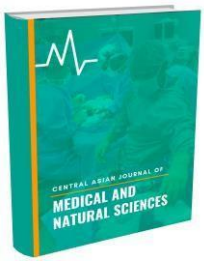

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Article

Evaluation of Rheumatoid Factor, Anti - Cyclic citrullinated peptide, Erythrocyte Sedimentation Rate, and Cell Reactive Protein in diagnosis of Rheumatoid

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Abstract: Rheumatoid arthritis (RA) is an autoimmune disease that causes synovial inflammation, which leads to joint erosion. A group of factors contribute to the occurrence of the disease, including ecological and hereditary features, which can lead to the disease's progress. This study investigated the association between rheumatoid disease and various biomarkers, including RF, anti-CCP, ESR, and CRP. The study aimed to determine the ability of these biomarkers to diagnose rheumatoid disease. Materials and Methods: 100 samples were collected from patients suspected of having rheumatoid arthritis from Al-Sadr Medical City in Najaf/Iraq, the samples were diagnosed by the consultant and the arthritis unit in the abovementioned hospitals from August 2023 to February 2024. Then 50 samples were collected from healthy people as a control group. five ml of intravenous blood was drawn from both patients and controls, and the sera were separated by centrifugation, kept at −20°C, and tested. RF was detected by agglutination of RF latex strips (Solarbio/China), ESR, and CRP. Antibodies to CCP were measured by (ELISA) kit from Genius/USA protocol. Results: The results showed that rheumatoid arthritis was significantly associated with age and gender. patients 24(24%) had a family history of genetic factors. while 76 (76%) were affected by other non-genetic factors. In this study, there is a highly significant difference (P<0.01) And the chi-square was 75. When comparing the RF group with the control group, the RF gave a positive result (84%), while 16 (16%) gave a negative result for the RF, compared with the control. The negative results for the RF were 45 (90%) and 5 (10%) gave positive results. a significant difference (P<0.01) and a chi-square of 122 when comparing between the anti-CCP group and the control group. Anti-CCP gave 93 (93%) positive results for RA compared to the control, 50 (100%) gave negative results (RF, Anti-ccp), (ESR, CRP) also significantly increased in RA. the sensitivity results showed that 84% of tested cases indicated positive results for RF. RF is found in cases of rheumatoid arthritis with a sensitivity of 84%. On the other hand, the specificity result indicates that 90% of healthy controls tested negative for RF. Therefore, RF may be a good indicator for monitoring RA, where positive findings are more prevalent. The susceptibility result indicates that all cases tested showed positive results for ANTI-CCP. ANTI-CCP is present in RA cases with a sensitivity of 93%. It is noteworthy that the specificity is also 100%, indicating that no positive results for ANTI-CCP were found in the healthy control group tested. Therefore, ANTI-CCP is a highly sensitive and specific indicator of RA. Conclusion: The research exposed a notable association between rheumatoid disease and age as well as gender. The occurrence of Anti-CCP demonstrated higher diagnostic accuracy for rheumatoid disease compared to RF alone, as indicated by their sensitivity and specificity. Moreover, ESR and CRP levels were meaningfully raised in individuals with rheumatoid disease, further supporting their role as dependable markers for measuring disease activity. These findings significantly contribute to our understanding of the analytic and prognostic value of these biomarkers in rheumatoid disease.

Keywords: ANTI-CCP, RF, ESR, CRP and Age

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1. Introduction

Rheumatoid arthritis (RA) is an autoimmune illness considered via symmetrical swelling of many joints, including the small joints of the fingers and feet [10]. Arthritis plays a role in causing disability for people afflicted with it, as gradual disability occurs

and then premature death, It also affects all ages, and the incidence increases in people over the age of 40, as it affects women more than men [8]. It spreads throughout global and affects about 0.5–1% of the adult population globally [14]. As well RA is a complex condition that is affected by many environmental and genetic factors. also, Chronic stress and emotional factors may production a role in the progress of rheumatoid arthritis. Including stress hormones may affect the immune system and contribute to inflammation. Smoking is a dangerous feature of rheumatoid arthritis (Romão & Fonseca, 2021). Arthritis is considered a hereditary family disease, as people with a family history are more susceptible to the disease, which is strongly linked to the hereditary related of danger alleles HLA-DRB1, which influences the progress of anti-citrullinated autoimmunity, which is considered by the occurrence of anti-citrullinated peptide antibodies (ACPAs)(Wen & Yu, 2023). An inflammatory reaction, autoantibody stimulation, and hurt to the synovial membrane and joints primarily illustrate the disease.

