



ISSN: 0067-2904

## Comparison of Some Physiological Parameters in Female Rheumatoid Arthritis Patients in Pre- and Postmenopausal Stages

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Received: 19/12/2019

Accepted: 5/4/2020

### Abstract

Rheumatoid arthritis is an autoimmune disorder that is highly prevalent, leading to gradual cartilage distraction, and therefore is important to diagnose in the early stage. This study aimed to estimate the level of rheumatoid factor (RF) and Anti-citrullinated protein (Anti-ccp) in the serum of female patients. We also investigated several female reproductive hormones in the patients and compared their levels in the premenopausal and postmenopausal phases. The study included 88 female subjects, 50 suffering from signs of rheumatoid arthritis who were attending AL-Yarmouk teaching hospital, Baghdad, Iraq, and 38 without clinical signs of RA as a control group. The ELISA technique was used to estimate all the studied parameters. The results showed a significant elevation in the levels of RF ( $103.6 \pm 227.0$  vs.  $22.1 \pm 111.0$  U/ml) and Anti-ccp ( $158.0 \pm 170.0$  vs.  $0.51 \pm 1.69$  U/ml) in patient's serum as compared to the control. The percentage of overweight and obese patients was higher than that of those with normal weight. Also, the current results showed significant differences in the serum concentrations of the reproductive hormones between the premenopausal and postmenopausal phases in the patients group. The level of FSH in postmenopausal female patients was higher than that in premenopausal ones ( $33.5 \pm 17.9$  vs.  $9.01 \pm 9.31$  U/ml). Also, the level of luteinizing hormone (LH) in the postmenopausal patients was higher than that in the premenopausal patients ( $26.4 \pm 12.3$  vs.  $12.7 \pm 10.2$  U/ml). While the level of prolactin (PRL) in RA postmenopausal female patients was lower than that in the premenopausal patients ( $8.60 \pm 7.07$  vs.  $14.8 \pm 10.6$  ng/ml). In addition, the level of Anti-mullerian hormone (AMH) in post-menopausal patients was lower than that in the pre-menopausal ones ( $0.034 \pm 0.023$  vs.  $0.635 \pm 0.683$  ng/ml). Finally, the concentration of estradiol (E2) in the post-menopausal female patients was lower than that in the pre-menopausal ones ( $32.9 \pm 18.6$  vs.  $76.5 \pm 43.6$  ng/ml).

**Keywords:** Rheumatoid arthritis; Anti-ccp; rheumatoid factor; FSH; LH; menopausal phase.

## مقارنة مستويات بعض المؤشرات الفسيولوجية في الإناث المصابات بالتهاب المفاصل الرثوي بين مرحلتي قبل وبعد سن اليأس

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الخلاصة:

يعد مرض التهاب المفاصل الرثوي من اكثر امراض المناعة الذاتية انتشارا، يؤدي الى تدمير الغضروف

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تدرجياً لذلك من المهم تشخيص المرض في مرحلة مبكرة جداً. تهدف هذه الدراسة الى تقدير مستوى العامل الرثوي و البروتين المضاد للستروليينات في مصلى مريضات التهاب المفاصل الرثوي وايضا تقييم مستوى بعض الهرمونات التكاثرية الانثوية ومقارنة مستوياتها في مرحلة ما قبل انقطاع الطمث ومرحلة ما بعد انقطاع الطمث.

