

Association of serum testosterone and dehydroepiandrosterone sulfate with rheumatoid arthritis: a case control studyMahin Lashkari¹, Akram Noori¹, Sonia Oveisi², Mohammadreza Kheirkhah³

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Type of article: Original**Abstract**

Background: It is supposed that hypoandrogenism may be involved in the pathogenesis of rheumatoid arthritis (RA). Testosterone and dehydroepiandrosterone sulfate (DHEAs) levels decrease in body fluids of patients with RA.

Objective: The aim of this study was to determine the association of serum testosterone and DHEAs with RA.

Methods: This case-control study was conducted on 59 patients with RA and 61 healthy gender- and age-matched controls at Qazvin University of Medical Sciences, Qazvin, Iran, in 2014. Serum free testosterone and DHEAs levels were measured and compared between two groups. Serum testosterone levels lower than 0.029 ng/ml in females and 2.49 ng/ml in males were considered as abnormal. DHEAs levels lower than 18.9 µg/dl in females and 88.9 µg/dl in males were considered as abnormal. Data were analyzed using independent sample T-test, Chi-square test, and logistic regression analysis by SPSS software, version 19.

Results: The mean testosterone level in females of the control group was significantly higher than females in the case group. The mean DHEAs in the control group was significantly higher than the case group. Abnormal testosterone and DHEAs level in the case group was significantly higher than the control group. Logistic regression analysis showed independent association only between DHEAs levels and RA, after adjusting for age and gender (OR: 0.966, 95% CI: 0.953-0.979; $p < 0.001$).

Conclusion: With regard to the results, abnormal testosterone and DHEAs level in patients with RA was significantly higher than the control group. This shows the anti-inflammatory effects of gonadal and adrenal androgens in RA.

Keywords: Rheumatoid arthritis, Dehydroepiandrosterone sulfate, Androgens, Humoral immunity, Cellular immunity

1. Introduction

Rheumatoid Arthritis (RA) is a chronic, systemic autoimmune disease characterized by inflammatory arthritis. The disease is characterized by a symmetric synovitis and tenderness of different joints, especially small joints of hands and feet. This disease is the most common form of inflammatory acute arthritis which usually causes joint damages and disability (1). The prevalence of RA is about 0.5%-1% in the world population. The disease is most prevalent in females and in the age range of 25-55 years (2-4). The RA symptoms at the time of referring to the clinics typically result from inflammation in joints, tendons and bursas (5). In a joint study by the American College of Rheumatology and the European League Against Rheumatism, the classification criteria of RA were revised (6), aiming to improve the process of early diagnosis of the disease in patients who can benefit from treatment with disease-modifying antirheumatic drugs (DMARD) (7). Some auto-antibodies, such as rheumatoid factor (RF) and

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antibodies to citrullinated protein antigens (ACPAs), are measured to diagnose RA disease (8, 9). The RF, secreted directly against immunoglobulin G (Ig G), is the most distinctive auto-antibody in the diagnosis of RA (10). Hypoandrogenism is a disorder associated with RA. The level of testosterone reduces at the onset of disease complications and returns to the normal state after relieving the acute symptoms (11). The severity of gonadotropin suppression is in association with the severity of disease. The pathophysiology of gonad dysfunction is not clear during the illness, but the effects of cytokines and/or glucocorticoids can be noted (12). The decrease of testosterone level is very common among patients with chronic diseases such as AIDS, RA, renal failure, different cancers and/or those who take glucocorticoids. Most of these people have a central defect in the hypothalamus-pituitary, or have defects in the testicles and in the hypothalamic-pituitary centers (13). Sexual hormones engage in immune responses; for example, estrogen hormones intensify humoral immune responses, and progesterone and androgens naturally act as immune inhibitors (14, 15); for example, the levels of testosterone and dehydroepiandrosterone sulfate (DHEAs) - one of the major circulating steroids in blood and as a central intermediate in the metabolic pathway of sex steroid hormone formation (16) - decrease in body fluids such as blood, synovial liquid (17) and saliva of males and females with RA (18). These hormones also strengthen the pathogenic potential role of the lower levels of androgens, which are the immune system inhibitors. The anti-inflammatory effects of androgens in physiological levels are proved (18). DHEAs exerts an immunomodulatory action, increasing the number of monocytes, T cells expressing T-cell receptor gamma/delta (TCR $\gamma\delta$) and natural killer (NK) cells. It improves physical and psychological well-being, muscle strength and bone density, and reduces body fat and age-related skin atrophy stimulating procollagen/sebum production (19). The mean testosterone and DHEAs serum levels in males and postmenopausal females with RA were lower than those of the control group; hence, it is supposed that hypoandrogenism may be involved in the pathogenesis of RA (20). In fact, some studies indicated that higher levels of testosterone in young males play protective roles against the incidence of RA (21). The idea that lower levels of serum testosterone associated with RA conditions result from chronic inflammatory situations relies on the observations indicating the clinical improvement associated with the level of serum testosterone after a successful treatment (22). It is clinically important that patients undergoing long-term treatment with glucocorticoids may experience hypoandrogenism due to the secretion of Luteinizing hormone (LH) and Follicle-stimulating hormone (FSH) by their hypophyses (23). If there is a relationship between the reduction of androgens and RA disease, further studies could be planned on the available therapeutic methods and testosterone. The aim of this study was to determine the association of serum testosterone and DHEAs with rheumatoid arthritis in a case control design.