The beginning of inflammation raises cytokine, chemokine, and inflammatory reactants such as C-reactive protein (CRP). So, CRP, RF, and anti-CCP, representing RA's inflammatory and immune reaction, are analytic blood biomarkers (Mun et al.,2021). (ACPA) which targets citrullinated proteins, as these antibodies were noticed 10 years earlier than the disease seemed [3] As a result of starting to produce ACCPA a long time before the appearance of RA, the process of self-immunization that occurs against these proteins, which are formed at the joint exit sites, can diagnose the disease very long before its appearance through ACCPA (Pope & Choy,2021). CRP is usually considered a marker of systemic inflammation in RA it shows an essential role in the body's attack against infectious mediators and making an inflammatory response. This protein is made in the liver and concealed into the circulation as pentameric CRP (pCRP), also identified as Original CRP. CRP is an immune regulator – not just an indicator of inflammation or infection (Shi et al.,2023). Erythrocyte sedimentation rate (ESR), is a test that measures the speed at which red blood cells settle at the bottom of the test tube. If the red blood cells settle rapidly, this indicates inflammation in the body [5.To develop the efficacy of RA analysis, anti-CCP is used with RF. Anti-CCP has more progressive positivity than RF positivity among RA patients; though, the sensitivity and specificity of the two markers do not significantly differ [12].

2. Materials and Methods

Study design

100 samples were collected from patients suspected of having rheumatoid arthritis from Al-Sadr Medical City in Najaf/Iraq The samples were diagnosed by the consultant and the arthritis unit in the abovementioned hospitals from August 2023 to February 2024 divided into 78 females and 22 males there aged <25-≥65. Then 50 samples35 females and 15 males ages <25-50 years were collected from healthy people as a control group.

Laboratory tests

five ml of intravenous blood was drawn from both patients and controls, and the sera were separated by centrifugation, kept at −20°C, and tested. RF was identified by agglutination of RF latex strips (Solarbio/China), ESR, and CRP. Anti-CCP was measured. By (ELISA) kit from Genius/USA protocol.

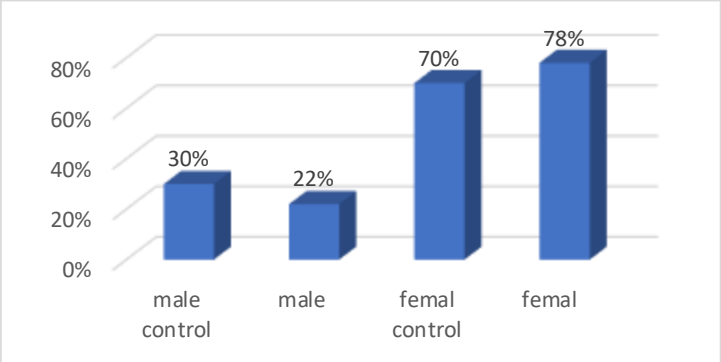
Statistical analysis

Data processing was performed using the software SPSS version 24. Chi-square was used to compare patients and controls at the level ($p>0.01$).

3. Results

Collection of Samples

Samples were collected from 100 patients suffering from rheumatoid arthritis which consisted of 78 (78%) females and 22(22%) males Diagram (1), 50 samples were collected from healthy persons35 (70%) females and 15(30%) as control.



Age group and RA

This study showed that RA is widespread in most ages, but in varying proportions, with the highest percentage recorded in the age group (45-54), 23 (29.4%) women and12(54.5%) men, followed by age group(35-44) 16(20.5%) women and 2(9.5%) men, while The percentage was lowest in the age group <25 for women 5(6.4%) and men 1(4.5%), the age group ≥65 appear 8(10.2%)females and 3(13.5%) males. There was diversity in the rest of the age groups, as exposed in the table (1)

Table 1. distribution of RA patients according to the age

Age		Gender(%)				control
	RA patients		Control		Total	
	Male	Female	Male	female	RA	

