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Evaluation Of Demographic, Serological and Hematological Features in patients diagnosed with Rheumatoid Arthritis in Wasit Province

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ABSTRACT

A case-control study was carried out at Wasit province, from July 2023 to June 2024, included 50 specimens of patients with rheumatoid arthritis were under treatment, and 50 specimens of control were collected. The serological tests were done including the Anti-ccp Abs test by an immunoenzymatic technique and RF, ESR, and CRP tests by a fluorescence Immunoassay (FIA) technique. Moreover, the ratio of females to males in the present study was 2.5:1. We found significant differences between patients and controls in all serological RF, Anticcp, CRP, and ESR tests. According to hematological tests, patients had a statistically significant lower mean Hb than controls, the mean WBC count was significantly higher in patients than in the control group, and platelets were not statistically different between the two study groups. In conclusion, the demographic, hematological, and serological characteristics of rheumatoid arthritis in Wasit province exhibited similarities to those observed in various national and international studies. The current research can help to increase understanding of the RA disease by analysing different clinical markers associated with the condition.

Introduction

Rheumatoid arthritis (RA) was a chronic, systemic inflammatory condition caused by the interaction between genes and environmental factors [1]. It was initiated in the smaller joints before affecting larger ones [2]. RA-related extraarticular manifestations (EAMs) have the potential to impact nearly every organ in the body [3]. It exhibited a global distribution that is widely dispersed, with an approximate prevalence of 0.5% and 2%. Moreover, it is predominantly observed in females [4]. In a medical context, early RA is characterized by a systemic disease. Common symptoms include flu-like feelings, fatigue, joint tenderness, swelling, and morning stiffness. Moreover, it is associated with elevated levels of C-reactive protein (CRP) and an elevated erythrocyte sedimentation rate (ESR) [5]. The condition had traditionally been recognized as a type III hypersensitivity disorder distinguished by the existence of autoimmune antibodies referred to as Rheumatoid factors (RF) and anti-citrullinated

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protein antibodies (ACPAs) [6]. Previous studies have demonstrated that the complete blood count (CBC) parameters can act as inflammatory biomarkers, possessing a predictive role in rheumatic disorders. This enables the assessment of disease activity within these conditions [7]. Additionally, several lifestyle-related factors have the potential to be modified, such as smoking and obesity. Furthermore, there are non-modifiable factors, including genetics, being female, and age, that have also been identified as potential risk factors for RA [8]. RA was currently categorized as either seronegative or seropositive based on the presence or absence of anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF). The seropositive group typically exhibits moderate to high disease activity, while the seronegative group tends to experience more remission and lower disease activity [9]. These citrullinated protein antigens were the dominant targets of the autoimmune response in RA. The detection of anti-cyclic citrullinated peptide (CCP)was precise (88–96%) and was about as sensitive as the rheumatoid factor (70–78%) in diagnosing RA [10].

Method

A case-control study was carried out to study the relationship between different characteristics of rheumatoid arthritis in a specific area, Wasit province/Iraq . it involved 100 participants, 50 RA patients (14(28%) male and 36(72%) female) and 50 control (34(68%) female and (16(32%) male. who participated in this study. Along with blood sampling, a questionnaire was also collected, composed of several questions, including information about the participants; all Samples were obtained from Al-Zahraa Teaching Hospital, Al-Karamaa Teaching Hospital, The rehabilitation center of the disabled, and prosthetics from Rheumatologists private clinics, during a period from July 2023 to June of 2024. Five milliliters of venous blood were extracted from a vein for the control and patient groups. The blood was then divided almost equally, with 2.5ml placed in EDTA tubes for CBC analysis and 2.5ml in Gel tubes for serological testing [11]. The serological testing includes the estimation of Anti-ccp by An immunoenzymatic technique by using the Chorus anti-ccp kit to qualitatively detect human IgG antibodies against cyclic citrullinated peptides (CCP) in human serum, estimation of RF by using the ichroma RF IgM Kit to fluorescence Immunoassay (FIA) that was utilized to analyze human RF IgM levels in human serum quantitatively, estimation of CRP by used AFIAS CRP Kit. to a fluorescence Immunoassay (FIA) for the quantitative determination of human C reactive protein in human serum, and ESR test was done to assess the rate at which erythrocytes settle in a blood sample. Blood samples collected in EDTA tubes were used to conduct White Blood Cell count, Hemoglobin level, and platelets by Complete Blood Count (CBC) Test using the Sysmex Automated Hematology Analyzer.

