study and filled up through direct interviews with the patient. The complete medical history was obtained, and any patients with other types of autoimmune disease or had systemic disease like hypertension, endocrine disease or cancer were excluded.

Control group: Includes 24 individuals whose ages ranged from 34 to 70 years. The group was selected after confirming they did not have any clinical signs of RA, or any other rheumatic disorders in addition to other exclusion criteria like cancer, pregnancy, endocrine disorder or any other autoimmune diseases. The study was established after obtaining ethical approval from the ethical committee, and participant consent obtained in the vernacular.

2. 3 Sample collection

Five milliliters of venous blood were obtained from all participants by a sterile a disposable syringe and transferred directly into a vacuum gel tube, left at room temperature for 30 minutes and after that centrifuged at 3000 rpm for 15 minutes to obtain serum. The sera were divided into four labeled sterile Eppendorf tubes and stored at -80ºC for measurement of rheumatoid factors (RF), anti-cyclic citrullinated peptides (Anti-CCP), C - reactive protein (CRP), and Angiotensin Converting Enzyme (ACE) concentration tests.

2. 4 Blood Laboratory tests

Serum quantitative concentration measurement for all RF, hs-CRP and Anti-CCP was done by using Cobas 6000 analyzer according to the manufacturer's instructions, except for ACE concentration, which was determined turbidimetrically by commercial kits from (Roche Diagnostics, GmbH). Normal range of each test was determined by the manufacturer (Roche Diagnostics, GmbH): RF ≤ 20 IU/ml, hs-CRP ≤ 10 mg/L, Anti-CCP ≤ 17 U/ml and Angiotensin enzyme (ACE) 8– 52 U/L.

2. 5 Body Mass Index (BMI)

It was calculated by using formula of BMI equals weight/height^2 in (kilogram/meter ^{2}). The National Institutes of Health (NIH) classified subjects according to their BMI: Normal weight: 18.5-24.9 kg/m2 Overweight: 25-29.9 kg/m2 Obese: ≥ 30kg/m2

2. 6 Ethical consideration

The study protocol was submitted to the ethics committee at Erbil Polytechnic University / Hawler Health Technical College, and official acceptance was obtained from the Erbil Directorate of Health and Rezgari Teaching Hospital in Erbil city. During interview with the participants, study details and its importance were explained verbally for them, and researchers kept the information as no names were mentioned by used codes only.

2. 7 Statistical Analysis

All obtained data were analyzed using the GraphPad Prism program, version 8. Normality t-test in addition to spearman`s correlation test were performed to determine the significance alterations and correlation status. The results were recorded as mean ± standard deviation (SD), and significant differences were reported when P value was less than 0.05. The receiver operating characteristics (ROC) curve was applied to analyze the results. An area under the curve (AUC) is frequently interpreted about thresholds, with good models defined at 0.7,0.8, or excellent models defined at 0.9 [14].

