ORIGINAL ARTICLE

17β -estradiol Hormone and Interleukin 1-beta Change Related to Menopause in the Women with Rheumatoid Arthritis

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Abstract

The depletion of 17β-estradiol-2 (17β-E2) is one of the factors that cause the risk of rheumatoid arthritis (RA) in females in the case of menopause. The aim of this study is to investigate whether the change in 17β-E2 levels and interleukin 1-beta (IL-1β) is associated with menopause in RA women and whether there is a relationship between them. 96 RA women were divided into three groups as follows: Group 1 (women of reproductive age) – 30, Group 2 (premenopausal women) – 32 (menstrual or normal menstrual period without menstruation for a period of not >6 months, and Group 3 (postmenopausal women) – 34 women in menopause (menopause for at least 12 months). Serum levels of 17β-E2, IL-1β, and anti-cyclic citrullinated peptide were evaluated by enzyme-linked immunosorbent assay. The results showed that a change in concentration of 17β-E2 resulted in excessive production of IL-1β in women during reproductive age, premenopausal, and postmenopausal compared to female control. Furthermore, there is a highly inverse correlation between IL-1β and 17β-E2 in the serum of pre- and post-menopausal RA women. On the other hand, the study showed a positive correlation between IL-1β and sex hormones 17β-E2 in women of reproductive age who suffer from RA. Moreover, the study confirmed that the most risk factor is 17β-E2. The study showed that a lack of 17β-2 concentration after menopause causes an increased concentration of IL-1β and this, in turn, stimulates the development of RA disease during menopause. Menopause-associated 17β-E2 deficiency plays the major role in the pathogenesis of RA.

Key words: 17β-estradiol, interleukin 1β, menopause, rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is the most common chronic inflammatory systemic autoimmune disease with multifactorial etiology.[1] It characterized by pain, swelling, stiffness, and synovial joint inflammation due to immune-mediated response.[2] Its prevalence of about 1% of the general population in western countries depends on age. RA is more prevalent among women,[3] but the difference is great during the reproductive years.[4] Interestingly, the highest incidence among women has been reported between 55 and 64 years of age, during the pre- or post-menopausal stages.[5] These gender differences seem to be highest before menopause,[6] which have led to the hypothesis that female hormonal factors play a role in RA disease development.[7] Menopause is a turning point in every woman's life. The onset of menopause is associated with a hormone deficiency, which is a contributory factor for the increased incidence of RA [8]. Alpizar-Rodriguez , suggesting that the acute decline in ovarian function might contribute to the development of autoimmunity associated with RA.[9]

Hormonal changes have assumed that they may act as a trigger for the evolution of certain diseases that occur around menopause.[10] 17β-estradiol is the primary female sex

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hormone and the most potent hormone.[11] The previous studies suggested that integral to the triggering of RA, especially in premenopausal women, may be polymorphism of the estrogen receptor.[12] RA is associated with an altered sex hormone balance characterized by higher amounts of estrogens and lower of androgens.[13] Aromatase inhibitors trigger the onset of RA and production of arthritis flares in both pre- and post-menopausal women because these compounds interfere with estrogen production.[14] The imbalance between pro-and anti inflammatory cytokine in rheumatoid joints , it is activities favors the induction of autoimmunity. It believed that cytokines are played multiple roles in the pathogenesis of RA beginning with the activation of antigen presenting cells.[15] The most important pro-inflammatory cytokines in RA are interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α).[16] IL-1β, a 17 kDa polypeptide produced by peripheral monocytes and macrophages, mediates a variety of immune responses.[17] Therefore, the biological response to IL-1β in the rheumatoid joint depends on a number of factors, one of these factors is the greatest number of IL-1RII which reduces the amount of IL-1, and anakinra competitively inhibits the binding of IL-1 to the IL-1 type I receptor. IL-1 blockade with anakinra efficaciously reduces the signs and symptoms of RA.[18]

MATERIALS AND METHODS

Study design

From a total of 150 patients suspected with RA, the study found that only 112 patients had confirmed RA by a rheumatologist according to the Revised 2010 American College of Rheumatology/ACR/ELARCI (An American College of Rheumatology/European League Against Rheumatism Collaborative Initiative) criteria for the classification of RA.[19] Patients with a score of ≥6/10 are classifiable as having RA. No patients fulfilling these criteria were excluded from the study. Patient approval was taken. 112 patients with RA including 96 female and 16 male referred to our patients to the clinic; 96 females of whom were divided into three groups as follows: Group 1 – 30 (women of reproductive age (aged 18–45 years); Group 2 (premenopausal women) – 32 women in normal menopause with regular menstruation or who were without menstruation for a period not exceeding 6 months between the ages of 46 and 53 years old; and Group 3 (postmenopausal women) – 34 women in natural menopause (menopause for at least 12 months) aged 54–70 years. Patients with surgical or radiation caused by menopause were excluded from the study. A questionnaire form was formulated to involve name, age, gender, clinical history, disease stage, disease duration, family history, morning stiffness, swollen joint (large and small joint), and any possible previous therapies. The study included 112 apparently healthy adults, with mean age (49.05 ± 13.3) years. Only 96 healthy non-pregnant women (with regular menstruation, pre and postmenopausal) were regarded as control group, was divided into three corresponding female groups for patients groups in terms of age and menstrual cycle.

