Correlation Between Menopause and Inflammatory Markers with Das28 Among Iraqi Patients with Rheumatoid Arthritis

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Abstract: RA is an autoimmune illness that is most common in women, suggesting that female hormone variations have a role in disease progression. Knowing how they affect the development of RA is clinically relevant and might help with specialized prevention initiatives in high-risk individuals. The goal of this study was to look at the relationship between menopause and inflammatory markers with RA disease progression. This is a case-control study carried out on 300 RA patients; they were diagnosed by rheumatology specialists during the period from September 2021 to January 2022 then tested for RF, CRP, ESR and ACCP. The findings revealed that 88% of patients had positive ACCP results, whereas 71% had positive RF results, and 97% had positive CRP values. The majority of rheumatoid arthritis patients 66.7% (200/300) were in their postmenopausal period, while 33.4% (100/300) were in their premenopausal stage when they were diagnosed. The findings revealed that postmenopausal RA women had more severe disease activity than premenopausal RA women, as evidenced by the elevated Das28 and ESR values, (4.63 ± 0.10) and (47.14 ± 0.69) respectively, in comparison to their lower levels (3.66 ± 0.09) and (36.12 ± 0.81) in premenopausal RA patients. Both groups, however, had elevated levels in contrast to the control group.

Keywords: ACCP, Das28, ESR, Menopause, Rheumatoid arthritis

1. Introduction

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Women are more likely to develop rheumatoid arthritis than men, which is the fourth major cause of disability in women [1].The illness mostly appears after menopause, with a peak incidence in the 35−75-years-old age group. Rheumatoid arthritis (RA) has the largest sex bias, while symptom intensity, illness course, responsiveness to medication, and overall survival may differ between males and females with autoimmune disorders, where the female to male ratio is 3:1. [2]. Women have higher immune responses than males, which are thought to have a role in their vulnerability to autoimmune disorders [3]. More research is needed on menopausal age as a predictor of illness severity before any conclusions can be drawn or utilized as a foundation for disease management.

2. Materials
Laboratory tests included: Anti-citrullinated peptide (ACCP), Rheumatoid factor (RF), C-reactive protein (CRP) antibody and Erythrocyte sedimentation rate (ESR). The anti-CCP cassette is ready to use in the CHORUS instruments (Italy).

3. Methods
Samples collection for this study was carried out from September 2021 until January 2022, which included 300 RA patients. They were diagnosed by physicians at Al-Karama Teaching Hospital using (MRI) examination and routinely used procedures. The patients were divided into two groups depending on menopause status (pre and post-menopause). Laboratory tests included: ACCP, RF, CRP antibody and ESR. The anti-CCP cassette is ready to use for the detection of antibodies of CCP in the CHORUS instruments, the test uses the ELISA principle. Rheumatoid factor (RF) in the blood causes agglutination of latex particles coated with human gamma-globulin on a slide. Serum C-reactive protein (CRP) at 6 mg/L or higher causes an observable agglutination on the slide of a suspension of latex particles coated with anti-C-reactive protein. ESR was accomplished by the Westergren method.

4. Statistical Examination
The SAS (2012) program was used to determine the effect of several study parameter components. To make a meaningful comparison between means, the LSD test (ANOVA) was performed. In this study, the Chi-square test was employed to compare percentages (0.05 and 0.01 probability) and the correlation coefficient was determined between variables.

5. Results and Discussion
 Only 70 women with rheumatoid arthritis (RA) were included in this research out of 300 women who were assessed. This research, however, comprised 300 women who were of the same age, lived in the same geographical location and had the

same living conditions. The findings revealed that 88 percent of women had positive anti-CCP results, 71 percent had positive RF values, and 97 percent had positive CRP results, Table (1). Only 70/300 (23.4%) of patients were subjected to experiments that yielded positive results for all inflammatory markers used in the current study to ensure sample homogeneity.

Table 1: Prevalence of RA in patients according to illness indicators.

Total No. = 300 RA female patients				
Indicators	**$ACCP^+$**	**RF^+**	**CRP^+**	**$ACCP^+$ RF^+ CRP^+**
No. of cases	264	213	291	70
Percentage %	88%	71%	97%	23.4%
Chi-Square -χ^2 (P-value)	10.084 ** (0.0001)			
** (P≤0.01)-H.S.				

