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Sex steroid hormones as marker of disease activity in Iraqi women infected with RA

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***Abstract**:*

RA is the autoimmune disease, with a higher prevalence among women, which indicates that female hormonal factors play a role in the disease development. Knowing their effect on the development of RA is clinically important and may contribute to specific preventive strategies in populations at high risk .The aim of this study was to analyze correlation of female hormonal factors with the disease activity of RA..

Samples collection of this study was started in October 2018 to January 2019.which included 225 RA women. The patients were divided into two groups depending on menopause status (pre and post menopause) .Laboratory tests are included: ACCP, RF, CRP, ESR and hormonal levels of progesterone and Estradiol.

The results showed that the percentage of women who had positive results with CCP was 88%,while those with positive RF results was 71% and who with positive CRP was 97%. Most of women diagnosed with rheumatoid arthritis were at the pre-menopausal period 32.5% (73/225) ,while the percentage of the patients women at post-menopause stage were 67.6% (152/225). The results showed that postmenopausal RA women had sever disease activity than premenopausal women and that declared by high levels of both das28 and ESR value (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) respectively in premenopausal RA women. However, both groups with high levels in comparison to control. A significant negative correlation between BMI and hormones levels in the studied groups, postmenopausal RA women had high BMI (29.71 ± 0.55 kg/m^2) than premenopausal women (26.58 ± 0.28 kg/m^2) and that characterized by low levels of both progesterone and E2 value (7.24 ± 0.12 ng/ml) and (0.330 ± 0.05 pg/ml) respectively in comparison to their higher levels (42.03 ± 0.59 ng/ml) and (3.49 ± 0.03 pg/ml) respectively in premenopausal RA women. Moreover, both patients groups were had low levels of hormones in comparison to control.

***Conclusion**: The results showed that there were a highly significant negative ($p < 0.01$) correlation between hormonal levels and RA disease severity (Das28).*

***Key words**: Rheumatoid arthritis, menopause, female hormonal factors, Das28*

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I. Introduction

Rheumatoid arthritis are typically more prevalent in women than in men and are considered the fourth leading cause of disability for women[1]. The disease presents most often after menopause, with peak incidence in the 35–75 year age group and the strongest sex bias is observed in rheumatoid arthritis (RA), the female to male ratio is 3:1, while Symptom severity, disease course, response to therapy and overall survival may also differ between males and females with autoimmune diseases[2].Women mount stronger immune responses than men and this is believed to have an effect on the different susceptibility to develop autoimmune diseases[3].Menopausal age as predictor of disease severity ,more studies need to be conducted to be able to draw any conclusions that may be used as a basis for disease management. The main factors affecting the differences between female and male immunity are the sex hormones, due to the presence of hormone receptors on immune cells[4].Sex hormones (estrogens, progesterone) can influence different aspects of immune system function and potentially affect the risk, activity and progression of rheumatoid arthritis [5].These hormones have different effects depending not only on the concentration but also on the type of target cell and the receptor subtype expressed on a given cell type [6]. Estrogen has been shown to modulate all subsets of T cells that include CD4+ (T-regs) [7]. Extensive studies have demonstrated that estrogen modulates IFNγ-secreting Th1 cells by enhancing IFNγ expression in human and has mainly anti-inflammatory effects, by inhibiting production and signaling of pro-inflammatory cytokines and enhances T-reg numbers and function [8].Progesterone as natural immunosuppressant's [8], that stimulates a switch from a predominantly pro-inflammatory to an anti-inflammatory immune response, favors T-regs differentiation [9], and exerts an inhibitory effect on natural killer (NK) cells[10]

II. Martials and methods

Samples collection of this study was carried out in October 2018 to January 2019.which included 225 RA women. They were diagnosed by physician at the Baghdad Teaching Hospital using Magnetic Resonance Imaging (MRI) examination and routinely used procedures .The patients were divided into two groups depending on menopause status (pre and post menopause). Laboratory tests were included: Anti-citrullinated peptide (ACCP), Rheumatoid factor (RF), C - reactive protein (CRP) antibody and ESR. The anti-CCP device is ready to use for the detection of antibodies against CCP in the CHORUS instruments (Italy)the test is based on the ELISA principle. Serum rheumatoid factor (RF) cause a visible agglutination on slide of a suspension of latex particles coated with human gamma-globulin. Serum C-reactive protein (CRP) at 6 mg/L or higher causes a visible agglutination on slide of a suspension of latex particles coated with anti-human C-reactive protein. ESR performed by the westergren method was adopted as the reference method for ESR. Progesterone and Estradiol estimation performed by kits supplied from ROCH, Germany determinations.