Samples source

The source of the samples was from the private laboratory as a routine work from March 2016 to March 2017. Venous blood samples (5 ml) were collected from both controls and patients. Serum samples were obtained and distributed in Eppendrof vials and saved in deep freeze at −20°C until testing.

Methods

Clinical and laboratory assessments

Enzyme-linked immunosorbent assay (ELISA)

The serum levels of 17β-estradiol were evaluated by competitive ELISA, according to the specifications provided by the manufacturer (BioCheck, Inc.) with respect to the corresponding concentration values in pg/ml.

The serum consecrations of IL-1β were determined in both RA and healthy controls using ELISA according to the manufacturer's instructions (BioVision's, USA). The minimum detectable dose of IL-1 beta is typically <0.3 pg/ml. Detection range: 0.3–100 pg/ml. The intra-assay reproducibility is coefficient of variation (CV) <10% and interassay is CV <12%.

Serum anti-cyclic citrullinated peptide (CCP) antibody was determined by ELISA (IMMULISA-CCP assay kit, IMMCO DIAGNOSTICS, USA), as described by the manufacture. A standard curve was established by plotting the optical density of each calibrator with respect to the corresponding concentration values in U/ml, and samples ≥25 U/ml are defined as positive.

Latex agglutination slide

Additional laboratory tests for general health assessment were evaluated including c-reactive protein (CRP) and rheumatoid factor (RF) tests by latex agglutination slide test which were done using RA and CRP latex test kits, Salucea, The Netherlands, according to the manufacturer's instructions.

Statistical analysis

In the present study, data are expressed as the group mean ± standard deviation. Independent samples Student's *t*-test was used to compare two groups. The corresponding 95% confidence interval (95% CI) was calculated with Statistical Package for the Social Sciences (SPSS) software version 16.0 (SPSS Inc., Chicago, IL, USA). The values were first analyzed using one-way analysis of variance. Bonferroni's *post hoc* analysis was then performed to compare among three group patients (reproductive age, premenopausal, and postmenopausal women). Analysis was performed using

GraphPad Prism software for Windows (version 6.0, GraphPad Software). Correlation between variables was performed using Pearson's. Odd ratio analysis (risk factor) was done. $P < 0.05$ was considered to be statistically significant.

RESULTS

The distribution of RA patients according to their age and sex

In this paper, we studied two main groups consisting of 112 total RA patients and 112 healthy controls to compare between RA patients and healthy people depending on their age and sex. Our results showed that there are no significant differences between the age of RA patients with mean age of 49.5 ± 11.8 years and control with mean age of 49.05 ± 13.3 as shown in Figure 1. In addition to their age, we classified them according to their sex. We observed that the majority of RA patients (96, 85.72%) were female, while males represent 16 (14.28%). The effect of sex on disease was statistically significant at $P = 0.05$ as shown in Figure 2.

The general characteristics of RA patients

The general characteristics of RA patients depending on questionnaire form are summarized in Table 1. Patients' assessment of morning stiffness was observed in 94.4% of RA patients. Joint involvement was found in 43.75% with large joint and 56.25% with small joint depending on physical and clinical examination, they are classified according to the location, and the number of the involved joints. The study noted a difference in the duration of illness among RA patients, (45.5%) of RA patients have duration of disease ranged from 2 to 10 years, (34.8%) of them less than 2 years and duration of disease more than 10 years have been found (19.6%).

The distribution of female RA patients and control groups according to their menstrual cycle and age

Ninety-six RA female patients were divided into three groups in terms of age and menstrual cycle. The first group represents the reproductive age women with 18–45 years of age, the second group represents premenopausal women

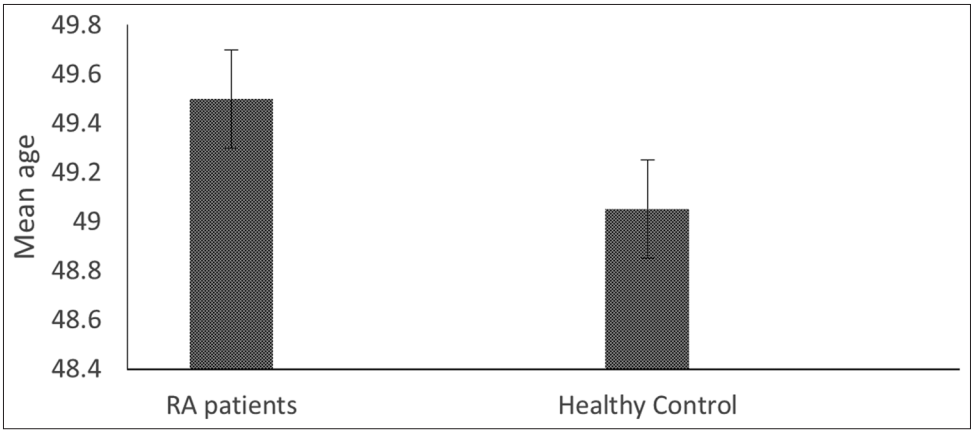

Figure 1: Distribution of rheumatoid arthritis (RA) patients according to their age. Bar charts displaying the distribution of RA patients and healthy groups according to their age. There is no significant difference

