SEX HORMONES AND RHEUMATOID FACTOR: A POSSIBLE LINK BETWEEN REPRODUCTIVE HORMONES PATTERNS AND THE ONSET OF RHEUMATOID ARTHRITIS AMONG WOMEN IN KHARTOUM, SUDAN.

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ABSTRACT

Background: Sex hormones are believed to contribute to the risk of rheumatoid arthritis (RA) because of the disease's female preponderance, especially during the child-bearing years, and because of the dramatic improvements seen during pregnancy. **Objective:** To investigate the association between reproductive hormones, follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL) patterns and the risk of developing rheumatoid arthritis disease among females in Khartoum, Sudan. **Participants and methods:** The study involved a total of 76 healthy females during their fertile years and age between 18-45years. Reproductive hormones (FSH, LH and PRL) were obtained using the enzyme-immunoassay method by Cobas e411or Tosho Biosciences AIA 360 full automated analyzers. Then RF levels were examined using latex card qualitative method and those which were confirmed positive were further estimated using semi-quantitative method. **Results:** 11 (14.86%) females participants showed positive RF results. The risk of RA appeared to be increased among women with elevated reproductive hormones levels. In

which the positive RF women showed mostly increased hormonal levels when compared to RF negative women. 12 females participants who have had an increased reproductive hormones levels have completed a life style related questionnaire and the data revealed that most of them were living unhealthy life style including unhealthy food, lack of exercise and high caffeine consumption. **Conclusion**: Further researches are required to explore the biological mechanisms behind these findings, but our results contribute to the knowledge of hormonal/reproductive factors, and their impact on the RA development.

KEY WORDS: Sex hormones, reproductive hormones, rheumatoid arthritis (RA), rheumatoid factor (RF).

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease which leads to joint damage and bone destruction, with a complex interplay of genetic and environmental factors involved in its etiology.[1] There is an abundant evidence that rheumatoid arthritis is immune mediated and the pathogenesis of the disease is complex involving both the innate and adaptive immune system.[5] RA is more common among women than men at all ages, but the gender difference seems to be highest before menopause.

It has been hypothesized that changes in female hormonal levels might have a role in RA pathogenesis.[2] There is an epidemiologic, clinical, and experimental evidence implicating hormones in the incidence and clinical expression of rheumatoid arthritis.[3] Overall, women have a more than two fold higher incidence of RA than men. This is mainly due to an increased risk for women during their reproductive years, when the incidence shows a female/male ratio of 4-6:1 and the peak incidence of RA in women occurs after the menopausal age.[4]

Sex hormones are believed to contribute to the risk of RA because of the disease's female preponderance, especially during the child-bearing years, and because of the dramatic improvements seen during pregnancy.[6] Hypothesis of sex hormone contributions to RA are tested by judgmental criteria for the causes or determinants of disease. Available data support hypoandrogenicity in RA patients, especially among premenopausal females and males.[7] Reports about the effect of reproductive factors on the development of RA have also been inconclusive and several studies have shown a peak incidence of RA in premenopausal or

early postmenopausal women, suggesting an influence of premenopausal hormonal changes; however, results of studies are inconsistent.[8]

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There is an increasing body of evidence that lifestyle factors can impact on reproductive performance. Life style factors have had a dramatic impact on general health and the capacity to reproduce. Lifestyle issues such as smoking, obesity, caffeine intake, psychological stress, alcohol consumption as well as eating and nutritional disorder can affect general health and well-being.[10]

As the prevalence of rheumatoid arthritis disease is still increasing among women in Khartoum, this study is intended to investigate whether females reproductive hormones could be used as marker to predict the diagnosis and prognosis of individual patients with RA, in which early diagnosis followed by aggressive treatment directed at controlling the disease process, is critical for the best outcome therefore leading to an active life, and patients are less likely to experience the type of damage that requires joint replacement.

MATERIALS AND METHODS

Design and participants

A cross-sectional study was conducted in three different private hospitals in Khartoum, Sudan. The study involved a total of 76 women during their fertile years and between the age of (18-45 years).