Statistical analysis

Performed using the Statistical Package of Social Sciences program (SPSS) version 26. Both descriptive and inferential statistics were used. Data were presented by frequencies and percentages if they are categorical and by mean, standard deviation (SD), median, and Interquartile range (IQR) if they are continuous numerical To assess the differences between two numerical variables, the independent samples t-test or Mann-Whitney U Test were used. For more than two groups, one-way Analysis of Variance (ANOVA) or Kruskal-Wallis Test was used according to a type of data distribution. Spearman's rho correlation test was used because the Pearson correlation assumptions were not met. A P-value less than 0.05 was considered significant for all conducted tests.

Results

In the current study, the sociodemographic features of the two groups were compared, and the description of age means ±SD for both groups were found the Mean of patients age (52.22±11.98) years old (age range 21-70 years) when compared to controls (43.78±15.37) years old (age range 18-70 years). A higher percentage of female patients with RA (72%) and the percentage of male patients with RA that lower percentage (28%). The female-to-male ratio was 2.5:1. Furthermore, the results show the body mass index (BMI) was also associated with the study groups with a P-value less than 0.001. Patients with RA are more likely to be obese (70%), while 80% of controls were presented with average body weight according to their BMI. The demographic description is shown in Table 1. The current study researches cigarette smoking (CS) as a lifestyle factor and found Smoking was not statistically

associated with disease status (P-value =0.629). A slightly similar percentage of smokers was found in both patients and control groups. The same is true for those who are non-smokers. The result is shown in Figure 1. The hematological test results for both patients and controls which demonstrated in Table 2. It was found that patients had a statistically significant lower mean Hb (11.14±1.55) gm/dl than controls (13.51±1.11) gm/dl with a P-value <0.001. The mean WBC count was significantly (P-value < 0.001) higher in patients (10.61±1.47) than in the control group (6.92±0.98) 1000/ml. The hematological test results also found that the mean platelet count was not statistically different between the two study groups. The result is seen in the Table 2. Studying the serological markers shown in Table 3, and was found significant differences between patients and controls in all serological tests, with a P-value less than 0.001 for each test. Patients had a significantly higher mean RF value (51.3±21.6) IU/mL than controls (3.7±1.1) IU/ml. Regarding Anticcp, patients had (90.0±53.2) U/ml compared to (5±2.2) U/ml for controls. Serogroups of RA among sample patients in this study found that most patients (92%) had a positive serogroup. RA patients who test positive for RF or ACPA are seropositive (SPRA) and believed to have a more aggressive disease progression when compared to seronegative (SNRA) patients who test negative for these markers, and the current results mean there was an anti-CCP or rheumatoid factor in the patient's blood. The same was demonstrated for the remaining two tests (ESR & CRP), with higher values in the patient group relative to the control group. Patients had a significantly higher mean ESR value (58.6±17.7) mml/h than controls (7.4±2.9) mml/h, and Patients had a significantly higher mean CRP value (52.5±21.4) IU/mL than controls (4.0±1.5) IU/ml.