Statistical Analysis:

SAS (2012) program was used to detect the effect of difference factors of study parameters. LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability), and correlation coefficient were estimated

between variables in this study.

III. Results

Out of 225 examined women infected with rheumatoid arthritis (RA), only 70 patients were included in this study. However, this study included thirty women chosen with the same age, geographical area and living conditions. The results showed that the percentage of women who had positive results with CCP was 88%,while those with positive RF results was 71% and who with positive CRP was 97%.Table (1) .To ensure the homogeneity of samples included in the current study only 70/225 (31%) of patients were subjected to experiments that had positive results to all inflammatory markers were used.

Table 1 Distribution of RA in patients women included in this study according to disease markers

Total No. = 225 RA women patients				
Markers	CCP+	RF+	CRP+	CCP+ RF+ CRP+
No. of cases	198	160	220	70
Percentage %	88%	71%	97%	31%
Chi-Square -χ2(P-value)	11.073 ** (0.0001)			
** (P≤0.01)-H.S.				

- The concentrations of CRP in patients with positive results are > 6 mg/dl
- Positive results for an RF concentration > 30 IU/ml.
- Positive results for anti-CCP are >20 IU /ml.

When performed blood tests for the inflammatory parameters, the results found that most clinically diagnosed rheumatoid arthritis had a high level of ACCP while RF was negative, Therefore ACCP is a preferred test for the diagnosis of rheumatoid arthritis.

RA prevalence in women before and after menopause

The results showed that most of women diagnosed with rheumatoid arthritis were at the post-menopausal period 67.6% (152/225), while the percentage of the patients women at pre-menopause stage were 32.5% (73/225).Table (2).

Table 2 The percentages of RA women according to menopausal stage.

	Total	Premenopausal	Postmenopausal
No.	225	73	152
Percentage%	100%	32.4%	67.6%
Chi-Square -$\chi 2$(P-value)	---	9.459 ** (0.0001)	
** (P≤0.01)-H.S.			

The mean of age in premenopausal women patients, postmenopausal women patients was 44.90 ± 1.12 years, 51.10 ± 0.99 a year's respectively, and control group (pre and post) (42.20 ± 0.85 years, 52.00 ± 0.64 years), respectively, Table 3 .

This study adopted Das28 score to measure the severity of the disease between different groups of patients. Table (3) showed that the total mean of Das28 scores (4.14 ± 0.16) in women infected with RA was significantly ($p<0.01$) increased in comparison to total control group (1.54 ± 1.13). However, postmenopausal women stored highly significant ($p<0.01$) increase of 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), it is a crude measure of inflammation in the study groups. In the current study ESR used to confirm the severity of RA in infected women along with Das28 score BMI and age, to determine the actual impact of menopausal stage on disease severity by their scores. The results showed that the total mean of ESR (41.36 ±1.12 mm/hr.) in women infected with RA was significantly ($p<0.01$) increased in comparison to total control group (11.1± 1.83 mm/hr.). However, postmenopausal women stored highly significant ($p<0.01$) increase of ESR (47.14 ± 0.69 mm/hr.) in comparison to premenopausal women (36.12 ± 0.81) and both stages declared a significant ($p<0.01$) increase in comparison to control group, This concludes that the disease is more severe in postmenopausal women, Table 3, Figure 1.