شملت الدراسة (88) حالة من النساء, (50) منها كانت تشكو اعراض التهاب المفاصل الرثوي في مستشفى اليرموك التعليمي في بغداد و (38) منها كانت بدون علامات سريرية سابقة تمثل مجموعة سيطرة. تم استخدام تقنية الاليزا في تقييم جميع المؤشرات المدروسة, وقد اظهرت الدراسة وجود ارتفاع معنوي في مستويات العامل الرثوي لدى المريضات مقارنة بمجموعة الاناث السليمات ( $22.1 \text{ vs. } 227.0 \pm 103.6$ ). بينما كان مستوى مضاد بروتين الستيرولين لدى المريضات مرتفع مقارنة بمجموعة الاناث السليمات ( $111.0 \pm 158.0 \text{ vs. } 170.0 \pm 158.0$ ) وقد بينت الدراسة ان نسبة الاناث المرضى اللاتي تعانين من زيادة في الوزن او من السمنة كانت اعلى من نسبة الاناث المرضى ذوات الوزن الطبيعي. وكذلك اظهرت النتائج وجود فرق معنوي في مستويات هرمونات التكاثر لدى المريضات في مراحل ما قبل وبعد انقطاع الطمث. كان مستوى هرمون FSH في المرضى الاناث بعد انقطاع الطمث اعلى من مستواه في المرضى قبل انقطاع الطمث ( $9.31 \pm 9.01 \text{ vs. } 17.9 \pm 33.5$  U/ml). وكذلك كان مستوى هرمون الاباضة (LH) في المرضى بعد انقطاع الطمث اعلى من مستواه في المرضى ما قبل انقطاع الطمث ( $26.4 \pm 12.3 \text{ vs. } 10.2 \pm 12.7$  U/ml). بينما مستوى البرولاكتين (PRL) في مرضى التهاب المفاصل الرثوي بعد انقطاع الطمث اقل من مستواه في المرضى الاناث قبل انقطاع الطمث ( $7.07 \pm 8.60 \text{ vs. } 14.8 \pm 10.6$  U/ml). وكذلك مستوى الهرمون المضاد للمولر AMH في مرحلة بعد انقطاع الطمث اقل من مستواه في المرضى الاناث قبل انقطاع الطمث ( $0.023 \pm 0.034 \text{ vs. } 0.683 \pm 0.635$  U/ml). كان تركيز E2 في المرضى الاناث بعد انقطاع الطمث اقل من تركيزه في المرضى الاناث قبل انقطاع الطمث ( $32.9 \pm 18.6 \text{ vs. } 43.6 \pm 76.5$  U/ml).

## Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disorder characterized by a continual joint inflammation leading to cartilage and bone destruction, disability, and eventually to systemic complications [1]. The causative factors are not known while a careful diagnosis has to be made. However, the knowledge of the disease pathogenesis has only led to many recent therapeutics with low impacts [2]. The disease affects women 2 to 3 times more often than men and occurs at any age. The peak prevalence is in the sixth decade. RA of pathophysiology involves chronic inflammation of the synovial membrane, which can destroy the articular cartilage and articular bone [3]. The key pathogenic markers of RA include the rheumatoid factor autoantibody which is directed against the Fc fragment of Immunoglobulin G (IgG) [4]. Anti-citrullinated protein antibody (ACPA) is prognostically and diagnostically useful, with ACPA being positive even years before the start of the disease. [5]. The best approaches for the management of RA are using synthetic or biologic disease-modifying anti-rheumatic drugs (DMARDs) [6].

Female hormonal factors play a vital role in the development of RA. The study of female hormonal factors is challenging because serum levels may differ throughout a woman's lifetime and interact with various environmental, genetic, immunological, and endocrine factors influencing the development of autoimmunity.[7]. Pregnancy may be related to lower disease activity in half of the women, but the postpartum period is associated with an increase in the activity in up to 90% of cases. The first symptoms of the disease have been related to the menopausal period and with an increased risk at the earlier stages of menopause. Some studies suggest that hormonal replacement therapy (HRT) reduces the risk of RA in postmenopausal women [6].

## Materials and methods

### Blood Samples collection

The study included 50 patients who suffered from joint pain and attended at AL-Yarmouk teaching hospital/Rheumatology clinic, Baghdad, Iraq. All patients were diagnosed by physicians.

An amount of 5 ml of blood was collected by a disposable syringe and placed in a gel clot activator tube at room temperature until the coagulant was forming. Then the samples were centrifuged at 3000

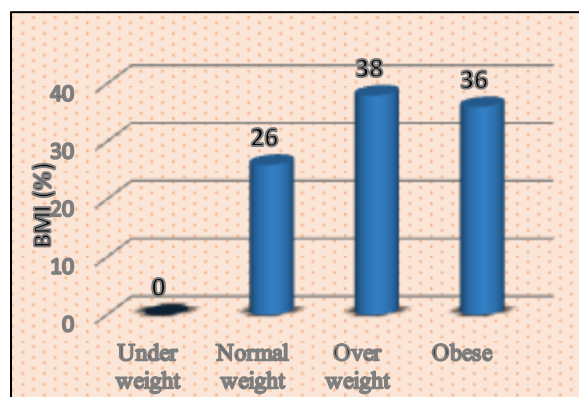
rpm for 15 minutes. Serum samples were separated on Eppendorf tubes. All samples were stored at  $-20^{\circ}\text{C}$  until examined.