2. Material and Methods

This case-control study was conducted on 59 patients with RA and 61 healthy subjects at Qazvin University of Medical Sciences from July to December 2014. The project was approved in the local medical researches ethics committee of Qazvin University of Medical Sciences. The case group included the patients with RA referred to the rheumatology clinic at Qazvin University of Medical Sciences with medical records. The RA disease was approved by a single rheumatologist using the American College of Rheumatology/European League criteria (6). The demographics, time of onset of illness symptoms, morning stiffness, extra-articular manifestations, type of joint involvement, time of menopause, and results of laboratory medical tests (RF, Anti-CCP) were recorded in the medical profile of the patients. The inclusion criterion was diagnosis of RA in men and postmenopausal women. The exclusion criteria were thyroid disorders, polycystic ovarian syndrome in women, adrenal tumors, congenital adrenal hyperplasia, hypopituitarism, liver disease, alcohol use, and taking medications including androgens, steroids, anticonvulsants, barbiturates, and clomiphene, adrenocortical tumors. The control group subjects were selected among the healthy people referred to the rheumatology clinic, and were matched for age and gender with the case group. The goals of the project were provided to the subjects in both groups and written informed consent was obtained from them, respectively. A 5 mL non-fasting blood sample at 8-10 am was obtained from each subject in both groups. The level of serum free testosterone and DHEAs were measured after preparing and centrifuging the blood samples by electrochemiluminescence (ECL) equipment (Cobas B 411 analyzer, Roche, Germany). Based on the reference range of Roche kit, the serum testosterone level lower than 0.029 ng/ml in females and 2.49 ng/ml in males were considered as abnormal. Based on the reference range of Roche kit, the DHEAs levels lower than 18.9 $\mu\text{g/dl}$ in females and 88.9 $\mu\text{g/dl}$ in males were considered as abnormal. Data were explained by central and diffusion indices. The association between the qualitative variables was measured using independent sample T-test and the association between the qualitative variables was evaluated using Chi-square test. The independent association between the variables and RA was evaluated by logistic regression analysis. $P < 0.05$ was considered as level of significance. All analyses were performed using the SPSS software, version 19.

3. Results

Fifty-nine patients with RA and 61 healthy subjects were enrolled into the study. The mean age was 55.3±9.5 years in the case group and 52.8±11.8 years in the control group. Of the two groups, 44.1% of subjects in the case group and 39.3% of the subjects in the control group were male. Mean time of menopause in female subjects was 9.98±8.01 in the case group and 10.38±8.13 in the control group. In the case group, the mean time of onset of illness symptoms was 5.68±5.24 years and the mean time of morning stiffness was 1.15±0.68 hours. In the case group, 6.8% had extra-articular manifestations. Of 59 patients with RA, 2 (3.4%) had oligoarticular arthritis and 57 (96.6%) had polyarticular arthritis. The minimum, maximum and mean numbers of joints involved in RA were 1, 20 and 10, respectively. Mean RF was 99.53 IU/ml and mean anti-cyclic citrullinated peptide (Anti-CCP) was 432.24 U/ml in the case group. The level of RF in 91.5% of the patients was ≥20 and the level of Anti-CCP in 98.3% of the patients was ≥30. Demographic and biochemical characteristics of the study subjects are presented in Table 1. Mean serum testosterone level was not significantly different between the groups. But in subgroup analysis, mean serum testosterone level in females of the control group was significantly higher than in females of the case group. Mean serum DHEAs level in the control group was significantly higher than the case group in both genders. The prevalence of abnormal testosterone and DHEAs level in the study groups is shown in Table 2. The prevalence of abnormal testosterone and DHEAs in the case group was significantly higher than the control group. Logistic regression analysis of factors and rheumatoid arthritis is shown in Table 3. Logistic regression analysis showed independent and significant relation only between the levels of DHEAs and RA, after adjusting the effects of age and gender (OR: 0.966, 95% CI: 0.953-0.979; p<0.001).

Table 1. Demographic and biochemical characteristics of the study subjects

Variable		Case group (n=59)	Control group (n=61)	p-value
Age ^a (year)		55.3±9.5	52.8±11.8	0.208
Gender ^b	Male	26 (44.1)	24 (39.3)	0.711
	Female	33 (55.9)	37 (60.7)	
Testosterone (ng/ml) ^a	Male	3.9±1.3	3.8±1.6	0.923
	Female	0.06±0.18	0.15±0.10	0.016
	Total	1.76±2.1	1.62±2.1	0.0708
DHEAs (µg/dl) ^a	Male	64.8±44.5	145.5±85.2	<0.001
	Female	16.1±18.7	95.9±18.7	<0.001
	Total	37.6±40.5	115.4±79.5	<0.001

^a Data are presented as mean±SD; ^b Data are presented as number (percent). DHEAs: dehydroepiandrosterone sulfate.