<25	1 (4.5%)	5(6.4%)	5(33.3%)	10(28.6%)	6(6%)	15(30%)
25-34	1(4.5%)	15(19.2%)	5(33.3%)	10(28.6%)	16 (16%)	15(30%)
35-44	2(9.5%)	16(20.5%)	3(20%)	8(22.8%)	18 (18%)	11(22%)
45-54	12(54.5%)	23(29.4%)	2(13.4%)	7(20%)	35(35%)	9(18%)
55-64	3(13.5%)	11(14.3%)	-	-	14(14%)	-
≥65	3(13.5%)	8(10.2%)	-	-	11(11%))	-
Total	22(22%)	78(78%)	15(30%)	35(70%)	100(100%)	50(100%)

family history association with RA disease

The study findings indicate that among patients) 2424%(have a family history of genetic factors contributing to their rheumatoid arthritis.76 (76%(are influenced by other non-genetic factors. While the control has a negative result. genetics play a role in RA susceptibility, various other factors also contribute to the development of this chronic inflammatory condition Diagram (2).

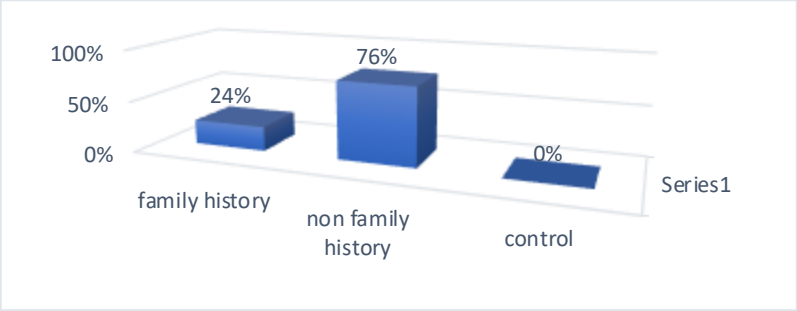


Diagram 2. spreading of the RA patients according to family history

RF and (Anti-CCP) in the study groups

In the present study, There is an extremely significant difference (P<0.01) and Chi-square 75 when compared between the RF and control group, the RF gave a positive result (84%) while 16(16%) gave a negative result for RF the control gave negative results for RF 45 (90%) and 5(10%) gave positive results, also the results show a significant difference (P<0.01) and Chi-square 122 when compared among Anti-ccp and control group, the Anti-ccp gave positive results93(93%) for RA compared to control 50 (100%) gave negative results table(3).

Table 3. show the percentage of RF and anti-ccp in RA patients compared with control

parameters	results	RA patients (%)	Healthy control(%)	Chi-square	P value
RF	Positive	84 (84%)	5 (10%)	75	p < 0.01
	negative	16(16%)	45(90%)		
Anti-ccp	Positive	93(93%)	0	122	p < 0.01
	negative	7(7%)	50 (100%)		

Specificity and sensitivity of RA and ANTI-CCP

The sensitivity result shows that 84% of the tested cases indicated positive results for RF. RF is existing in cases of RA with a sensitivity of 84%. On the other hand, the specificity result proposes that 90% of the healthy controls tested negative for RF. Therefore, RF may be a good pointer for noticing rheumatoid arthritis, as positive results are more prevalent. The sensitivity result indicates that all tested cases revealed positive results for ANTI-CCP. ANTI-CCP is present in cases of RA with a sensitivity of 93%. It is value noticing that the specificity is also 100%, significant that no positive results for ANTI-CCP were found in the healthy control who were tested. Therefore, ANTI-CCP is a highly sensitive and specific indicator for RA, It is a more important diagnostic factor than RF. table(4)

Table 4. Sensitivity and specificity of RF and anticcp

parameters	Sensitivity	Specificity
RF	84%	90%
ANTI-CCP	93%	100%

Association of CRP and ESR in RA patients

There is a statistically significant difference in the percentage of positive CRP tests between RA patients 80(80) and the healthy control group 6(12%), with a chi-square value of 63 and a p-value less than 0.01. This shows that CRP levels are significantly higher in RA patients. There is a significant difference in the percentage of positive ESR tests between RA patients 85(85%) and the healthy control group 4(8%), with a chi-square value of 81.9 and a p-value less than 0.01. This recommends that the ESR is significantly higher in RA patients.