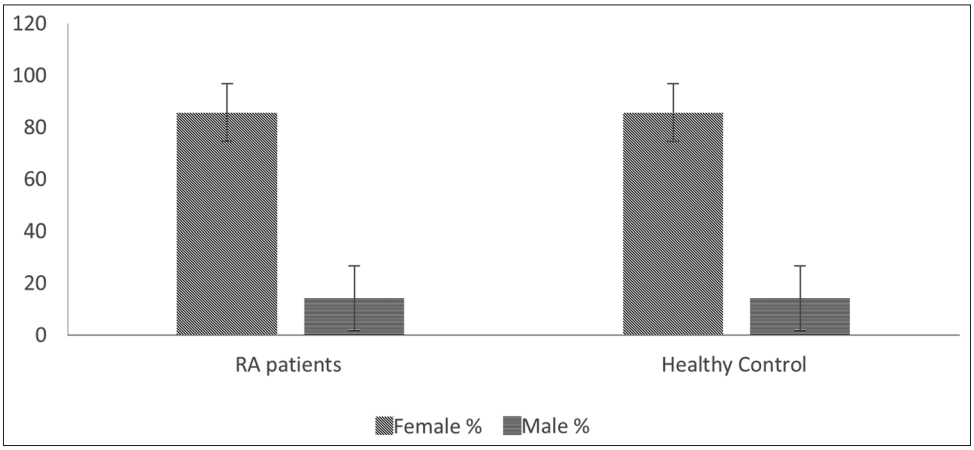

Figure 2: Distribution of rheumatoid arthritis (RA) patients and control groups according to their sex. Bar graph showing the distribution of RA patients and healthy groups according to their sex. There is a significant difference between female and male; *$P < 0.05$ one-way analysis of variance, Bonferroni's post-test with error bar representing standard error of the mean

Table 1: The general characteristics of RA patients

Character	*n* (%)
Joint involvement	
Large joint	49 (43.75)
Small joint	63 (56.25)
Moring stiffness	
Present	106 (94.6)
Absent	6 (5.4)
Score of ≥6–10	
Positive	112 (100)
Score of ≤6	
Negative	Non
Duration disease (year)	
≤2	39 (34.8)
2–10	51 (45.5)
≥10	22 (19.7)

*Data are presented as number and percentage. RA: Rheumatoid arthritis

Table 2: Estimation laboratory parameter of all RA patients and controls

Groups	*n*	Parameter	*n* (%)	*P*
		CRP		≤0.0001*
RA patients	112	Positive	90 (80.4)	
		Negative	22 (19.6)	
Control	112	Negative	112 (100)	
		RF		
RA patients	112	Positive	99 (88.4)	
		Negative	13 (11.6)	
Control	112	Negative	112 (100)	

P value is significant at level $P \leq 0.0001$ compared to healthy control. No: Number, RF: Rheumatoid factor, RA: Rheumatoid arthritis, CRP: C-reactive protein

with 46–53 years of age, and the third group represents postmenopausal women with 54–70 years of age. The present study showed significant differences in the reproductive age women compared to their control, and also, our results showed that there is a significant difference in Group 3 which represent postmenopausal compared to their control, $P < 0.05$ [Figure 3].

Estimation of CRP and RF laboratory parameter of all RA patients and controls

The results of laboratory parameter measured in this study for both patients and control are shown in Table 2. Our finding showed that the positive value of RF was 88.4%, while the negative value was 11.6% in RA patients. However, CRP positive was 80.4% and its negative value was 19.6%. Interestingly, our data found that there was significant differences in patients with RFs when compared to healthy group and also there was a significant difference between CRPs of RA patients when compared to healthy group at $P \leq 0.0001$.

Comparison of serum levels of 17β-estradiol-2 (17β-E2), IL-1β, and anti-CCP in the female patient groups compared to their controls

Our results proved that the serum concentrations of 17β-E2, IL-1β, and anti-CCP were detectable in all RA women with reproductive age, premenopausal, and postmenopausal women when compared to their healthy group. The study observed high concentrations of both IL-1β and anti-CCP in serum patients of reproductive age, premenopausal, and postmenopausal women compared to their healthy group, and the differences were statistically significant at $P \leq 0.001$, while the lowest significant value of 17β-E2 (2.53 ± 0.21 pg/ml) was detectable in patients with postmenopausal when compared to the premenopausal, reproductive age, and control group as shown in Table 3.