When blood tests for inflammatory parameters were done, it was shown that most clinically diagnosed rheumatoid arthritis patients had a high level of ACCP whereas RF was negative. As a consequence, ACCP is the recommended test for rheumatoid arthritis diagnosis.

6. RA prevalence in women before and after menopause

According to the data, the majority of women diagnosed with rheumatoid arthritis (66.7% (200/300)) were in the postmenopausal period, while 33.4 percent (100/300) were in the premenopausal stage, Table (2).

Table 2: The ratios of RA women according to menopausal stage

	Pre-menopausal	**Post-menopausal**	**Total**
No.	100	200	300
Ratio%	33.4%	66.7%	100%
Chi-Square -χ^2 (P-value)	9.459 ** (0.0001)		----
** (P≤0.01)-H.S.			

The average age in premenopausal patients, post menopause patients was 44.91± 1.13 years, 52.11 ± 0.99 years correspondingly, and the control group (pre and post) (42.20 ± 0.85 years, 52.00 ± 0.64 years), respectively, Table (3).
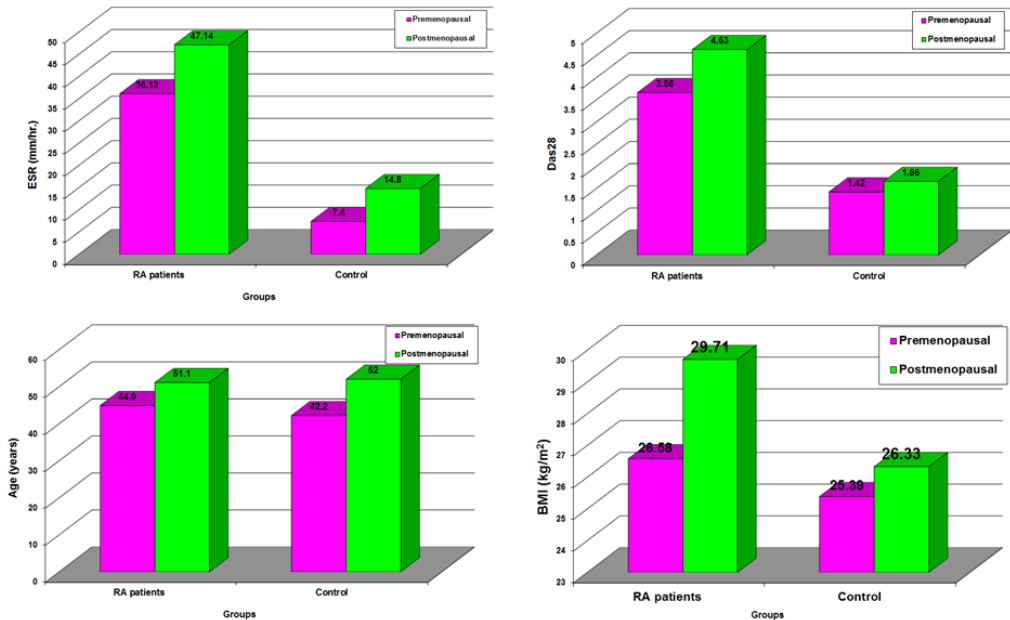
Table 3: Comparison between different groups in Das28, ESR and Age in women with different menopausal stages

Groups		Mean ± SE		
		Das28	ESR (mm/hr.)	Age (years)
Total patients		4.14 ± 0.16 b	41.36 ±1.12 ab	48 ± 2.11 ab
Total control		1.54 ± 1.13 c	11.1± 1.83 cd	47.1 ± 1.49ac
RA patients	Premeno pausal	3.66 ± 0.09 b	36.12 ± 0.81 b	44.91 ± 1.12 b
	Postmeno pausal	4.63 ± 0.10 a	47.14 ± 0.69 a	51.10 ± 0.99 a
Control	Premeno pausal	1.42 ± 0.06 c	7.40 ± 1.01 d	42.20 ± 0.85 c
	Postmeno pausal	1.66 ± 0.07 c	14.80 ± 082 c	52.00 ± 0.64 a
LSD value		0.246 **	2.429 **	2.641 **

This study used the Das28 score to compare the severity of illness in various patient groups. Table (3) demonstrated that the whole mean of Das28 scores in women with RA (4.14 ± 0.16) was significantly (p<0.01) higher than the entire control group (1.54 ± 1.13).