Table 1. Sociodemographic features of the study groups (n=100)

Sociodemographic features		**Study groups No. (%)**		**Total**	**P-value**
		Patient 50 (50%)	**Control 50 (50%)**	**No. (%)**	
Sex	Female	36(72%)	34(68%)	70(70%)	0.663
	Male	14(28%)	16(32%)	30(30%)	
Age (years)	≤ 35	5(10%)	16(32%)	21(21%)	0.007
	36 - 53	18(36%)	20(40%)	38(38%)	
	≥54	27(54%)	14(28%)	41(41%)	
Age mean ±SD		52.22±11.98	43.78±15.37	-	0.003
BMI (Kg/m^2)	Under weight	0 (0%)	1(2%)	1(1%)	<0.001
	Normal	1(2%)	40(80%)	41(41%)	
	Over weight	14(28%)	9(18%)	23(23%)	
	Obese	35(70%)	0(0%)	35(35%)	

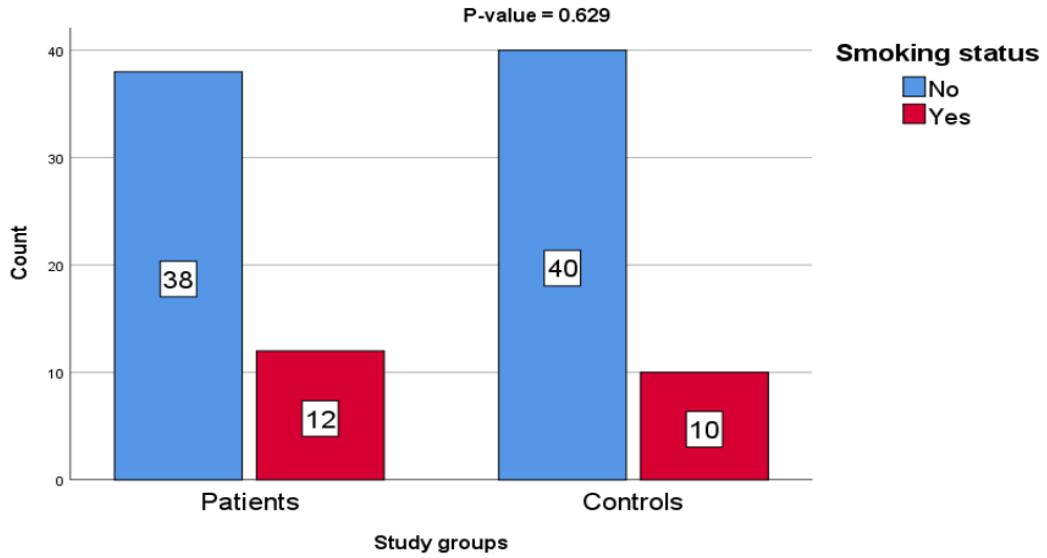

Fig. 1. Frequency distribution of smoking status between the study's two groups (n=100)

Table 2. Hematological test among the study groups (n=100)

Hematological test	Study group	No	Mean	Standard Deviation	Min-Max	P-value
Hb (gm/dl)	Patient	50	11.14	1.55	8.9-15.6	<0.001
	Control	50	13.51	1.11	10.6-13.51	
WBC (1000/ml)	Patient	50	10.61	1.47	7.8-14.2	<0.001
	Control	50	6.92	0.98	4.8-9.8	
PLT (1000/ml)	Patient	50	255.70	55.89	168-395	0.366
	Control	50	245.87	52.15	29-351	

Table 3. Mean difference between patient and control groups in serological tests (n=100)

Serological test	Study group	No	Mean	Standard Deviation	Min-max	P-value
RF (IU/mL)	Patient	50	51.3	21.6	9-107	<0.001
	Control	50	3.7	1.1	2-8.2	
Anticcp (U/ml)	Patient	50	90.0	53.2	11.8-300	<0.001
	Control	50	5.0	2.2	1.5-10.6	
ESR	Patient	50	58.6	17.7	34-110	<0.001
	Control	50	7.4	2.9	2-14	
CRP	Patient	50	52.5	21.4	11-102	<0.001
	Control	50	4.0	1.5	2-7	