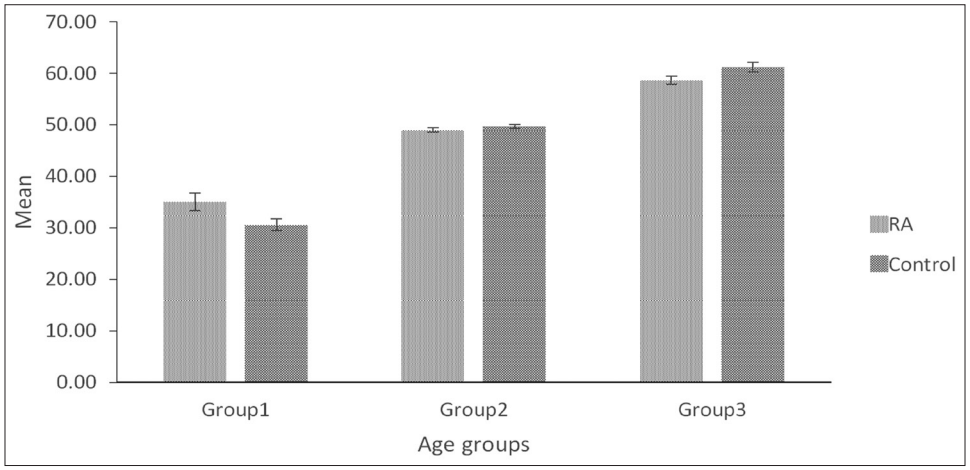

Figure 3: Distribution of female rheumatoid arthritis patients and control groups according to their menstrual cycle and age. **P* value is significant at level $P < 0.05$. Group 1 (patient women of reproductive age), Group 2 (premenopausal women); and Group 3 (postmenopausal women)

Table 3: Comparison of serum levels of 17β-E2, IL-1β, and anti-CCP in study groups

Compared groups	*n*	17β-E2			IL-1β			Anti-CCP		
		Mean	SEM	*P*	Mean	SEM	*P*	Mean	SEM	*P*
[a]Group 1 versus control	30	319.7	21.3	≤0.001	237.3	13.9	≤0.001	344.5	59.5	≤0.001
	30	77.4	2.8		1.4	0.15		2.35	0.27	
[b]Group 2 versus control	32	110.8	5.42	≤0.001	140.5	1.7	≤0.001	217.1	22.1	≤0.001
	32	47.18	47.18		1.2	0.08		2.17	0.24	
[c]Group 3 versus control	34	2.53	0.21	≤0.001	88.7	0.84	≤0.001	181.8	58.1	≤0.003
	34	12.2	0.7		0.54	0.07		3.26	0.34	

IL-1β: Interleukin 1-beta, SEM: Standard error of the mean, CCP: Cyclic citrullinated peptide, 17β-E2: 17β-estradiol-2

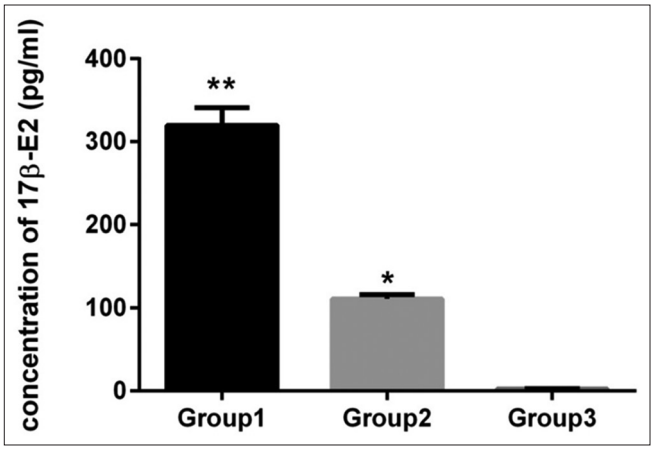

Figure 4: The levels of 17β-estradiol-2 (17β-E2) in reproductive age, premenopausal, and postmenopausal rheumatoid arthritis women. Group 1: Women of reproductive age**;** Group 2: premenopausal women; Group 3: Postmenopausal women. Bar graph showing the comparison of serum level of 17β-E2 among patients with reproductive age, premenopausal, and postmenopausal women. There is a significant difference among groups; ***P*<0.0001 one-way analysis of variance, Bonferroni's post-test with error bar representing standard error of the mean

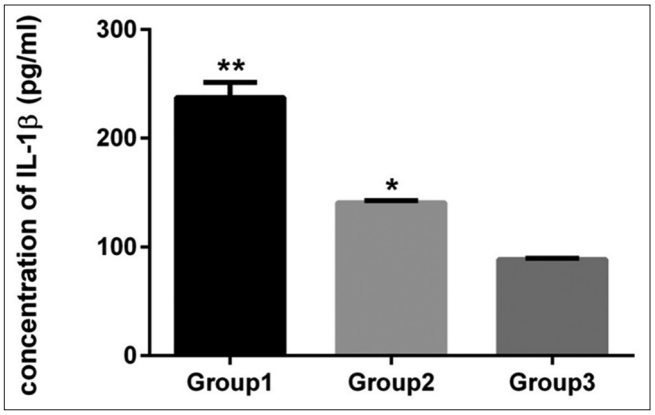

Figure 5: Interleukin 1-beta (IL-1β) levels in reproductive age, premenopausal, and postmenopausal women. Group 1: Women of reproductive age; Group 2: Premenopausal women; Group 3: Postmenopausal women. Bar graph showing the comparison of serum level of IL-1β among three rheumatoid arthritis women groups. The highest concentration of IL-1β was found in reproductive age women. There is a significant difference among groups; ***P*<0.0001 one-way analysis of variance, Bonferroni's post-test with error bar representing standard error of the mean

The levels of 17β-E2 in reproductive age, premenopausal, and postmenopausal RA women

The level of 17β-E2 was detectable in all women Groups 1, 2, and 3, but its level in Group 3 – postmenopausal – was detectable (2.53 ± 0.21 pg/ml) below the minimum detectable levels when compared to the premenopausal and reproductive age (110.8 ± 5.42 and 319.7±21.3 pg/ml). Our results observed that the mean differences between Group 1 and Group 3 were 317.2 pg/ml, while the mean differences between Group 2 and Group 3 were 108.3 pg/ml. The results showed significant differences among patients' group at $P \leq 0.0001$ as shown in Figure 4.

