Hormonal and Immunological Disturbances in Patients with Rheumatoid Arthritis and Systemic Lupus Erythematosus

Numman Hamed. Salih* , Majid Mohammed. Mahmood Al-Jewari, Khalil Ismail.Abid. Mohammed*****

ABSTRACT:

BACKGROUND:

Rheumatoid arthritis and systemic lupus erythematosus are multifactorial autoimmune diseases. Some recent reports indicated a hormonal disturbances affected the balance between Th1 and Th2 lymphocyte response.

OBJECTIVES:

To investigate the immunological and hormonal disturbance in patient with *rheumatoid arthritis* (RA) and *systemic lupus erythematosus* (SLE).

METHODS:

Serum samples, were collected from patients with RA, SLE and control, then the tests for antinuclear antibodies, anti double strand DNA, anticardiolipin antibodies are done by using Enzyme linked immuno assay (ELISA) method. Also, hormonal studies including estrogen, progesterone and prolactin level are done by using the Radio immunoassay technique (RIA).

RESULTS:

The results indicated the increasing of ANA, anti ds-DNA, and anticardiolipin antibodies. Also, elevation in the levels of estrogen, progesterone and prolactin in patients with RA and SLE comparing with control.

CONCLUSION:

Immunological and hormonal disturbances in patients with RA and SLE were documented through through the increasing of ANA, anti dsDNA anticardiolipin antibodies and elevation of the level of estrogen, progesterone, and prolactin.

KEW WORDS: rheumatoid arthritis , systemic lupus erythematosus, ana, anti dsdna , progesterone , estrogen, prolactin.

INTRODUCTION:

Rheumatoid arthritis (RA) and *systemic lupus erythematosus* (SLE) are multifactioral auto immune diseases that originate from patient's excessive immune and inflammatory response to a pathogenic agent that is, an infective antigens[1]. The patho-physiological mechanisms are activated after combination of several predisposing factors, which include the relations between histocompatability epitopes and epitopes of the pathogenic antigen. The altered status of the stress response system (hypothalamic – pituitary – adrenocortical axis) and gondal hormone pattern (hypothalamic – pituitary – gondal axis) with oestrogen principally implicated as inducer of the immune response, and androgen, progesterone as a natural suppressor's[2,3].

Steroid hormones influence autoimmunity and may entail a further balance between Th1 and Th2 lymphocyte response[4]. The lymphocytes produce mainly interleukin-2 (IL-2) and interferon-γ (IFN-γ) and are primarily responsible for cell mediated immunity, while Th2 lymphocyte produce mainly IL-4, IL-5, IL-13 and IL-10, and are responsible for humoral immunity[5]. These observation suggest that the cytokine secretion abnormalities may have a paramount importance in the triggering or maintenance a different pathophysiological mechanism and clinical aspects characterizing RA and SLE[6]. Increased concentration of steroids, as obtained after ovulation induction therapy, have been described to enhance a fatal exacerbation of the disease in several women

*Depth of Biology, College of Science, Tikrit University

**Depth of Biology, College of Science, Al-Mustansyria University

***Depth of Basic Science, College of Dentistry, Baghdad University

with SLE, the concomitant presence of antiphospholipid antibodies being considered a risk factor[7].

In general, the clinical observation that hormones play a role in the immune response suggests that the endocrine system is an important factor for the development and maintenance of that response, therefore, the hormonal and immunological abnormalities in patient with RA and SLE were evaluated.

MATERIALS AND METHODS:

Samples: the study included (180) serum samples, were collected by taking blood from patient attending Baghdad Teaching Hospitals and Al-Falluja General Hospitals. The samples were taken from (89) patients with RA, and (51) patients with SLE while (40) samples of healthy peoples were collected as a control. All sera separated from the blood samples and were stored in freezer at -20°C until needed.

Immunological Study:

Antinuclear Antibodies ANA Enzyme linked immuno asorbent assay for patients and control specimen were tested according to methods of Tan (1982)[8] the ANA Index Calculated using the following formula.

The ANA Index less than (1.0) considered negative or more than (1) considered as positive.