3 Result and Discussion

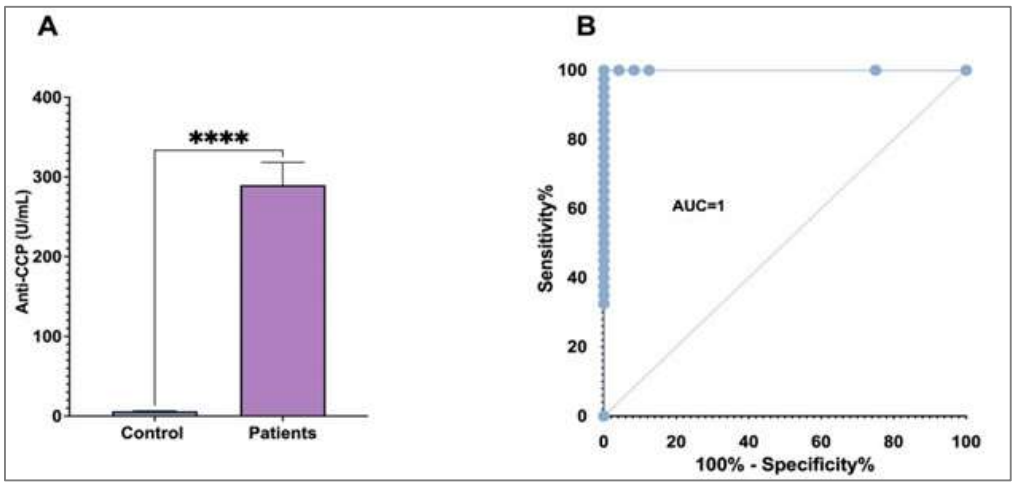
The high incidence of RA made it a hot spot for research, and it is important to mention that this study is the first conducted in Iraq for assessment Angiotensin Converting Enzyme (ACE) among RA patients, and assess its relationship with the levels of serum RF, Anti-CCP Ab and hs-CRP. In this study, several factors were examined to assess their effects on RA, including age and MBI. The current study's results show that the participating patients were over fifty (Table 1), which aligns with Khusainova's findings MA [15,16] as the inflammation of joints will progress with age towards joint damage, physical disruption in patients associated with elevated disease activity and the presence of bone erosions that will lead to increased patient disability. In other hand body weight or body mass index is one of the points that enhance patient state as it was increased with significant differences in patients who suffer from rheumatoid arthritis rather than health control individuals with same age, so the estimation of BMI can be considered one of the issues that must be put in mind for reducing signs and symptoms[17,18]. The results of BMI for participants in this study can be seen in (Table 1) and showed most patients above 30 were considered obese. This result agrees with Shand and his team in 2003, who reported obesity had a high prevalence rate among RA patients and it associated with adverse effect impact on the course of RA [19] and the same result obtained by Dey and his time [20].

Table 1: Demographic characteristics of the studied groups about age and BMI

Categories	Patients 71 Mean ± SD	Control 24 Mean ± SD	*P value*
Age	50 ± 11.7	53 ± 10.7	0.29 *
BMI (kg/cm^2)	30 ± 6.5	26 ± 2.8	0.002 **

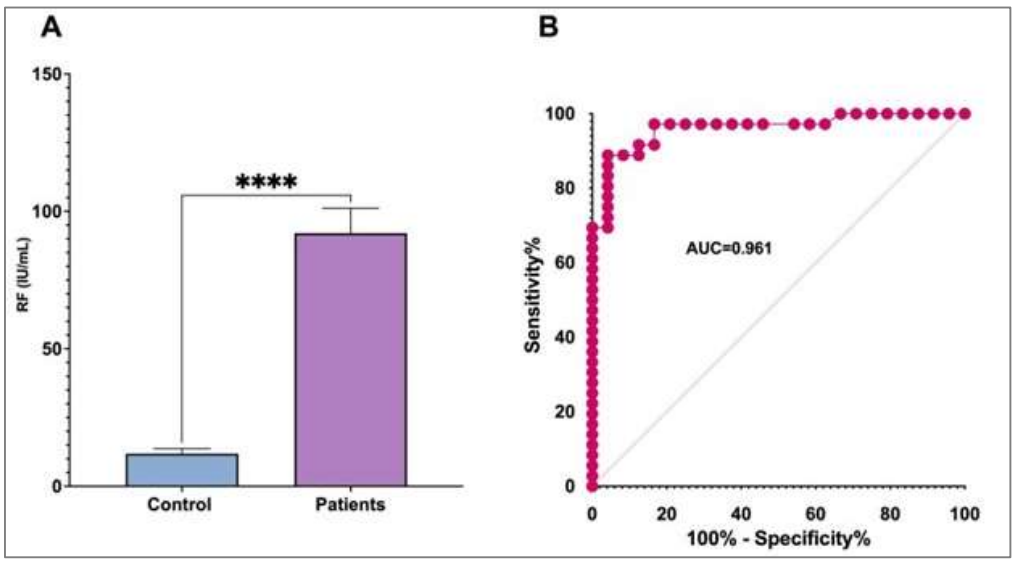
* No-significant.
** P<0.05 is a Significant