However, postmenopausal women scored a highly significant (p<0.01) increase in Das28 score (4.63 ± 0.10) in comparison to premenopausal women (3.66 ± 0.09) and both stages declared a significant (p<0.01) increase in comparison to control group.

Erythrocyte sedimentation rate (ESR) is a rudimentary indicator of inflammation [4]. In this study, ESR was utilized to confirm the severity of RA in infected women, as well as Das28 score, BMI and age, to assess the true influence of the menopausal stage on disease severity using their scores. The overall mean ESR (41.36 ± 1.12 mm/hr.) in women with RA was substantially (p<0.01) higher than the entire control group (11.1 ± 1.83 mm/hr.). However, postmenopausal women had a highly significant (p<0.01) increase in ESR (47.14± 0.69 mm/hr.) when compared to premenopausal women (36.12± 0.81) and both stages had a significant (p<0.01) increase when compared to the control group, implying that the disease is more severe in postmenopausal women. Table (3), Figure (1). Means in the same column with different letters vary significantly. ** (P≤0.01).

Figure 1: Comparison between different studied groups according to age, BMI, Das28 score and ESR levels

Anti-citrullinated protein antibodies (ACPA) are a more specific marker for RA than RF in many previous studies. Unlike RF, ACPA is seen in only approximately 2% of healthy people and is similarly uncommon in other inflammatory diseases [5]. The synovium of persons who were seropositive for RF and ACPA but had not developed any clinical manifestations of arthritis revealed generally normal synovium with modest T-cell infiltration on magnetic resonance imaging (MRI) and biopsy examination. [6]. These findings suggest that RF, and particularly ACPA, may have a critical pathogenic role in the initiation and propagation of RA, emphasizing the severity and potentially destructive nature of the disease's clinical development.

Although women are 2–4 times more likely than males to develop rheumatoid arthritis (RA), circumstances linked with normal estrogen and progesterone levels in women usually appear to protect joints. [7]. Women with RA report decreased joint symptoms throughout the postovulatory period of the menstrual cycle and during pregnancy, when progesterone concentrations are high, and in some studies oral contraceptives containing progesterone were protective against RA, according to Roomruangwong *et al.* (2019). [8] The earlier research, on the other hand, stated that RA symptoms frequently worsen during the postpartum period, when estrogen

and progesterone levels drop. Women have a lower chance of getting RA during pregnancy, but the first few months after delivery are a high-risk period. [9]

Many studies on the link between menopause and the onset and progression of RA disease have focused on four main areas: the effect of menopause age on disease onset and course, subject, disease characteristics in women with late onset typically after menopause, and the impact of menopause on disease course. Hormonal changes after menopause, together with a slew of physical and psychological changes and stress, may contribute to an increase in the prevalence of rheumatoid arthritis after menopause. Previous research from Iowa University in the United States found that women who reached menopause after the age of 51 had a higher chance of developing RA than those who had menopause before the age of 45. [10]

Menopausal status, body mass index (BMI), bone mineral density (BMD), and a range of other variables affect the degree of illness reflected by Das28 and ESR [4]. A previous study found that premenopausal women have greater BMD than postmenopausal women (Al-Hafidh and Goral, 2018) [11]. The current study found a significant increase in disease severity among postmenopausal subjects when compared to the control group and premenopausal women. These findings were consistent with several studies that found a significant link between menopausal status and RA, and they reported that the risk of bone formation is reduced as a result of decreased bone mineral density (BMD), and thus the severity of RA is increased. [12]

Many previous researches have discovered that pregnancy has a significant influence on the severity of RA. According to reports, 50 percent of RA patients heal during pregnancy, compared to disease activity before conception, and roughly 40% experience illness flares post-partum when the female hormones E2 and progesterone increase [13].

According to the findings of this study, there is a substantial negative link between a BMI and a drop in estradiol and progesterone levels in women with RA. A higher BMI produces a hormonal imbalance in the female reproductive system, notably a reduction in E2 and progesterone. As a result, the severity of the condition worsens. The first option for reducing the severity of RA caused by obesity and sex hormone imbalance is to lose weight. Increased radiographic joint degeneration is highly connected with a high BMI, according to several studies. They discovered that blood indicators of cartilage collagen breakdown and proteoglycan turnover were linked to joint deterioration in RA, and hence BMI was regarded as a sensitive and inflammation-independent predictor of RA radiographic prognosis [14].