Anti double strand DNA**.** Anti ds DNA (ELISA method) for patients (or control) specimens were tested according to methods of (9). Then the result up to (20 IU/ml) considered as positive.

Anti cardiolipin antibodies class IgG, IgM (ELISA method). The test were done according (10) then the result up to 7 IU/ml consider as positive for IgG and IgM.

Hormonal Study:

Determination of Estrogen level in serum:

The methods was done by using radio immunoassay technique (RIA), then the results considered as positive or negative as following according to (11).

- Males up to 55 pg/ml
- Female30 – 200 pg/ml in middle follicular phase 100-450 pg/ml in pre ovulantory phase 50-250 pg/ml in middle luteal phase Less than 55 pg/ml post menopausal phase.

Determination of progesterone level in serum:

The method was done by using (RIA) technique, then the results considered as positive or negative as following according to (11).

Males 0 – 0.6 ηg/ml

Femal 0.1 – 0.2 ηg/ml in middle follicular phase 2.5 – 29 ηg/ml in middle luteal phase

Determination of prolactin level in serum:

The method was done by using the RIA technique then the results considered positive or negative as following according to (11).

Males 90 – 370 µIU/ml

Females 130-700 µIU/ml

85-490 µIU/ml post menopausal phase.

Statistical Analysis:

Analysis of variance (ANOVA) test was used to compare the results according to Stites & Torri (1987)[12].

RESULTS:

Estimation of ANA in patients of RA & SLE. The ANA titer in male patients with RA & SLE higher than control ($P < 0.05$) as shown in Table (1). Also, ANA titers in female patients with SLE, were higher but the difference was statistically non significant with RA patients ($P>0.05$). The sensitivity and specificity test (Table 2) revealed the specificity and sensitivity with female more than male for RA and SLE.

Estimation of anti ds-DNA antibodies in patients with RA & SLE.

The anti-ds DNA antibodies titers in male and female patients with SLE & RA were higher than control ($P<0.05$) Table (3). Also, the sensitivity, specificity tests for female with RA & SLE more than males Table (4).

Estimation of anticardiolipin – antibodies class (IgG, IgM) in RA, SLE.

The anticardiolipin antibody (ACA) (class IgG) level in male patients with RA & SLE were not differe with control ($P>0.05$), Table (5) while the ACA in female patients were higher in patients with RA but the difference was non significant when compared with control.

Also, the difference of ACA (class IgM) in male & female patients with SLE & RA were statistically non difference ($P > 0.05$) compared with control (Table-6), significant sensitivity in females reach to (47.5%) in RA patients and reached 37.2% in male patient with SLE.

Determination of Estrogen Level in ρg/ml in RA, SLE;

Estrogen level increased ($P < 0.05$) in patients with SLE & RA compared with control. The levels of hormones were (46.96 ρg/ml) in male patients with RA and 25.88 ρg/ml in male patient with SLE compared with control which reached (23.97 ρg/ml) (Table 8) while the level of hormones were (73.38ρg/ml) and (98.66ρg/ml) for female patients with RA & SLE respectively

compared with the control which were (40.93ρg/ml).

Determination of Progesterone level in ηg/ml in patients with RA and SLE:

Progesterone level increased (P < 0.05) in patients with RA & SLE compared with control. The level in male patients (0.79) ng/ml, (0.82) ηg/ml for RA & SLE respectively compared with the control which were (0.07) ηg/ml (Table 9) while the level of hormones in female patients were (1.73) ηg/ml, (2.20) ηg/ml in RA, SLE respectively compared with 0.5 ηg/ml for the control.

Determination of Prolactin level /µIU/ml in patients with RA and SLE:

Prolactin levels increased (P < 0.05) in patient with SLE & RA compared with the control, the levels were in male patients (228.97, 241.84) µIU/ml for RA & SLE respectively compared with the control which were (125.7) µIU/ml (Table 10). Also, the level of hormone were in female patients (295.70, 310.15) µIU/ml for RA & SLE respectively compared with the control which were (146.76) µIU/ml.

Table (1): ANA-antibodies in patients with RA & SLE.