Discussion

The current study found that the development of rheumatoid arthritis is influenced by age. However, rheumatoid arthritis can manifest in people across all age groups, and its occurrence continues to increase with age, persisting at least in the elderly individual. Numerous theories have been put in to explain the aging process, including the accumulation of DNA damage and subsequent alterations in biological mechanisms [12]. Also, RA progression advances with the aging process, mainly due to systemic inflammation. Overproducing reactive oxygen species (ROS) can enhance cell destruction and

decrease antioxidant defense [13]. The increased susceptibility rate of females to rheumatoid arthritis (RA) in comparison to males can be attributed to the impact of sex hormones. Extensive research indicates that autoimmunity is influenced by genetic factors, and genes situated on the sex chromosomes may play a role in the elevated occurrence of RA among females [14]. Furthermore, individuals diagnosed with rheumatoid arthritis (RA) have a higher propensity for obesity. The result agreed with a study at Al-Nahrain University / Iraq [15]. They showed that patients with RA who were obese were more likely to have elevated inflammatory markers and a rising prevalence of RA. Obesity was commonly viewed as a state marked by systemic inflammation, resulting in increased concentrations of inflammatory cytokines like tumor necrosis factor-alpha and interleukin-6. These cytokines could potentially contribute to inflammatory responses within the body [16]. Also the adipose tissue of obese RA patients released adipokines, which were biologically active substances that influence both the innate immune system (increasing cytokines such as IL6 and IL12 and TNF-alfa) and the adaptive immune system by increasing T-helper 1 lymphocytes and decreasing regulatory T cells [17]. Regarding Smoking a slightly similar percentage of smokers was found in both patients and control groups. The same is true for those who are non-smokers, and the result agreed with a study in Baghdad, Iraq (alkaaby et al., 2023) [18]. That showed Smoking is not linked to rheumatoid arthritis. Further studies are required to determine the smoking effect in patients with RA, and larger groups of patient subjects need to investigate a correlation between Smoking and RA disease. Furthermore, The results of the Hematological marker showed a significant decrease in Hb in the rheumatoid disease group compared to the control group. Low hemoglobin levels in RA due to Methotrexate treatment can lead to decreased hemoglobin levels, resulting in anemia due to reduced red blood cell count. It may also decrease platelet count and white blood cell abnormalities [19]. The WBC was significantly higher in patients than in the control group. At the same time, the platelets (PLT) count was not statistically different between the two study groups, and this result agrees with the study in Sulaymaniyah /Iraq [20]. They found a strong connection between RA and low hemoglobin levels, hematocrit, and high leucocyte count. The result agrees with [21]. They showed a significant decrease in platelet mean count after treatment. Anemia in patients with rheumatoid arthritis (RA) can have various causes based on the disease stage and the treatments they are undergoing. The most prevalent type is iron deficiency anemia, which can result from malabsorption or, more commonly, iron loss. Individuals with RA might also develop hemolytic anemia, anemia associated with myelodysplastic syndrome, folate deficiency anemia (which is typically managed in patients receiving the antifolate drug methotrexate), vitamin B12 deficiency anemia (although concurrent Biermer anemia is rare), or anemia induced by medications like leflunomide, salazopyrine, or methotrexate through various mechanisms [22]. In addition , high count of Neutrophil(leucocyte) implicated with inflammation and destruction of host tissues such as cartilage in RA conditions by Reactive oxygen species (ROS) and granule proteases originating from neutrophils. In contrast, the serological markers for RA patients' sera showed a substantial rise in median RF, CRP, anti-CCP Abs, and ESR levels. The result agreed with the study in Baghdad/Iraq [23]. They showed a significant difference ($P<0.01$) in the anti-CCP antibodies, RF, ESR, and CRP levels between the RA and control groups. The specific pathogenic role of ACPA in rheumatoid arthritis (RA) remains uncertain. The reason for the elevated ACPA levels in RA is believed to be influenced by genetic factors such as HLA-DR, as well as environmental factors, and smoking. However, research has indicated that immune complexes containing ACPA can induce the production of inflammatory cytokines like TNF. Peptidylarginine deiminases (PADs) are expressed, leading to the presence of citrullinated proteins in the synovium of RA patients, with evidence suggesting that ACPA deposition may contribute to osteoclastogenesis and subsequent bone loss [24]. Anti-CCP had been added as one of the criteria in the 2010 (ACR)/(EULAR) classification of RA [25]. So, this study's findings confirm that measuring anti-CCP is a valuable test for diagnosing rheumatoid arthritis. The seropositive patients are higher among RA patients compared with seronegative patients [26].