Table 3 Comparison between difference groups in Das28, ESR and Age in womens 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

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RA patients	Premenopausal	3.66 ± 0.09 b	36.12 ± 0.81 b	44.90 ± 1.12 b
	Postmenopausal	4.63 ± 0.10 a	47.14 ± 0.69 a	51.10 ± 0.99 a
Control	Premenopausal	1.42 ± 0.06 c	7.40 ± 1.01 d	42.20 ± 0.85 c
	Postmenopausal	1.66 ± 0.07 c	14.80 ± 082 c	52.00 ± 0.64 a
LSD value		0.246 **	2.429 **	2.641 **
Means having different letters in same column differed significantly. ** (P≤0.01).				

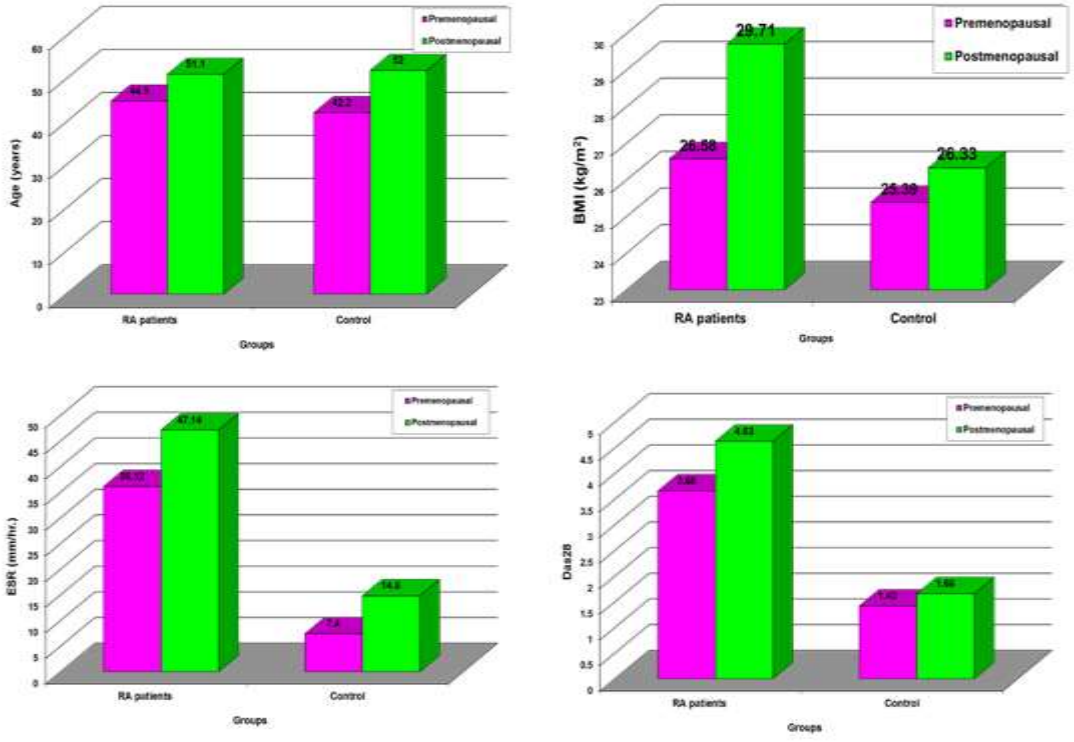

Figure 1 comparison between different studied groups according to age, BMI, Das28 score and ESR levels.

Effects of Hormonal levels on the disease severity in RA women

Progesterone

The results showed that the total mean concentration of progesterone (1.91± 0.08 ng/ml) in women infected with RA in the middle of their menstrual cycle was significantly ($p<0.01$) decreased in comparison to total control group (2.86 ± 0.23ng./ml). However, progesterone hormone results demonstrated that there was a significant difference between the studied groups. The mean of progesterone concentration in premenopausal women patients,

showed high significant ($p<0.01$) increase (3.49 ± 0.03 ng/ml) in comparison to postmenopausal women patients (0.330 ± 0.05 ng/ml), however both types of patients were showed high significant ($p<0.01$) decrease in comparison to control group (pre and post) menopause women (4.50 ± 0.10 ng/ml and 1.22 ± 0.13 ng/ml) respectively, Table 4, Figure 4

Table 4: Comparison between different groups according the levels of Progesterone, E2 and BMI in RA women with different menopausal stages correlated with Das28