The collected serum was used to determine some biochemical and immunological parameters according to the manufacturer's protocols; RF (IgG) and Anti- ccp (IgG) antibodies were measured as diagnostic tests for the disease using commercially available enzyme-linked immunosorbent assay (RF and Anti- ccp kits, Aeskulisa / Germany). FSH, LH, PRL, Estradiol kits (Monobind / USA) and AMH kit (Anshlab, Germany) were measured using enzyme-linked immunosorbent assay (ELISA).

## Result and Discussion

### Distribution of the patient groups according to Body Mass Index (BMI)

The current study showed that the majority of rheumatoid arthritis patients were found to be overweight (38%) followed by obese (36%) and the normal weight (26%) patients, as illustrated in Figure-1.



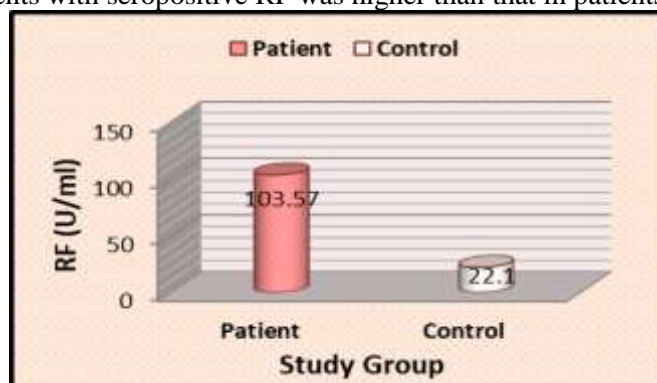
**Figure 1-**Weight categories percentage for the patient groups. Under weight (below 18.5), normal weight (18.5-24.9), over weight (25-29.9) and, obese (30 and above)

These results agree with those reported by Abass and Ramadhan [8] who noted that overweight patients had the highest percentage among RA patients. Similar observations were reported in two cohort studies [9, 10]. Excess body weight was considered as a potential contributor to the development of RA [11]. The excess production of adipocytokines, developed primarily by adipose tissues, creates a loop of chronic inflammation due to continuous activation of macrophages, while the white adipose tissues produce  $\text{TNF-}\alpha$  and IL-6. These findings reveal the connection between the chronic inflammatory conditions and the adipose tissue [12].

### The level of rheumatoid factor (RF) in the sample groups

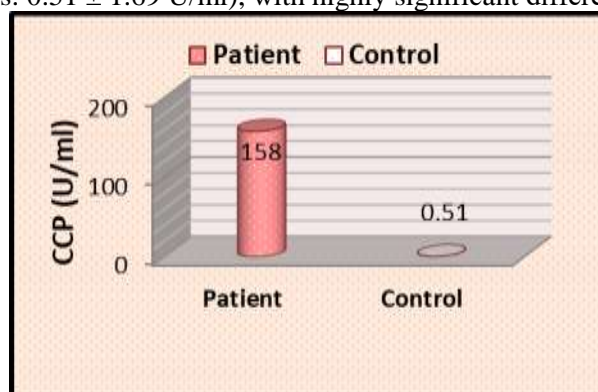
In the current study, the level of rheumatoid factor (RF) in the patient groups was higher than its level in the control group. Statistically, a significant difference ( $p < 0.05$ ) was found in RF level between patient and control groups ( $103.6 \pm 227.0$  vs.  $22.1 \pm 111.0$  U/ml) as illustrated in Figure-2.

The percentage of patients with a positive value of RF was 56%, while that in the healthy control was 2.6%. This result agrees with that of Mohammed and coworkers [13] who illustrated that the percentage of RA patients with seropositive RF was higher than that in patients with negative RF.



**Figure 2-**The level of RF in the study groups.

The percentage of patients with a positive value of Anti-ccp was 78%, while that in the control was 0%, which agrees with the results reported by Jasim and Ibrahim [14]. A combination of anti-CCP and RF levels is important in the diagnosis of disease and result in the highest sensitivity and specificity. As shown in Figure- 3, the level of Anti-ccp was higher in the patients as compared to controls ( $158.0 \pm 170.0$  vs.  $0.51 \pm 1.69$  U/ml), with highly significant differences ( $p \leq 0.001$ )



**Figure 3-**The level of anti-ccp antibodies in the study groups.

There is a proof that early aggressive clinical action in RA patients will delay the progression of the disease and joint damage, leading to a better prognosis. Thus, differentiating between RA and other types of arthritis is important early after the onset of symptoms.

CCP ELISA could be clearly shown to provide the best combination of sensitivity and specificity for detecting RA, with a significant difference between the anti-ccp and RF.