Smoker females and those whom were previously diagnosed with any type of autoimmune diseases were excluded from the study.

This study was not sufficiently large enough and the small number of samples collected was due to lack of financial support.

Sample collection

Between 4–8 ml of venous blood was collected from each women, with most collections done between 9:00 a.m. and 9:00 p.m. The blood was collected into plain containers and

allowed to clot. Each sample was centrifuged at 1000 rpm for 10 minutes to achieve separation. The serum obtained was labeled and stored at –20°C.

Serum samples were analyzed initially for reproductive hormones analysis on either Cobas e411 (Roche Diagnostics, Australia) or Tosoh Biosciences AIA 360 (Japan), full automated analyzer. Three reproductive hormones follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin were measured.

Furthermore, all samples were subjected for rheumatoid factor (RF) detection by latex card technique.

Data collection

An identical questionnaire was given to only 12 females participants shortly after sample collection, in order to collect information on a broad range of environmental and life-style factors and informed consent was obtained from each women.

Rheumatoid factor testing

Latex technique (qualitative method)

50µl (approximately one drop) of each women serum was transferred into the test circle on the latex card using a commercially-purchased kit (BioSystems S.A., Spain), and the test was performed according to manufactured instructions. One drop of the latex reagent was added. Then the drops were mixed using disposable strrier and the test circle was covered by the mixture. Gently and evenly, the test card was rotated for 5 minutes. After that, agglutination was examined under a strong light source after 2 minutes. Positives samples which showed visible agglutination were further investigated by latex semi quantitative method.

Latex technique (semi quantitative method)

Using isotonic saline solution serial dilution of women positive samples was prepared (1/2, 1/4, 1/8, 1/16, 1/32, 1/64). 50 µl of each sample was transferred and one drop of the CRP latex reagent was added. The drops were mixed and gently rotated for 5 minutes. After that, agglutination was examined under a strong light source after 5 minutes.

Statistical analysis

Results are expressed as mean SD, and The Fisher's test of the two-tailed p value was used to measure the significance of association between groups and outcomes.

RESULTS

Table 1: Association between FSH hormonal levels and RF semi-quantitative result:

Sample ID	FSH hormone level	Rheumatoid factor semi quantitative result
1	12.2	1/2
2	5.5	1/2
3	9.0	1/2
4	19.1	1/4
5	157.4	1/4
6	13	1/32
7	4.6	1/2
8	7.4	1/16
9	7.9	1/2
10	6.8	1/4
11	30.9	1/4

normal range= 1.37-9.9 mIU/ml.
Mean SD 24.8 $\pm$ 44.6

P value= 0.02

In total, the study samples consisted of 76 females whom were included in the hormonal analysis and RF evaluation. Of those 69 samples were subjected for hormonal analysis using Tosho analyzer and the rest 7 samples were analyzed by Cobas machine. Although all of the study women were not previously diagnosed of any type of autoimmune diseases, 11 (14.86%) of them showed positive RF results by latex technique.

The risk of RA appeared to be increased among women with elevated levels of (FSH, LH and PRL) reproductive hormones. In which the positive RF samples analyzed by Tosho analyzer showed FSH levels within the range of 5.5 to 157 mIU/ml, (table 1), while LH levels were within the range of 0.9 to 30.9 mIU/ml (table 2.), whereas prolactin hormones were within the range of 4.9 to 128 uIU/ml (table 3). In the other hand there was no association revealed between RF titre by semi quantitative method and increased hormonal levels (table 1,2,3).

Calculation of the P values results of FSH hormones were considered to be statistically significant, (table 1) while LH were considered not statically significant, table (2) and PRL hormones showed statistically significance, (table 3).

Apart from that the negative RF samples analyzed by Tosho showed lower hormonal levels compared to the positive ones. In which FSH hormones levels ranged between 0.1 to 31.0

mIU/ml, while LH ranged between 1.1 to 18.8 mIU/ml and Prolactin showed a range of 2.1 to 128 uIU/ml.