Groups		**Mean ± SE**		
		Prog. (ng/ml)	**E2 (Pg./ml)**	**BMI (kg/m2)**
Total RA patients		1.91± 0.08 bd	24.6 ± 0.72 bc	28.14± 0.78 ab
Total control		2.86± 0.23 ac	52.70 ± 8.26 ac	25.86± 0.61 bc
RA patients	Premenopausal	3.49 ± 0.03 b	42.03 ± 0.59 b	26.58 ± 0.28 b
	Postmenopausal	0.330 ± 0.05 d	7.24 ± 0.12 c	29.71 ± 0.55 a
Control	Premenopausal	4.50 ± 0.10 a	88.80 ± 1.26 a	25.39 ± 0.33 c
	Postmenopausal	1.22 ± 0.13 c	16.61 ± 8.10 c	26.33 ± 0.28 bc
LSD value		0.267 **	11.789 **	1.094 **
Means having different letters in same column differed significantly. ** (P≤0.01).				

Variables	**Correlation coefficient-r with Das28**	**Level of Sig .**
BMI	0.73	**
Progesterone	-0.46	**
E2	-0.52	**

Estradiol (E2)

The results showed that the total mean concentration of E2 in the middle of their menstrual cycle (24.6 ± 0.72 pg./ml) in women infected with RA was significantly ($p<0.01$) decreased in comparison to total control group (52.70 ± 8.26 pg./ml). However hormone results demonstrated that there was a significant difference between the studied groups. The mean of progesterone concentration in premenopausal women patients, showed high significant

(p<0.01) increase (42.03 ± 0.59 pg/ml) in comparison to postmenopausal women patients (7.24 ± 0.12 pg/ml), however both types of patients were showed high significant (p>0.01) decrease in comparison to control group (pre and post) menopause women (88.80 ± 1.26 pg/ml and 16.61 ± 8.10 pg/ml) respectively, Table (4).

The current study have been investigated the links between RA and hormonal levels for women with different stages of menopause , maintaining normal levels in of the reproductive hormones estrogen and progesterone especially in premenopausal stage appear to have a protective effect against RA by presenting lower Das28 score (3.66 ± 0.09) than postmenopausal women (4.63 ± 0.10),Table 3.Figure 4.

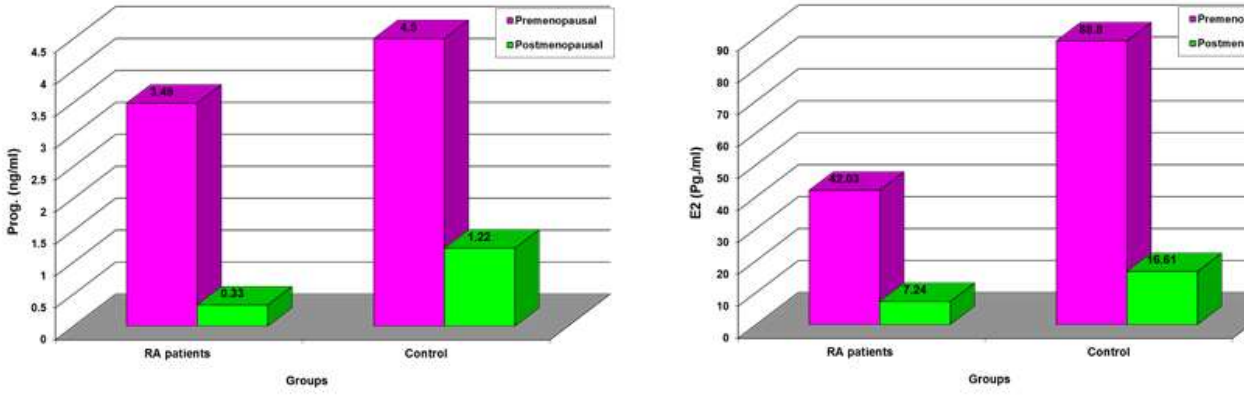

Figure 2 Comparison between different studied groups according to hormonal levels (progesterone and E2)

According to the disease severity the results showed that highly negative correlation between sex hormones levels (progesterone and E2) with Das28 score as showed in Table 3, Figure 3