In our research, we discovered that overweight participants had a higher risk of RA than those who were of normal weight. In a previous population study, it was

discovered that the RA risk for obese vs. normal weight individuals was greater in women than in men. Furthermore, the aggregate research revealed a substantial nonlinear association between BMI and RA [15]. A Study done by Qin *et al.* (2015) suggested that there is a favorable link between overweight/obesity and RA when compared to normal weight people [16]. According to the Meta analysis, every 5 kg/m2 increase in BMI increased the risk of RA by 9% [17].

In both control and patients group trials, we discovered a substantial link between BMI and the severity of RA. The findings showed that age, smoking, and drinking may interfere with the relationship between BMI and the incidence of RA, thus future epidemiological research should take these factors into account. The current study deemed BMI to be one of the most inflammatory indicators, with obesity or a higher BMI increasing the likelihood of RA, and so providing a signal to anticipate the disease's development. As a result, increasing BMI lowered the concentrations of numerous sex hormones, such as estrogen and progesterone, and influenced the immunological response of RA patients, especially since estrogen has been hypothesized to have an immunomodulatory function [18, 19].

7. Conclusions

Antibodies to citrullinated proteins (ACPA) are a more specific marker for RA than RF. In addition, the findings revealed that menopausal age is a predictor of illness severity. There is a substantial relationship between BMI and the severity of RA. Obesity produces an imbalance in sex hormones, which leads to menstrual cycle issues and an increased risk of RA. As a result, reducing body weight is the first step toward resolving RA women's obesity-related health issues.

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العلاقة بين سن اليأس وبعض المؤشرات الالتهابية على شدة المرض في النساء العراقيات المصابات بالتهاب المفاصل الرثوي

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المستخلص: التهاب المفاصل الرثوي هو أحد أمراض المناعة الذاتية الأكثر شيوعًا عند النساء، مما يشير إلى أن الاختلافات الهرمونية الأنثوية لها دور في تطور المرض. لذلك فان معرفة كيفية تأثيرها على تطور التهاب المفاصل الرثوي وهو على صلة سريرية وقد يساعد في الاليات الممكنة لتقليل فرص الإصابة عند الافراد المعرضين للمرض. كان الهدف من هذه الدراسة هو النظر في العلاقة بين انقطاع الطمث وعلامات الالتهاب مع تطور مرض التهاب المفاصل الرثوي. هذه الدراسة أجريت على 300 مريضة التهاب المفاصل الرثوي. تم تشخيصهم من قبل متخصصين في أمراض الروماتيزم خلال الفترة من سبتمبر 2021 إلى يناير 2022 ثم تم ادراء لهم فحوصات تتضمن (RF - CRP - ESR و ACCP) كشفت النتائج بأن 88٪ من المرضى كانت لديهم نتائج إيجابية لـ ACCP ، بينما 71٪ كانت لديهم نتائج RF إيجابية ، و 97٪ كانت لديهم نتائج CRP إيجابية. حيث ان غالبية المرضى النساء المصابات بالتهاب المفاصل الرثوي 66.7٪ (300/200) كانوا في فترة ما بعد انقطاع الطمث ، بينما كان 33.4٪ (300/100) هم في مرحلة ما قبل انقطاع الطمث في لحظة تشخيصهم. و كشفت النتائج أيضا أن النساء المصابات في فترة ما بعد انقطاع الطمث لديهن شدة مرض أكثر من النساء قبل انقطاع الطمث ، كما اتضح من قيم Das28 و ESR المرتفعة (4.63 ± 0.10) و (47.14 ± 0.69) على التوالي ، مقارنة بمستوياتهن المنخفضة (3.66 ± 0.09و (36.12 ± 0.81) في المريضات فب فتره ما قبل انقطاع الطمث. ومع ذلك ، كان لدى كلتا المجموعتين مستويات مرتفعة على عكس مجموعة السيطرة. الاستنتاج: يعتبر ال ACPA هو اكثر علامة أكثر تشخيصية للمرض من ال RF ، و فترة سن اليأس تساعد على زيادة شدة المرض.

الكلمات المفتاحية: التهاب المفاصل الرثوي، شدة التهاب المفاصل الرثوي، سن اليأس

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