Specific laboratory tests for RA are measured such as Rheumatoid factor (RF) and anti-CCP antibodies, which consider the immunological parameters that are strongly related to Rheumatoid arthritis and were highly elevated with significant differences among patients compared with the control group (figure 1,2). This result resembles many studies done previously in different parts of the world [21,23]. According to the results of this study, especially the result of ROC analysis, the estimation of anti-CCP Ab is considered one of the most useful laboratory investigations for the diagnosis and for determining the severity and progression of rheumatoid arthritis, and this finding agrees with [24,25]. It's important to mention here that the estimation of Anti-CCP Ab has a vital role in monitoring the treatment and controlling the prognosis of the disease as Anti-CCP was not associated with disease activity but had associated with increased radiographic damage [26]. Concerning RF, it is less specific for diagnosis as it is found in only about 80% of RA patients, and approximately 30% to 45% of patients with early RA do not have RF, though some may develop RF later in the course of the disease [27].



******** P<0.000 high significant increase in RF

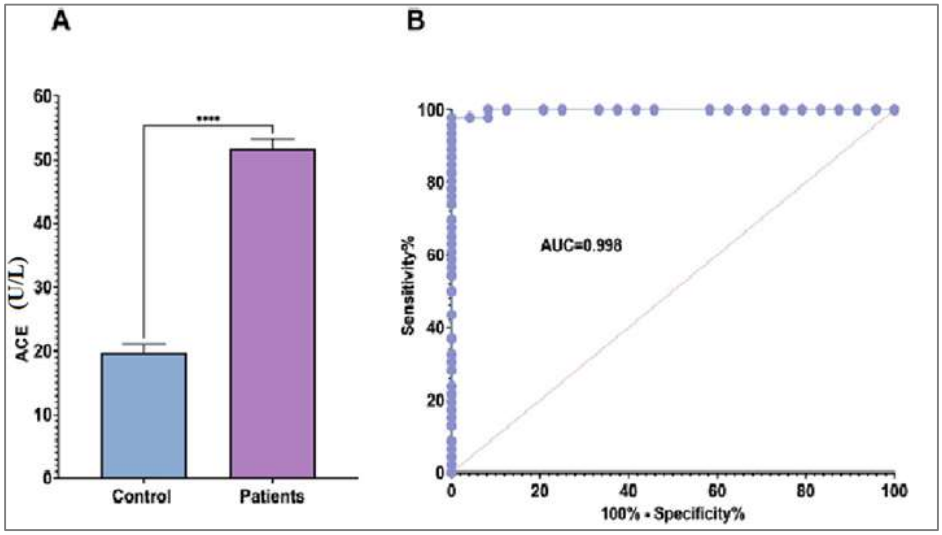
Figure 1: Mean concentration of Anti-CCP Ab (U/ml) in the sera of patients and control group.



**** P<0.000 high significant increase in RF

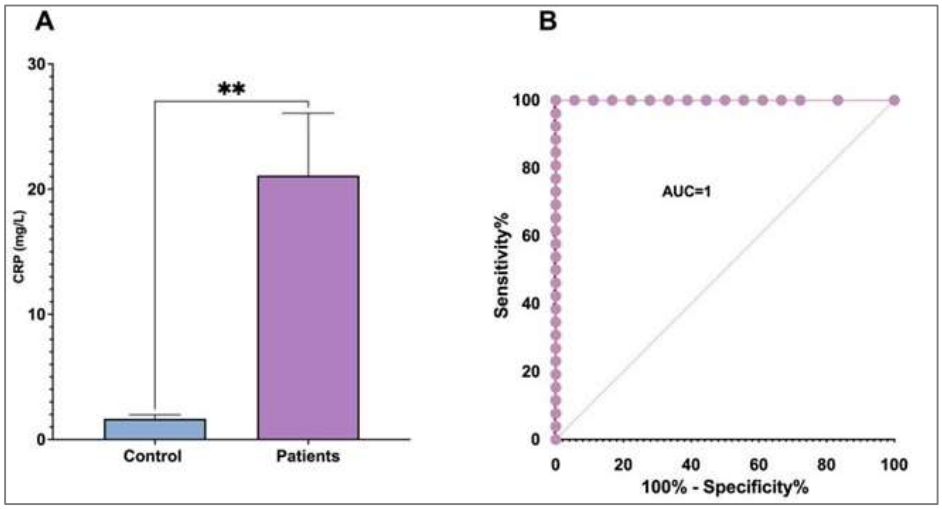
Figure 2: Mean concentration of RF (IU/mL) in sera of patients and control group.