Table 2. Prevalence of abnormal testosterone and *dehydroepiandrosterone* sulfate level in the study groups

	Case group (n=59)	Control group (n=61)	p-value
Abnormal Testosterone < 0.029 ng/ml for females < 2.49 ng/ml for males	33 (55.9)	14 (23.0)	<0.001
Abnormal DHEAs < 18.9 µg/dl for females < 88.9 µg/dl for males	44 (74.6)	8 (13.1)	<0.001

DHEAs: dehydroepiandrosterone sulfate. Data are presented as number (percent).

Table 3. Logistic regression analysis of factors and rheumatoid arthritis

	OR*	95% CI	p-value
Age	0.986	(0.942, 1.03)	0.551
Gender	0.283	(0.025, 3.19)	0.308
Testosterone	1.12	(0.62, 2.0)	0.710
DHEAs	0.966	(0.953, 0.979)	<0.001

*OR: Odds ratio

4. Discussion

Hypoandrogenism may play a role in RA and/or appear as a complication of chronic inflammatory reaction. The higher levels of testosterone in young males may somewhat play protective roles against RA (25). Clinical improvement after a successful treatment is followed by an increase in the level of serum testosterone in RA (26). It

should be noted that patients undergoing long-term treatment with glucocorticoids may experience hypoandrogenism (27). In a case-control study conducted in Sweden (28), out of 151 male cases with RA, the RF was only found in 73% of the cases; the level of serum testosterone in patients with RA was significantly lower than the control group; and abnormal serum testosterone level was significantly lower in patients with RA and positive RF. It means that hormonal changes may occur during the onset of RA and can affect the phenotype of the disease (28). There are few findings regarding the importance of androgen levels in the incidence of RA, especially in males. Lower levels of testosterone can result from primary gonadal dysfunction, and also may be due to dysfunction in hypothalamic-pituitary-gonadal axis, or be caused by an inflammation which led to lower secretion of testosterone (28). In a study by Pikwer et al., there was no significant difference regarding the serum indicators of inflammation or health status between the cases with early rheumatoid arthritis and those of the control subjects (28). Therefore, it cannot be concluded that secondary inflammation (ESR, CRP) in early rheumatoid arthritis is the main cause of differences between the groups. Lower levels of serum testosterone may be caused by RA or may indicate the role of androgens in the pathogenesis of RA. In a study by Dessein et al. in South Africa on 38 patients with RA, the activity of hypothalamic-pituitary-adrenal axis was reduced and DHEAs levels in females and males with RA was low (24). Another study in the USA by Masi et al. (29) compared 36 females with RA with 144 subjects in the control group and reported lower levels of adrenal androgens - DHEAs and androstenedione - in the females with RA. Tengstrand et al. (30) evaluated 41 patients with early RA and found that the mean level of testosterone in the patients was lower than the control subjects, and the inflammatory process of hypothalamic-pituitary-adrenal axis in RA had significant effects on gonads. Geenen R et al. pointed to the inflammatory process of hypothalamic-pituitary-adrenal axis in RA (31).

The prevalence of RA in males is lower than females and sexual hormones play a significant role in the pathogenesis of this disease. Males with RA may have lower levels of serum testosterone. Testosterone also has anti-inflammatory effects which can suppress the cellular and humoral immune systems (32, 33). On the other hand, the male sex hormone is considered as an independent predictive factor to treat early stages of RA; it can prove the hypothesis that the replacement of androgens can be useful in the treatment of RA (34). However, using testosterone to treat RA is under investigation and different results are reported in this regard (35). Results of the current study showed that serum testosterone levels in females with RA and DHEAs in postmenopausal females and males with RA were lower compared with the control group. Also, the current study showed that the normal level of DHEAs can have protective effects on RA. Considering that gonadal and adrenal androgens have anti-inflammatory effects which specifically reduce in inflammatory tissues, some studies showed that with lower activity of the disease resulted from treatment, the hypothalamic-pituitary-adrenal axis improved, which led to increase in the level of serum testosterone (36). It is noteworthy that lower levels of testosterone can cause osteoporosis; males with serum levels of testosterone lower than normal range should be treated with androgens (37).

5. Conclusions

The current study showed that abnormal testosterone and DHEAs level in patients with RA was significantly higher than the control group. After adjusting the effects of age and gender logistic regression analysis showed DHEAs has independent and significant association with RA. This shows the anti-inflammatory effects of gonadal and adrenal androgens in RA. On the other hand, lower activity of the disease resulted from treatment which led to an increase in the level of serum testosterone after improvement in hypothalamic-pituitary-adrenal axis. It may prove the hypothesis that the replacement of androgens can be useful in the treatment of RA.

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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