The levels of IL-1β in reproductive age, premenopausal, and postmenopausal RA women

The study confirmed that there was a significant increase in the levels of IL-1 β among Groups 1, 2, and 3, in general, and this increase was highly significant among Group 1 reproductive age women in particular. Our data showed that the mean differences between Groups 1 and 3 were 148.7 pg/ml and the mean differences between Groups 1 2 were 96.80 pg/ml. The highest significant concentration of IL-1β was 237.4 ± 13.97 pg/ml which was observed in reproductive age women when compared to other groups, whereas the lowest concentration of IL-1β was 88.7 ± 0.84 pg/ml which was found in Group 3 postmenopausal women as shown in Table 3 and Figure 5.

Comparison of serum level of anti-CCP among RA patients with reproductive age, premenopausal, and postmenopausal women

Our results revealed that there was a significant increase in the levels of anti-CCP among Groups 1, 2, and 3, in general. However, there were no significant differences among women groups as shown in Figure 6. There were statistically significant differences among patients' groups

when compared to their control groups ($P \leq 0.001$) as shown in Table 3.

Correlation between 17β-E2 and other assessment parameters sex, RF, age and anticcp in RA patients

Pearson correlation showed that there were different correlations between the factors adopted in the study among the groups of RA patients. The present study demonstrated a strong positive correlation between 17β-E2 with both sex and RF (r = 0.269*, P = 0.01, and r = 0.269*, P = 0.01), respectively, while it turned out to be an inverse correlation with age (r = −0.877**, P = 0.001). Furthermore, IL-1β also showed strong positive correlation with both sex and RF (r = 0.318**, P = 0.004, and r = 0.268*, P = 0.01), respectively, and reverse with age (r = −0.0.839**, P = 0.001). In addition, the study found that the anti-CCP does not have any association with the study factors, except a positive correlation with inflammatory marker CRP (r = 0.525*, P = 0.01). Finally, the study found an inverse correlation between the duration of disease and each of different parameters such as sex, RF, IL-1β, and 17β-E2 (r = −0.355, P = 0.001*; r = −0.226*, P = 0.04; r = −0.575**, P = 0.001 and r = −0.630**, P = 0.001) as shown in Table 4.

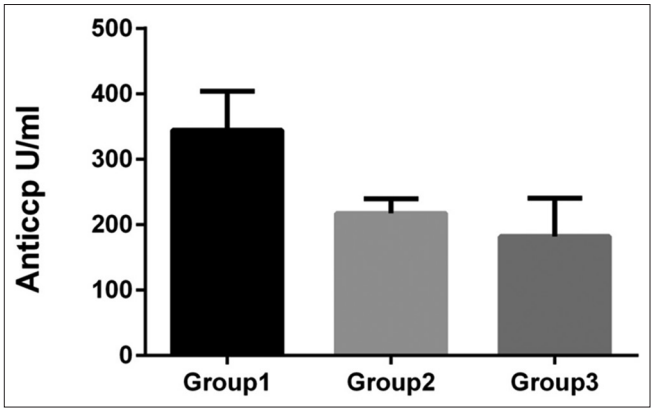

Figure 6: Comparison of serum level of anti-cyclic citrullinated peptide among rheumatoid arthritis patients with reproductive age, premenopausal, and postmenopausal women. Group 1: Women of reproductive age; Group 2: Premenopausal women; Group 3: Postmenopausal women. Bar graph showing that there were no significant differences among women groups. One-way analysis of variance, Bonferroni's post-test with error bar representing standard error of the mean

Correlation between 17β-estradiol and IL-1β in reproductive age, premenopausal, and postmenopausal women

The results showed that the relationship between the 17β-estradiol hormone and IL-1β in RA women groups varies from one group to another depending on the menstrual cycle and their age, where the study noted a strong positive correlation (r^2 = 0.933, P = 0.0001) between 17β-E2 and IL-1β in the reproductive age of RA women. In contrast, in pre- and post-menopausal RA women, we noticed an inverse correlation between them (r = −0.942, P = 0.0002, and r = −0.830, P = 0.001) respectively, as shown in Table 5.

Risk factor with 95% confidence interval

The risk factor with 95% CI was calculated to determine which factors were more serious in exacerbating the disease. It has been shown that risk factor of 17β-E2estrodial (odds ratio [OR] 4.333; 95% CI confidence interval 0.256–73.290) was more dangerous than other factors OR of IL-1β (OR 0.702; 95% CI 0.078–6.312, and OR 0.222; 95% CI 0.27–1.824) for anti-CCP as shown in Table 6.