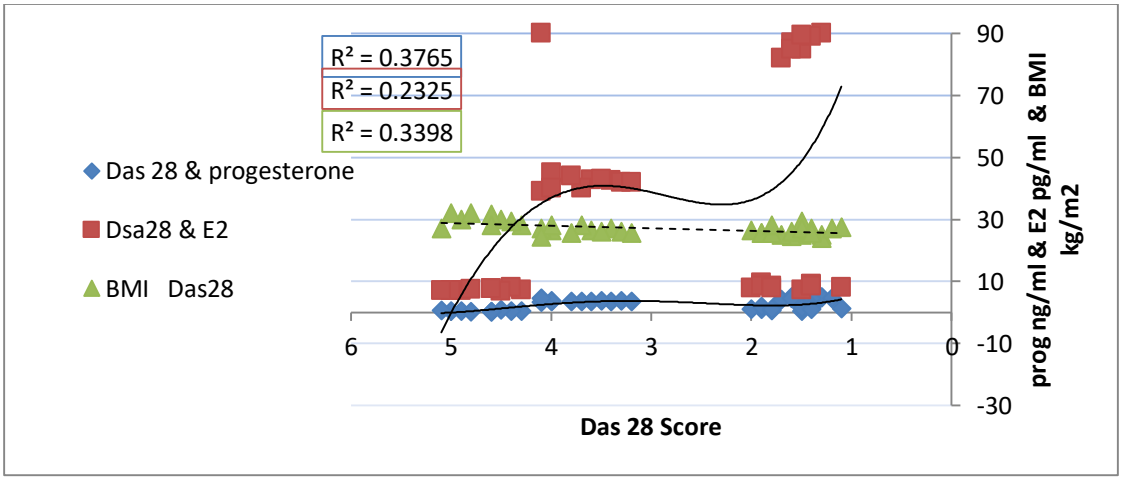

Figure 3 correlation of Das 28 with hormonal levels

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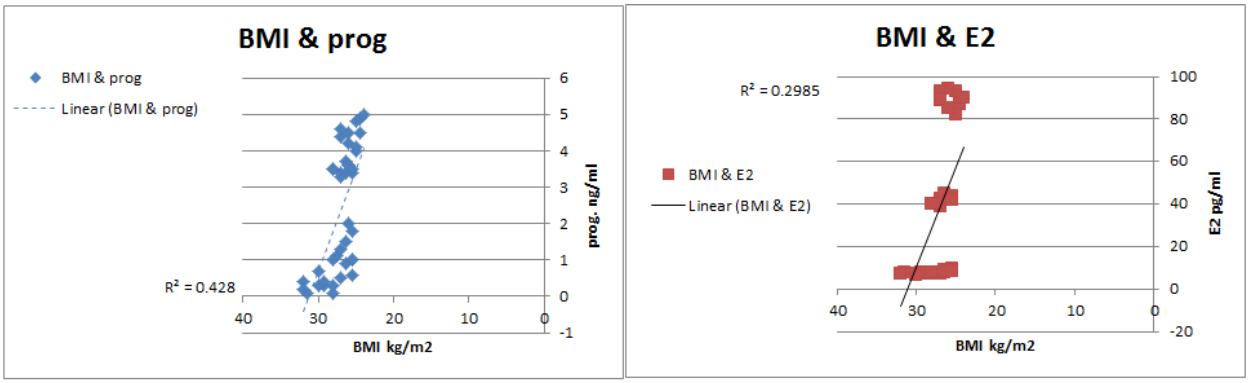

Figure 4 correlation of BMI with sex hormones

The results showed that the postmenopausal RA women scored high significant (P<0.01) increase of BMI (29.71 ± 0.55 kg/m^2) in comparison to premenopausal one (26.58 ± 0.28 kg/m^2),However, both groups showed paralleled significant increase (p<0.01) in comparison to control groups (26.33 ± 0.28 kg/m^2) and (25.39 ± 0.33 kg/m^2) respectively. Table 4,Figure1

This increase of BMI in postmenopausal stage affects the level of hormones (progesterone and E2) in women by a significant negative correlation which affected directly on severity of the disease due to scoring high level of Das28 score. Table 4, Figure 3

Also, the results showed that highly negative correlation between sex hormones levels (progesterone and E2) with BMI as showed in, Figure 4.