Sex	ANA index	Mean ± SD					
		Control	No.	RA	No	SLE	No.
Males	< 1	0.3 ± 0.23	17	0.44 ± 0.04	10	0.58 ± 0.07	5
	> 1	1.45 ± 0.15	2	1.57 ± 0.14	19	1.91 ± 0.3	11
Females	< 1	0.27 ± 0.03	18	0.21 ± 0.02	19	0.31 ± 0.04	6
	> 1	1.31 ± 0.15	3	1.60 ± 0.23	41	2.01* ± 0.29	29

* P < 0.05 statistically significant

Table (2): Specificity and sensitivity of ANA antibodies in serum of patients with RA, SLE.

Disease	Sex	Sensitivity (%)	Specificity (%)
RA	Males	65.5	89.5
	Females	68.5	85.7
SLE	Males	68.75	89.5
	Females	82.9	85.7

Table (3): Anti ds-DNA antibodies in serum of patients with RA & SLE.

Sex	Anti ds-DNA IU/ml	Mean ± SD					
		Control	No.	RA	No	SLE	No.
Males	< 20	6.07 ± 1.26	19	8.84 ± 1.2	24	9.82 ± 1.8	4
	> 20	----	----	41.77 ± 8.7	5	114.2 ± 29.7	12
Females	< 20	5.71 ± 1.0	20	6.2* ± 0.87	46	12.8 ± 0.8	6
	> 20	23.68	1	48* ± 6.6	14	80.2* ± 14.4	29

* P < 0.05 statistically significant

Table (4): Specificity and sensitivity of anti ds-DNA antibodies in serum of patients with RA & SLE.

Disease	Sex	Sensitivity (%)	Specificity (%)
RA	Males	17.24	100
	Females	23.3	4.7
SLE	Males	75	100
	Females	82.8	4.7

Table (5): Anti Cardiolipin antibodies (ACA) class IgG in serum of patients with RA & SLE.

Sex	ACA level IU/ml	Mean ± SD					
		Control	No.	RA	No	SLE	No.
Males	< 10	0.99 ± 0.2	19	0.93 ± 0.2	29	1.38 ± 0.6	16
	> 10	---	---	----	---	-----	---
Females	< 10	0.38 ± 0.13	21	0.88* ± 0.18	33	0.46 ± 0.11	35
	> 10	----	---	13.04* ± 1.31	27	------	----

* $P \leq 0.05$ statistically significant.

Table (6): Anticardiolipin antibodies ACA class IgM in serum of patients of RA & SLE.

Sex	ACA IU/ml	Mean ± SD					
		Control	No.	RA	No	SLE	No.
Males	< 7	4.88 ± 0.37	15	5.63 ± 0.41	14	4.35 ± 0.84	4
	> 7	10.41 ± 1.65	4	10.11 ± 1.46	15	9.12 ± 0.79	12
Females	< 7	5.24 ± 0.29	10	5.36 ± 0.25	30	4.89 ± 0.41	12
	> 7	8.83 ± 0.36	11	11.47 ± 2.31	30	8.43 ± 0.61	23

Table (7): Specificity and sensitivity of ACA (class IgG + IgM) in serum of patients with RA & SLE.

Disease	Sex	Sensitivity (%)	Specificity (%)
RA	Males	25.86	10.53
	Females	47.5	26.19
SLE	Males	37.5	10.53
	Females	32.86	26.19

Table (8): Estrogen level in ρg/ml in serum patients of RA & SLE

Sex	Mean ± SD					
	Control	No.	RA	No	SLE	No.
Males	23.97 ± 4.29	19	46.96** ± 5.43	29	25.88 ± 3.01	16
Females	40.93 ± 4.56	21	73.38* ± 14.21	60	98.66* ± 21.17	35

* $P < 0.05$ statistically significant

Table (9): Progesterone level ηg/ml in serum patients of RA & SLE.

Sex	Mean ± SD					
	Control	No.	RA	No	SLE	No.
Males	0.07 ± 0.03	19	0.79* ± 0.22	29	0.82* ± 0.37	16
Females	0.54 ± 0.16	21	1.73** ± 0.41	60	2.20** ± 1.01	35

* $P < 0.05$ statistically significant.