DISCUSSION

The present study confirmed that RA is a systemic autoimmune disease which affects women more than men.

Table 4: Correlation between 17β-E2 and other inflammatory markers in RA patients

Variables		Sex	Age	RF	IL-1β	CRP	17β-E2
17β-E2	r	0.269*	−0.877**	0.269*	0.957**	0.127	1
	P	0.01	0.001	0.01	0.0001	0.262	-
IL-1β	r	0.318**	−0.839**	0.268*	1	0.178	0.957**
	P	0.004	0.001	0.01	-	0.114	0.0001
Anti-CCP	r	0.05	0.106	0.083	0.09	0.525*	0.123
	P	0.658	0.351	0.457	0.425	0.01	0.277
RF	r	0.14	0.215	1	0.268*	0.123	0.269
	P	0.215	0.056	-	0.01	0.279	0.01
Duration of disease	r	−2.84	0.835*8	−0.226*	−0.575**	0.148	−0.630**
	P	0.001	0.001	0.04	0.001	0.19	0.001

*Correlation is significant at level 0.05 (2-tailed). *Correlation is significant at level 0.01 (two-tailed). IL-1β: Interleukin 1-beta, SEM: Standard error of the mean, CCP: Cyclic citrullinated peptide, CRP: C-reactive protein, RF: Rheumatoid factor, 17β-E2: 17β-estradiol-2

Not surprisingly, patients with RA are the majority of female due to hormonal factors such as estrogen and its receptors. Our study believed that sex hormones contribute to the risk of RA due to the disease's female preponderance. This is due to the harmful effects of estrogen and its ability to reduce apoptosis of B cells. Furthermore, hormones have a complex effect on the balance of T-cell subunits with distinct cytokine profiles.[19,20] The average age of patients in the current study was slightly higher than those reported by Al-Salem[21] and younger than that observed by Kobak.[22] This difference can be explained by the demographic characteristics of the region. However, the age distribution of the patient group showed that the majority of patients were between 46 and 53 years. Other observations suggest that changes in sex hormone levels may cause a possible effect on the pathogenesis of RA, especially the strong age and sex distribution of disease.[23] Our data further showed that the investigation of various laboratory parameters in assessment of RA patients as anti-CCP, CRP, and RF reflect a general agreement that anti-CCP antibodies are an important serologic diagnostic marker for RA.[24] This finding was corresponding with a previous Study by Kroot *et al*.[25] Moreover, the high concentration of anti-CCP that found in women of reproductive age RA patients and other groups due to the high concentration of 17β-E2 induced the humoral immunity to produce high concentrations of autoantibodies. This is confirmed by our results in the existence of a positive relationship between anti-CCP and CRP. The current study noted differences in anti-CCP concentrations between female groups, and this is due to that these antibodies are directed against different epitopes in citrulline-containing peptides and sera from individual patients may contain different subsets of anti-CCP antibodies.[26] In the present study, 88.4% of RA patients were RF positive, and our finding is similar to a study done by Krishnan *et al*.[27] while RF seropositive varies among RA patients in different studies ranged from 57%[28] to 90.3%.

Table 5: Correlation between 17β-estradiol and IL-1β in each of reproductive age, premenopausal, and postmenopausal women

Correlation between		Group 1	Group 2	Group 3
17β-E2 and	r	0.933**	−0.942**	−0.830**
IL-1β	P	0.0001	0.0002	0.001

Correlation is significant at level 0.05 (two-tailed). **Correlation is significant at level 0.01 (two-tailed). IL-1β: Interleukin 1-beta, 17β-E2: 17β-estradiol-2

This may be attributed to the size of the study population and the method of assessment used besides the time of patient's selection. As matter of fact, the prevalence of CRP reported in the present study was 80.4%. Similarly, to other studies,[29,30] this due to the pro-inflammatory response results in the increased secretion of IL-1-β and TNF-α, which then results in the release of the messenger cytokine, IL-6, which stimulates the liver to secrete CRP.[31] Furthermore, the current study showed that the typical large symptoms are arthritis in the affected area, and 94.6% mild stiffness in the joints due to persistent symptom exacerbation over a long period can lead to deformities in the feet and hands; this finding is consistent with recent study, suggesting that the destruction of rheumatoid joints begins with cell-cell interactions between complex antigen presenting cells and $CD4^{+}$T cells which lead to activation of the macrophages and induction of the inflammatory process contributing to degradation and dissatisfaction of cartilage and bone. The crucial element in this process is the high concentration of IL-1β.[32] The current study confirmed that there were differences in the concentration of both the 17β-E2 and IL-1β among the RA female groups adopted in the study due to the different age, menstrual cycle, and menopause which led to a heterogeneity in the nature of the relationship between two factors per group. High 17β-E2 concentrations were found, especially, in women of reproductive age with RA patients, which is most probably due to increased conversion of estrone to estradiol seemed to be the cause for the high levels due to the effect of the 17-beta-hydroxysteroid dehydrogenase.[33] As the same time, the level of serum pro-inflammatory cytokines IL-1β was significantly higher in RA female groups of reproductive age, premenopausal, and postmenopausal plus male group compared with healthy control. The study also showed a strong positive relationship between 17β-E2 and IL-1β in women of reproductive age with RA patients. Appropriate interpretation may arise from previous study, showing that IL-1β inflammatory cytokines, particularly increased in RA, are able to stimulate marked aromatase activity in peripheral. This result agrees with the recent study by strongly indicating that IL-1β is the secondary mediator responsible for the arthritic changes,[34] while the other study indicated that the destructive aspect of RA disease is IL-1β responsible for it.[35] Interestingly, the study indicates that 17β-E2 estrogen level in postmenopausal women decreased significantly compared to premenopausal levels in RA women patients and that the least concentration has the potential to stimulate the secretion of IL-1 β at very high concentrations after menopause as does