All the remaining 7 samples analyzed by Cobas machine showed negative RF results and all hormones were within the normal reference ranges.

Table 2: Association between LH hormonal levels and RF semi-quantitative result:

Sample ID	LH hormone Level	Rheumatoid factor semi quantitative result
1	6.8	1/2
2	18.1	1/2
3	16.4	1/2
4	2.4	1/4
5	30.9	1/4
6	5.2	1/32
7	9.9	1/2
8	6.7	1/16
9	1.3	1/2
10	0.9	1/4
11	8.3	1/4

Normal range= 1.68-15 mIU/ml.

Mean SD 9.7 $\pm$ 8.9

P value= 0.3

Table 3: Association between PRL hormonal levels and RF semi-quantitative result:

Sample ID	Prolactin hormone level	Rheumatoid factor semi quantitative result
1	21.3	1/2
2	98.6	1/2
3	128.1	1/2
4	110.8	1/4
5	39	1/4
6	15.6	1/32
7	15.6	1/2
8	4.9	1/16
9	6.5	1/2
10	5.9	1/4
11	65.1	1/4

Normal range= 3.8-19.3 ng/ml.

Mean SD 46.4 $\pm$ 46.29

P value= 0.006

12 females participants whom had high reproductive hormones levels completed an extensive questionnaire, collecting information about life-style/environmental exposures which could affect hormonal levels. All females declared that they never exercise and unhealthy food with high carbohydrates uptake was the most type of food consumed. Also all of them pointed to the importance of drinking coffee, tea and soft drinks several times during their regular day and 75% reported that they are moderate consumers while 15% indicated that they are heavy caffeine consumers.

DISCUSSION

There is a growing support for the theory that an interaction between the immune and reproductive/endocrine systems underlies the pathogenesis of autoimmune rheumatoid diseases.[11,12] A variety of laboratory and clinical observations indicated that hormonal variations are associated with reproductive events may either be a risk factor for rheumatoid arthritis or modify disease activity.[12]

The hormone prolactin is known to have a number of pro-inflammatory actions and may play a role in the pathogenesis and persistence of several autoimmune diseases, including rheumatoid arthritis.[13] It has been previously stated by numerous studies the role of prolactin in autoimmune response of rheumatoid arthritis.[14,15,16] These reports agrees with the results of this study in which 6 (54%) of positive RF females had an elevated prolactin hormonal levels among them 4 (36%) had unexplained dramatic increased PRL levels [17,18] . In addition we reported that 3(27%) and 5(45%) of positive RF participants showed high LH and FSH hormonal levels respectively. There are, however, several studies which pointed to the fact that RA disease development is associated with hormonal imbalances of increased FSH and LH levels. This results confirm a previous study which revealed a significant associations between percentage changes in LH and FSH and percentage changes in key cytokines and several cross-sectional markers of disease activity may indicate that LH and FSH influence crucial points of the cytokine cascade in RA. This may help to explain, partially, why disease activity initiates or worsens during periods of increased LH and FSH, such as the postpartum period and the menopause.[19]

Lifestyle issues such as smoking, exercise, caffeine intake, diet and nutrition can affect general health and well-being.[20] In this study according to data collected from females with high reproductive hormonal levels they all declared that they never exercise and unhealthy food with high carbohydrates uptake was the most type of food consumed. These results are

consistent with previous studies which suggests that moderate regular exercise positively influences fertility and assisted reproductive outcomes.[20] Whereas high intensity exercise or law energy availability may lead to altering the endocrine pathway an menstrual cycle.[21] In the other hand in a recent study from Iran investigated the influence of dietary fat intake on oocyte competence and embryo quality and the study concluded that fat rich diet might induce the oxidative stress in oocyte environment and negatively influence embryonic development which agrees with other researches proven by other researches that the content of diet affects fertility capacity of a women by affecting ovulation.[22] It was also determined by previous researches that caffeine is thought to affect particularly female fertility and also it was determined that, with daily high caffeine consumption, the time for occurrence of pregnancy exceeds the normal time expected.[22]

CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest.

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