Conclusions

The current study found that rheumatoid disease induces alterations in some hematological markers and raises levels of some serological markers within the RA patient's body; clinicians and researchers should thoroughly evaluate the demographic feature, hematological markers, and serological markers of RA patients, consider these elements as potential predictors in

diagnosing the condition. This analysis may assist in developing effective therapeutic intervention strategies and increasing consciousness about disease management among RA patients in the studied population**.**

Ethical considerations

Before sampling, all participants who took part in the study were (already informed about the aim of the study, agreed, and verbal consent was obtained from all). The study was conducted following the principles of the Declaration of Helsinki.

Ethical Issues

This study was approved by the ethical Committee of the Council of College of Medicine /University of Wasit in July 2023 and by the "Wasit Health Directorate (1201)" on 24/7/2023. All individuals in this study gave written consent to participate in the Rheumatoid arthritis questionnaire.

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تقييم الخصائص الديموغرافية والمصلية والدموية لدى المرضى الذين تم تشخيص إصابتهم بالتهاب المفاصل الرثوي في محافظة واسط

استبرق داود غالي *، هيثم قاسم محمد

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الملخص

أجريت الدراسة في محافظة واسط خلال الفترة من تموز 2023 إلى حزيران 2024، وشملت 50 عينة من مرضى التهاب المفاصل الروماتويدي الذين كانوا تحت العلاج و50عينة من الاشخاص الاصحاء .الهدف من الدراسة هو تقييم الخصائص الديموغرافية والسيرولوجيه والدموية لدى مرضى التهاب المفاصل الرثوي ومقارنتها مع خصائص الاشخاص الاصحاء .حيث شملت اختبار الاجسام المضادة للببتيد الحلقي المضاد للسترولين بواسطة تقنية الإنزيمات المناعية وحساب عامل الروماتويد ومعدل ترسيب كريات الدم الحمراء ومستوى بروتين سي التفاعلي بواسطة الاختبارات المناعية الفلورية. حيث وجد ان الفئات العمرية الأكبر سنًا (≤ 54 عامًا) لديها نسبة أعلى من المرض (54٪). و تم ايجاد نسبة الإناث إلى الذكور في المرضى وكانت 2.5:1 في هذه الدراسه. وجدنا فروقًا ذات دلالة إحصائية بين المرضى والضوابط في جميع الاختبارات المصلية. اما في اختبار الصورة الكاملة للدم فوجد انخفاض هيموغلوبين الدم لدى المرضى وارتفاع متوسط عدد خلايا الدم البيضاء عند المرضى عنه في الاصحاء واما عدد الصفائح الدموية فلم يوجد اختلاف احصائي بين المجموعتين. حيث نستنتج ان السمات الديموغرافية والصورة الدموية والسيرولوجية لمرضى التهاب المفاصل الروماتويدي في محافظة واسط اظهرت تشابه مع مختلف الدراسات الوطنية والدولية. مما يمكن أن يساعد البحث الحالي في زيادة فهم مرض التهاب المفاصل الروماتويدي من خلال تحليل العلامات السريرية المختلفة واجراء الاختبارات المرتبطة بالحالة.

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