Table 6: Risk factor with 95% confidence interval

Odd ratio for normal patients/abnormal patients	Cohort	Risk factor	95% confidence interval	
			Lower	Upper
	17β-E2	4.333	0.256	73.290
	IL-1β	0.702	0.078	6.312
	Anti-CCP	0.222	0.027	1.824

IL-1β: Interleukin 1-beta, CCP: Cyclic citrullinated peptide, 17β-E2: 17β-estradiol-2

the physiological response to this cytokine, which explains the inverse relationship between them. This result is similar to the general agreement.[36,37] This result is interpreted based on the previous study, and there was a decline after menopause in CD4 T and B lymphocytes cytotoxic activity.[38] Furthermore, this finding was also approved by other study which found that a significant reduction occurs in the proportion of positive monocytes during menopause.[39] Moreover, the study demonstrated that 17β-estradiol is one of the most risk factors because it is considered the onset of the disease in the case of autoimmune diseases such as RA. The study assumed that it plays a major role in regulating the immune response in RA patients, which affects the concentration of IL-1β in the case of elevation, which leads to increase in the severity of disease and damage to bone. The study believed through the results that pre- or post-menopausal serum 17β-E2 sex hormone balance is a crucial factor in the regulation of immune and inflammatory responses in RA disease.

CONCLUSION

Immunological evidence in this study suggests that the depletion of the 17β-E2 hormone plays a major role in development rheumatoid arthritis during menopause. Leading to increased levels of pro-inflammatory IL-1β cytokines due to low concentration of 17β-E2, which is considered the most dangerous factors during the menopause. Therefore, we find that women are more likely to develop RA than men. Further research is required to explore the biological mechanisms behind our findings.

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REFERENCES

1. Firestein GS. Evolving concepts of rheumatoid arthritis. Nature 2003;423:356-61.
2. Shivanand P. Arthritis an autoimmune disorder: Demonstration of *in-vivo* anti-arthritic activity pandey. Int J Pharm Life Sci 2010;1:38-43.
3. Hegen M, Keith JC Jr., Collins M, Nickerson-Nutter CL. Utility of animal models for identification of potential therapeutics for rheumatoid arthritis. Ann Rheum Dis 2008;67:1505-15.
4. Kvien TK, Uhlig T, Ødegård S, Heiberg MS. Epidemiological aspects of rheumatoid arthritis: The sex ratio. Ann N Y Acad Sci 2006;1069:212-22.
5. Humphreys JH, Verstappen SM, Hyrich KL, Chipping JR, Marshall T, Symmons DP, *et al.* The incidence of rheumatoid arthritis in the UK: Comparisons using the 2010 ACR/EULAR classification criteria and the 1987 ACR classification criteria. Results from the Norfolk arthritis register. Ann Rheum Dis 2013;72: 1315-20.
6. Pozo CO. The Association between Hormonal/ Reproductive Factors and the Risk of Developing Rheumatoid Arthritis. Stockholm, Sweden: Karolinska Institutet; 2015.
7. Alpízar-Rodríguez D, Pluchino N, Canny G, Gabay C, Finckh A. The role of female hormonal factors in the development of rheumatoid arthritis. Rheumatology (Oxford) 2017;56:1254-63.
8. Malutan AM, Dan M, Nicolae C, Carmen M. Proinflammatory and anti-inflammatory cytokine changes related to menopause. Prz Menopauzalny 2014; 13:162-8.
9. Alpizar-Rodriguez D, Mueller RB, Möller B, Dudler J, Ciurea A, Zufferey P, *et al.* Female hormonal factors and the development of anti-citrullinated protein antibodies in women at risk of rheumatoid arthritis. Rheumatology (Oxford) 2017;56:1579-85.
10. Neugarten BL, Kraines RJ. "Menopausal symptoms" in women of various ages. Psychosom Med 1965;27:266-73.
11. Wluka AE, Cicuttini FM, Spector TD. Menopause, oestrogens and arthritis. Maturitas 2000;35:183-99.
12. McKay LI, Cidlowski JA. Molecular control of immune/ inflammatory responses: Interactions between nuclear factor-kappa B and steroid receptor-signaling pathways. Endocr Rev 1999;20:435-59.
13. Cutolo M, Villaggio B, Seriolo B, Montagna P, Capellino S, Straub RH, *et al.* Synovial fluid estrogens in rheumatoid arthritis. Autoimmun Rev 2004;3:193-8.
14. Ngo ST, Steyn FJ, McCombe PA. Gender differences in autoimmune disease. Front Neuroendocrinol 2014; 35:347-69.
15. Babushetty CM. The role of sex hormones in rheumatoid arthritis. Int J Pharm Pharm Sci 2012;4:15-2.
16. Yasui T, Uemura H, Tomita J, Miyatani Y, Yamada M, Kuwahara A, *et al.* Association of interleukin-8 with hot flashes in premenopausal, perimenopausal, and postmenopausal women and bilateral oophorectomized women. J Clin Endocrinol Metab 2006;91:4805-8.
17. Bouman A, Moes H, Heineman MJ, de Leij LF, Faas MM. The immune response during the luteal phase of the ovarian cycle: Increasing sensitivity of human monocytes to endotoxin. Fertil Steril 2001;76:555-9.
18. Kay J, Calabrese L. The role of interleukin-1 in the pathogenesis of rheumatoid arthritis. Rheumatology (Oxford) 2004;43 Suppl 3:32-9.
19. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, BinghamCO3rd,*etal.*2010rheumatoidarthritisclassification criteria: An American college of rheumatology/European