#### **Levels of physiological parameters in pre- and post-menopausal patients:**

The current study revealed no significant differences ( $p > 0.05$ ) in RF and Anti-ccp levels between pre- and post-menopausal female patients, but the concentration of RF in postmenopausal patients was higher than that in premenopausal ones ( $87 \pm 194$  vs.  $121 \pm 256$ ). While the Anti-CCP concentration was higher in premenopausal patients as compared to that in postmenopausal ones ( $195 \pm 177$  vs.  $133 \pm 163$ ), as shown in Table-1. This result may reveal that RF increases in postmenopausal female patients due to the hormonal changes in this stage. These hormonal changes may have an effect on the Anti-ccp serum level in patients.

Statistically, there were highly significant differences in the levels of FSH between pre- and postmenopausal patients ( $p < 0.01$ ), as shown in Table-1. The level of FSH in postmenopausal female patients group was higher than its level in premenopausal one ( $33.5 \pm 17.9$  vs.  $9.01 \pm 9.31$  U/ml). This result is consistent with that reported by Ginting *et al.*[15] who observed that FSH level was increased with aging. In addition, there were highly significant differences ( $p < 0.01$ ) in the level of LH between both stages ( $26.4 \pm 12.3$  vs.  $12.7 \pm 10.2$  U/ml) as shown in Table-1. This result agrees with that of Cevik *et al.*[16] who reported that LH had statistically lower concentrations in pre-menopausal RA women. However, the level of prolactin (PRL) in RA postmenopausal female patients was significantly ( $p < 0.05$ ) lower than that in premenopausal ones ( $8.60 \pm 7.07$  vs.  $14.8 \pm 10.6$  ng/ml).

The anti-mullerian hormone is the best marker for ovarian functional reserve. With aging, the functional capability of ovaries is decreasing, therefore, the serum level of AMH falls continuously in the post-menopausal phase. In this study, there were highly significant differences ( $p < 0.01$ ) in the level of AMH, with lower levels in post-menopausal than in the pre-menopausal patients ( $0.034 \pm 0.023$  vs.  $0.635 \pm 0.683$  ng/ml), as shown in Table-1.

**Table 1-**A comparison between pre- and postmenopausal patient groups in RF and Anti-ccp levels

Variable	Phases		
	Premenopausal	Postmenopausal	P - value
RF (U/ml)	87 ± 194	121 ± 256	0.602
Anti-CCP (U/ml)	195 ± 177	133 ± 163	0.212
FSH ml (U/ ml)	9.01 ± 9.31	33.5 ± 17.90	0.001**
LH ml (U/ml)	12.7 ± 10.2	26.4 ± 12.3	0.001**
PRL (ng/ml)	14.8 ± 10.6	8.60 ± 7.07	0.024*

<b>E2 (pg/ml)</b>	<b>76.5 ± 43.6</b>	<b>32.9 ± 18.6</b>	<b>0.001**</b>
<b>AMH (ng/ml)</b>	<b>0.635 ± 0.683</b>	<b>0.034 ± 0.023</b>	<b>0.001**</b>

Data presented as mean  $\pm$  SE. NS: No significant differences ( $p > 0.05$ ), (\*) significant differences ( $p < 0.05$ ), (\*\*) highly significant differences ( $p < 0.01$ ). Independent *t*-test is statistically used to compare between patients and control group.

It is known that estradiol level is decreasing with age in females, corresponding to hormonal changes and loss of functional ovaries. In this study, there were highly significant differences ( $p < 0.01$ ) between the level of E2 in pre and post-menopausal female patients. As shown in Table-1, the concentration of E2 in the postmenopausal female patients was lower than its concentration in the pre-menopausal female patients ( $32.9 \pm 18.6$  vs.  $76.5 \pm 43.6$  ng/ml). Ginting [15] reported similar results to the current ones.

The reason for the difference in the level of hormones between the two stages of pre- and post-menopause is that some hormones are regulated according to feedback mechanisms. For example, elevated FSH and LH levels lead to inhibition of follicles development and reduction in the function of the ovaries, so that the AMH level falls down. Also the increase FSH level reflects a decline of estrogen and progesterone production. This study found that RA in women does not affect the rate of changes in the levels of reproductive hormones between the pre- and postmenopausal stages.

### Conclusions

Rheumatoid arthritis is more prevalent in overweight and obese female patients and in those aged 50-60 years. The increase in the level of disease indicators (RF and Anti-CCP) among the post-menopausal female patients, even if it was not significant, indicates that the hormonal changes accompanying this stage may have a role in the development of the disease and the stimulation of the inflammatory condition,

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