Cobankara and his team, in a study was done in 2005, mentioned that the angiotensin-converting enzyme is strongly linked to joint destruction in rheumatoid arthritis [11]. So, the estimation of ACE was important to find or detect the link in the sera of RA patients. The finding of Angiotensin-converting enzyme concentration (ACE) in the sera of patients in the current study (figure 3) showed a significant elevation in patients compared with the control group. That means the presence of a strong relationship between rising ACE concentration and the destruction of joints among RA patients as a result of systemic autoimmune disease represented by rheumatoid arthritis [27,28]. Also, other study mentioned that ACE was higher in the synovial fluid of rheumatoid arthritis [29]. This finding appears as another indicator shows a link between the rising of ACE concentration and the destruction of joints among RA patients. hs-CRP is another inflammatory marker commonly used for rheumatoid arthritis and many other systemic diseases. In the current study, hs-CRP was measured in the sera of rheumatoid patients and healthy control group; the results show the concentration of hs-CRP was significance differences between patients and the control group (figure 4) and this is due to the patients with RA who participated in current study generally suffering from the severity of inflammation which directly related to more than one causes such stress and environmental factors. It was also established that most RA patients with higher hs-CRP have a higher degree of inflammation and are more prone to develop CVD than those with lower hs-CRP [30]. Another study done in Baghdad also showed significant differences in hs-CRP and that agreed with the result of the current study [31]. Furthermore, the result of the ROC curve shows that the AUC value was 1, which indicates that the hs-CRP test can be used as a specific test for the diagnosis or for determining the severity of Rheumatoid arthritis



**** P<0.000 high significant increase in RF

Figure 3: Mean concentration of Angiotensin-converting enzyme (ACE) U/L in the sera of participants.