Table 5. percentage of CRP and ESR in RA patients compared with control

parameters	results	RA patients (%)	Healthy control	Chi-square	P value
CRP	Positive	80 (80%)	6 (12%)	63	p < 0.01

	negative	20(20%)	44(88%)		
ESR	Positive	85 (85%)	4 (8%)	81.9	$p < 0.01$
	negative	15(15%)	46 (92%)		

4. Discussion

[7] found in their study that RA is more prevalent in women than men. (Murata et al.,2020) explain the Occurrence of RA in this populace (N=27,371) was 1.9% among men and 2.8% among women. [11] found that 56 people had RA, and their average age was 51.3 ± 13.2 years. The percentage was approximately 25.0% older than 60 years, and 19.6% each in the age group 41-50. and 5.4% were less than 30 years old. (Murata et al.,2020) showed in his study that 31 patients (11.9%) out of 260 patients diagnosed with RA were due to a family history of RA up to the second degree. [1]found that measuring (anti-CCP) is additionally specific and less sensitive

1. as a serological indicator for diagnosing RA. [15] found that of 9135 patients diagnosed with active rheumatoid arthritis, 58% did not have raised ESR or C-reactive protein (CRP);16% were found to have elevated ESR and CRP and 26% had elevated ESR or CRP. RA is a greater rate in females, signifying female hormonal features production a role in the progress of the disease[2]. Epidemiological information regarding endogenous and exogenous hormonal factors is unpredictable, but it is clear that low estrogen and/or progesterone levels in menopause and postpartum raise the danger and severity of RA. (Raine & Giles,2022). A family history of RA is a sign of an individual's genetic risk and is a good guide to the occurrence of the disease, and an increased risk of developing rheumatoid arthritis due to common environment and genetics [9]. [17] showed that a family history of other autoimmune rheumatic diseases such as lupus and connective tissue diseases predicts rheumatoid arthritis. RA is diagnosed based on clinical symptoms and the diagnosis is made based on RF. However, its analytical specificity for RA is weak because it is common in the diagnosis of many other rheumatic and non-rheumatic diseases and even in a noticeable percentage of normal healthy people, especially in persons of advanced age. Attention has turned to another test to identify RA, which is anti-CCP, as it has a high degree of specificity in diagnosing RA, as it is used to diagnose it in patients who suffer from the disease early (Samanci et al.,2005). The relationship between anti-CCP and the severity of the disease in early arthritis. Elevated ESR levels indicate inflammation, but are not specific to rheumatoid arthritis As some patients have a normal level of ESR and CRP, it is found that there are factors that affect it, such as disease activity, individual differences, and concurrent medications. Other conditions can also cause a high level of ESR. production of cytokines, which stimulate the liver to produce certain proteins called acute phase reactants. Such as fibrinogen, which increases the erythrocyte sedimentation rate by helping red blood cell clumping. Which leads to increased blood viscosity. This increased viscosity can hinder red blood cell flow and lead to an elevated ESR (Jassim et al., 2024). CRP is produced by the liver in response to inflammation. It is a more sensitive marker than ESR and tends to rise more quickly CRP is one of the reactant substances whose production leads to increased inflammation, which activates the immune system, prominent to the release of many pro-inflammatory cytokines, stimulates the liver to produce CRP. Therefore, increased inflammation in RA leads to raised (CRP) levels [6].

5. Conclusion

The studies reviewed indicate several key findings about rheumatoid arthritis (RA). Firstly, RA is more prevalent in women than in men, with varying occurrence rates reported across different populations. Age distribution shows a notable percentage of RA cases occurring in individuals older than 60 years, while familial history of RA increases the risk significantly.

Diagnosis of RA relies on clinical symptoms and specific biomarkers like rheumatoid factor (RF) and anti-CCP. However, RF lacks specificity and can be found in other conditions, whereas anti-CCP is more specific to RA. Elevated ESR and CRP levels indicate inflammation associated with RA, although some patients may present with normal levels due to individual differences or concurrent medications.

Hormonal factors, particularly low estrogen and progesterone levels post-menopause or postpartum, contribute to increased RA risk and severity in women. Genetic predisposition, environmental factors, and other autoimmune diseases in the family history also play significant roles in RA development.

In conclusion, while RA diagnosis and understanding of its biomarkers and risk factors have advanced, challenges remain in accurately diagnosing and managing the disease, particularly in diverse patient populations and across different stages of life.

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