IV. Discussion

The present results agree with many previous results that found the anti-citrullinated protein antibodies (ACPA), more specific marker for RA than RF .In contrast to RF, ACPA only exist in about 2% of healthy individuals and are also very rare in other inflammatory conditions [12].Magnetic resonance imaging (MRI) and biopsy investigation from the joints of individuals who were seropositive for RF and ACPA but had subsequently not established any clinical manifestation of arthritis, showed a relatively normal synovium, with minor infiltration of T cells. [13].Collectively, these evidences propose that RF and especially ACPA may play a key pathogenic role in initiating and propagating RA, impressing the severity and possibly destructive nature of its clinical progression.

Women are 2– 4 times more likely than men to develop rheumatoid arthritis (RA), yet conditions associated with normal levels of estrogen and progesterone in women frequently appear joint protecting [18]. Current results agree with Roomruangwong *et al.*(2019) who stated that Women with RA report decreased joint symptoms throughout the postovulatory period of the menstrual cycle and during pregnancy, when progesterone concentration are high, and in some studies oral contraceptives contains progesterone were protective against RA [20]. In contrast, the older study mentioned that RA symptoms often flare in the postpartum period, when estrogen and progesterone levels fall. Women are at a decreased risk of developing RA during pregnancy, but the first few months of the postpartum period are a time of increased risk [21]

Many investigations conducted on the association between menopause and RA disease onset and course have placed around four main spaces of includes the effect of age at menopause on disease onset and course, subject , disease characteristics in women with late onset typically after menopause and impact of menopause on disease course. Hormonal changes that occur after menopause and the accompanying by series of physical and psychological changes and stress it may play a role to increasing the incidence of rheumatoid arthritis after menopause. This endocrine changes occurring at menopause induce changes in immune function in addition to those associated with immune-senescence. Also, the incidence of RA also rises with age. Among women, peak RA incidence appears to be between 45 and 49 years of age, suggesting an influence of premenopausal hormonal changes[14].Previous study from Iowa University, USA, displayed that women with menopause after the age of 51 had a relative risk to develop RA, compared to women with menopause before the age of 45[15].

The disease severity that represented by Das28 and ESR is changed with menopausal status, body mass index (BMI), bone mineral density (BMD) and other a variety of factors. Previous study confirmed a higher BMD in premenopausal than postmenopausal women(Al-Hafidh and Goral, 2018).The current study showed significant increasing of disease severity among postmenopausal subjects in comparison to control group and premenopausal women this results was in agreement with several studies that concluded significant association between menopausal status and RA, and they reported that the risk of decreasing in bone mineral density(BMD) leading to the bone formation is reduced and therefore the severity of RA are increased [17].

Given the female prevalence with RA, the influence of sex hormones on many other autoimmune diseases is often discussed. The fact that the peak of incidence of RA in women coincides with the drop of estrogen, whereas for men the incidence of RA continues to increase with age when testosterone levels decrease, indicates that gonadal steroids play a role in RA pathogenesis [22].

Women are more prone to development of autoimmune disease the female hormone estrogen is affect the immune system and estrogen receptor contribute to T-cell mediated autoimmune inflammation by promoting T-cell activation and proliferation [23],while previously coincide red a key provider to maintaining normal immune function in women [24]. The present result agree with previous study found that a significant positive linear correlation was observed between estrogen and soluble (s -IgA) in healthy pre-menopausal women , with the pattern of (s -IgA) mimicking serum estradiol through the various phases of the menstrual cycle [25], [26].

The rapid drop in ovarian estradiol production resulting in menopause might influence RA predisposition and the peak incidence of RA is around 45–55 years of age, coinciding with menopausal stage. Furthermore, RA incidence increases after menopause [27],this fact was agreed with the present results in the current study. Several previous studies have indicated that sex hormones can act on a variety of immune cells and interfere with the expression and production of proinflammatory cytokines, thereby affecting the development of RA.