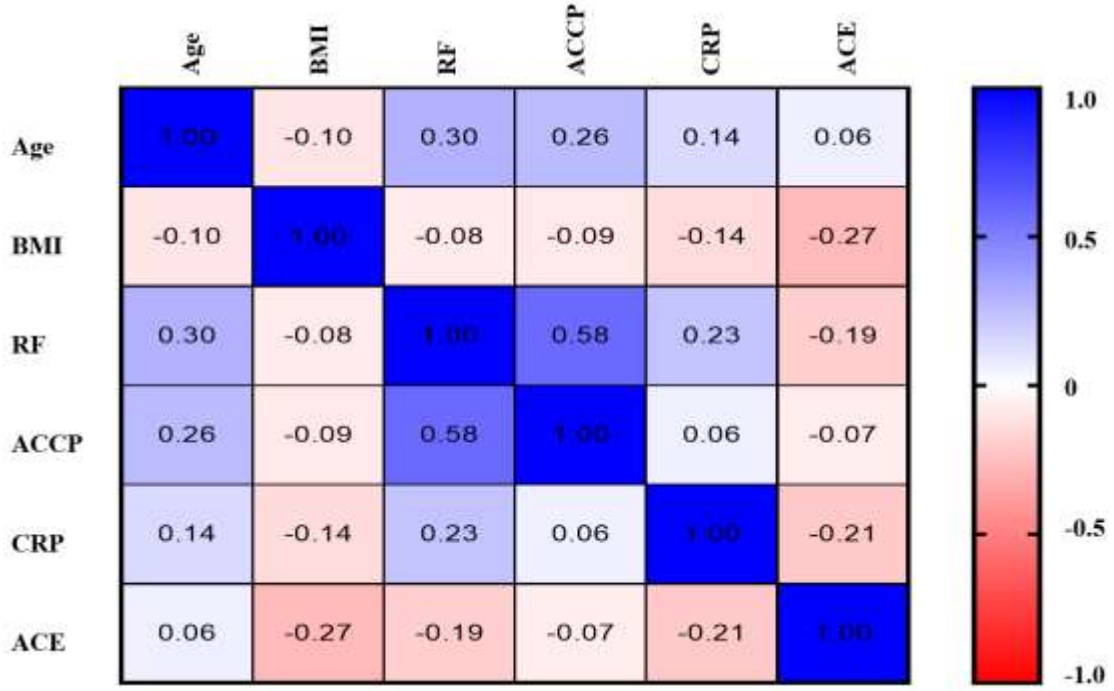


** P<0.001 high significant increase in RF

Figure 4: Mean concentration of hs-CRP mg/L in sera participants.

The correlation coefficients between variables including Age, BMI, RF, ACCP, CRP, and ACE, that their values range from -1 to 1, where a positive value indicates a positive correlation (as one variable increases, the other tends to increase) and a negative value indicates a negative correlation (as one variable increases, the other tends to decrease). While the values closer to 0 suggest a weaker correlation. and a zero (0) value means there was no correlation between the variables. The diagonal line of 1.00 values represents the correlation of each variable with itself, which is always 1.

Table 2: provides statistical information about the correlation between the variables in the current study: Age, BMI, RF, ACCP, CRP, and ACE.



The statistical analyses comparing three pairs of variables: ACE versus anti-CCP, CRP, and RF are shown in Table 3). The statistical analysis is based on Pearson correlation coefficients. First, the Pearson r (correlation coefficient) shows that the (r) value for ACE versus Anti-CCP was -0.07 with p = 0.596 ; (r) value for "ACE versus CRP" was -0.21 with p= 0.100, and(r) value ACE versus RF was -0.19 with p = 0.148 respectively. Regarding the (P value), the statistical analysis finding shows that there is statistically no significant appearance between ACE versus Anti-CCP, ACE versus CRP , and ACE versus RF respectively. In addition , there is no correlation was found between ACE versus Anti-CCP with a very weak negative correlation in both ACE versus CRP and ACE versus RF respectively, which means ACE will increase in RF patients' independent to other variable. A high concentration of ACE was found in the sera of RA patients who have a high level of Anti-CCP, hs-CRP and RF, and these findings go with the outcomes of disease [28].

Table 3: provides statistical information about the correlation between three pairs of variables: ACE vs. Anti-CCP, ACE vs. CRP, and ACE vs. RF.

	ACE vs. Anti-CCP	ACE vs. CRP	ACE vs. RF
***P* value**	**0.596**	**0.100**	**0.148**
r	**-0.07**	**-0.21**	**-0.19**
Type of correlation (C.)	**No C.**	**Weak –ve C.**	**Weak –ve C.**

4 Conclusion

The current study concluded that there is a link between ACE concentration in participants sera and numerous immuno-inflammatory markers in RA patients. Results found significant elevations in ACE concentration in the sera of RA patients compared to a control group, suggesting a strong relationship between the concentration of ACE and joint destruction in RA. The current study, supports that the ACE test can be used as an inflammatory biomarker in RA, highlighting its potential role in monitoring disease severity and progression.

Authors contribution

1. Prof Dr. Najat Jabbar Ahmed Berwary.
   General supervision and ideas, writing, aim and Objective of the study.
2. Assist Prof Dr. Raghed Mohammed Jassem
   Rewrite the manuscript , decrease the plagiarism and arranged the paper according to journals format
3. Assist Prof Dr. Zaid Nabeel Elia
   Laboratory working and performing the investigations.
4. 4. Dr. Mahdi Qadr Khalid4
   Diagnosis of RA cases and selection of patients.
5. 5.IT. Sana Najat Jabbar
   Participate in primary data collection from patients.

Conflict of interest:

None

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