league against rheumatism collaborative initiative. Arthritis Rheum 2010;62:2569-81.
20. Firestein GS, Budd RC, Gabriel SE, McInnes IB, O'Dell JR, Kelley WN. Etiology and pathogenesis of rheumatoid arthritis. In: Kelley's Textbook of Rheumatology. 9th ed. Philadelphia PA: Elsevier/Saunders; 2009. p. 1035-86.
21. Al-Salem IH, Al-Awadhi AM. The expression of rheumatoid arthritis in Kuwaiti patients in an outpatient hospital-based practice. Med Princ Pract 2004;13:47-50.
22. Kobak S. Demographic, clinical, and serological features of Turkish patients with rheumatoid arthritis: Evaluation of 165 patients. Clin Rheumatol 2011;30:843-7.
23. Wilder RL. Adrenal and gonadal steroid hormone deficiency in the pathogenesis of rheumatoid arthritis. J Rheumatol Suppl 1996;44:10-2.
24. Avouac J, Gossec L, Dougados M. Diagnostic and predictive value of anti-cyclic citrullinated protein antibodies in rheumatoid arthritis: A systematic literature review. Ann Rheum Dis 2006;65:845-51.
25. Kroot EJ, de Jong BA, van Leeuwen MA, Swinkels H, van den Hoogen FH, van't Hof M, *et al.* The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. Arthritis Rheum 2000;43:1831-5.
26. Pruijn GJ, Wiik A, van Venrooij WJ. The use of citrullinated peptides and proteins for the diagnosis of rheumatoid arthritis. Arthritis Res Ther 2010;12:203.
27. Krishnan E, Sokka T, Hannonen P. Smoking–gender interaction and risk for rheumatoid arthritis. Arthritis Res Ther 2003;5:R158.
28. Bagchi D, Moriyama H, Raychaudhuri SP. Arthritis: Pathophysiology, Prevention, and Therapeutics. Boca Raton: Taylor and Francis; 2011. p. 577.
29. Fiedorczyk M, Klimiuk PA, Sierakowski S, Gindzienska-Sieskiewicz E, Chwieckо J. Serum matrix metalloproteinases and tissue inhibitors of metalloproteinases in patients with early rheumatoid arthritis. J Rheumatol 2006;33:1523-9.
30. Milovanovic M, Nilsson E, Järemo P. Relationships between platelets and inflammatory markers in rheumatoid arthritis. Clin Chim Acta 2004;343: 237-40.
31. Rani HS, Madhavi G, Srikanth B, Jharna P, Rao U, Jyothy A. Serum ADA and C-reactive protein in rheumatoid arthritis. Int J Hum Genet 2006;6:195-8.
32. Feldmann M, Brennan FM, Maini RN. Role of cytokines in rheumatoid arthritis. Annu Rev Immunol 1996; 14:397-440.
33. Cutolo M, Sulli A, Straub RH. Estrogen metabolism and autoimmunity. Autoimmun Rev 2012;11:A460-4.
34. Abramson SB, Amin A. Blocking the effects of IL-1 in rheumatoid arthritis protects bone and cartilage. Rheumatology (Oxford) 2002;41:972-80.
35. Miossec P, van den Berg W. Th1/Th2 cytokine balance in arthritis. Arthritis Rheum 1997;40:2105-15.
36. Gameiro CM, Romão F, Castelo-Branco C. Menopause and aging: Changes in the immune system-a review. Maturitas 2010;67:316-20.
37. Afzal S, Khanam A. Serum estrogen and interleukin-6 levels in postmenopausal female osteoarthritis patients. Pak J Pharm Sci 2011;24:217-9.
38. Gameiro C, Romao F. Changes in the immune system during menopause and aging. Front Biosci (Elite Ed) 2010;2:1299-303.
39. Ben-Hur H, Mor G, Insler V, Blickstein I, Amir-Zaltsman Y, Sharp A, *et al*. Menopause is associated with a significant increase in blood monocyte number and a relative decrease in the expression of estrogen receptors in human peripheral monocytes. Am J Reprod Immunol 1995; 34:363-9.

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