Many older studies found that the Pregnancy has strong impact on RA severity As the female hormones E2 and progesterone increases reported that 50% of RA patients recover during pregnancy, compared with disease activity before conception, and around 40% have disease flares post-partum [28].While , Østensen and Wallenius,(2016) reported that up to 75% of RA patients experience improved disease symptoms during pregnancy. However, rise in estrogens is not the only hormonal alteration that occurs during pregnancy, *e.g.* progesterone also increases,

which might influence RA disease.Study done by Gameiro and Castelo-Branco, (2010)find that falling levels of estrogen and other female hormones lead to increased production of proinflammatory cytokines (IL1, IL6, TNF-alpha), and increased physiologic response to these cytokines and decreased secretion of anti-inflammatory cytokines , decreased lymphocyte levels (CD4+ T cells) and decreased cytotoxic activity of NK cells.

RA patients getting Hormone replacement therapy (HRT) had lower serum concentrations of pro-inflammatory molecules such as soluble receptor for advanced glaciation end product (SRAGE), soluble IL-6 receptor (sIL-6R), compared with non-HRT RA group [32].In fact, in one of the above-mentioned studies with HRT in RA, improved disease activity (Das28 and ESR) were found only in those patients with serum E2 levels of >100 pmol/L (around 60% of the patients). Thus, this suggests that conventional HRT results in borderline E2 levels, with respect to anti-arthritic efficiency[39].

The result of this study finds that there is a significant negative correlation between a BMI with the decrease of estradiol and progesterone level in RA women. Higher BMI causes an imbalance in sexual hormones, specifically a decrease in E2 and progesterone. This induces increases in severity of disease. Lowering the body weight is the first option to reduce the severity of RA caused by obesity and imbalance in sex hormons.Many studies showed that increased radiographic joint damage is significantly correlated with high BMI. They found the concentration of serum biomarkers of cartilage collagen breakdown and proteoglycan turnover to be correlated with joint destruction in RA, and hence considered BMI to be a sensitive and inflammation-independent predictor of radiographic outcome of RA [44].

In our study, we found increased risk of RA in overweight participants compared with normal weight contributors. Previous analysis in population found that the RA risk for obese versus normal weight participants was higher in women compared to men. The meta analysis exposed that each 5 kg/m^2 rise in BMI resulted in an 9% increase in the risk of RA. In addition, a significant nonlinear relationship between BMI and RA was found in the overall studies [45].Study by Qin *et al.*(2015) reported a positive association between overweight/obesity and RA compared to the association in normal weight individuals[46]. Furthermore, Qin *et al.* explored the non-linear relationship between RA and BMI. Another study also reported a positive relationship between BMI and RA only in females, and the increased risk of RA had a linear relationship for every 5 kg/m^2 increased in BMI [47].

We also found a significant correlation between BMI and RA severity, in both controls and groups studies. The results suggested that the association between BMI and risk of RA might be interfered by age, smoking, and alcohol, so the future epidemiological studies should acknowledge them. The present study considered BMI is one of the most infalammatory markers and the obesity or a higher BMI increases the risk of RA and this gives a signal to predict the occurrence of the disease.The adipose tissue of obese individuals discharges inflammatory cytokines such as TNF-α, IL-6,leptin, interleukin-1β, and monocyte chemotactic protein-1 (MCP-1) and These adipo-kines induce an inflammatory response in individuals [48]. So increased BMI significantly reduced the concentrations of several sex hormones, such as estrogen and progesterone and affected the immune response of RA patients especially when estrogen has been proposed to play a role as an immunomodulation [49,50].

V. Conclusion

The anti-citrullinated protein antibodies (ACPA), more specific marker for RA than RF. also, the results showed that the menopausal age as predictor of disease severity and a highly significant correlation between BMI and sex hormones were found that increased adiposity among postmenopausal women lead to increase disease severity of RA. The results of this study, it is concluded that there is a significant link between a BMI with the decrease of estradiol and progesterone level in postmenopausal women. Obesity causes an imbalance in sexual hormones; This prompts complications in menstrual cycles and increase predisposition to RA. For this reason lowering the body weight is the first choice to restore RA women's health problems caused by obesity.

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