Table (10): Prolactin level / μIU/ml in serum patients with RA & SLE

Sex	Mean ± SD					
	Control	No.	RA	No	SLE	No.
Males	125.7 ± 9.62	19	228.9* ± 35.58	29	*241.84 ± 49.25	16
Female	146.7 ± 25.18	21	*295.7 ± 44.78	60	310.15* ± 56.89	35

* $P < 0.05$ statistically significant.

DISCUSSION:

The antinuclear antibody test used to screen for the presence of autoantibodies in human serum as an aid in the diagnosis of certain rheumatic disease, therefore the ANA are increased (Table 1) in patient with RA & SLE. The results were consistent with Stenier, Smolon, 2002[14] when demonstrated that the ANA. was Positive in 50% of RA, and 90% with SLE patients and with rare precent in healthy control. Also, the sensitivity of ANA in female(Table 2) which were 68.3%, 82.9% in RA & SLE more than in male patients are consistent with Whitecare, (2001)[15] , when demonstrated that the female animal laboratory produce a high titers of antibodies after immunization or infections with pathogens. Antibodies to dsDNA in patients serum are increased (Table-3) comparing with the control. The results were consistent with Giasuddin et al., (1991)[16] when demonstrated that the anti ds-DNA antibodies are found in 85.3% of patients with SLE. The high levels of antibodies with SLE exhibit a positive correlation to the severity of the disease. Also, the sensitivity of anti ds-DNA in patients with RA, SLE reach 23.3% , 82.86% for females respeactively and 17.24%, 75% for male respeactively, the result, consistent with Tzioufas et al. (1987)[17] when demonstrated the sensitivity reach 91% and specificity reach 96% to the disease. In a general most autoantibodies to ds-DNA are directed against the phosphate unit of the DNA, Thus, these autoantibodies also bind to DNA single strand, while the autoantibodies against single strand DNA are mainly directed against the basic compound which in the native DNA masked inside the helical structure.

The anticardiolipin antibodies in patients serum are increased (Table 5, 6) for IgM and IgG, the results indicated the level increased with the severity of RA and consistent with Grommica et al., (1990)[18]. Also, the sensitivity of anticardiolipin antibodies (Table – 7) reach 47.5%, 32.86% for RA, SLE female patients respeactively and 25.86%, 37.5% for RA, SLE male patients respeactively. The results consistent with Lovea Santoro (1990)[19] when demonstrated the sensitivity reach 50% of patient with SLE. In a general the presence of anticardiolipin antibodies in systemic lupus erythematosus can be related to the development of thrombosis, thrombocytopenia, fetal loss and some neurological disorders[7].

Estrogen level increased ($P < 0.05$) in patients with RA & SLE comparing with the control (Table–8), the result consistent with Salem et al.,[20] when demonstrated the high level of estrogen enhance the progressing of RA. New explanation for the different mechanism involved that ostrogen stimulate synovial cells to proliferate and increasing synovial fluid in RA patient. On the basis of these driven mechanisms the gradual decline of oestrogen around menopause might explain the high incidence of RA at that time, with mechanism similar to those realized in the postpartum period. Also, the same hormonal change might explain the high incidence of SLE observed during a fertile ages and the decline around menopause[21]. Progesterone level increased ($P < 0.05$) in patient with RA & SLE the role of progesterone with autoimmune disease unclear currently, but the disturbance in the regulation of progesterone and estrogen develop the severity of autoimmune disease like RA & SLE. especially the hormones that increase the IL-4 production (Th2-type cytokine) by human T-cells[22]. Prolactin level increased ($P<0.05$) in patients with RA & SLE, the results consistent with Blanco – Favela et al., (1999)[23] when demonstrated the elevation of hyperprolactemia with autoimmune disease like RA & SLE but the current mechanism was not clearly understood. Possible mechanism involved increased the sensitivity to the hormones[24].

CONCLUSION:

The results illustrated the Immunological and hormonal disturbances in patients with *Rheumatoid arthritis* and *systemic lupus Erythematosus* were documented through the increasing of ANA, anti dsDNA, anti cardiolipin anti bodies and the elevation of the level of estrogen , progesterone and prolactin.

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