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Antimullerian Hormone Levels In Premenopausal Systemic Lupus Erythematosus Patients

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ABSTRACT

Background: The use of antimullerian hormone [AMH] to reflect the ovarian reserve help guide the treatment decisions, especially by medications with gonadotoxic effects. This study aimed to assess ovarian reserve by measuring AMH level in premenopausal SLE patients. M**ethods**: A case control study, included 60 female subjects [30 SLE patients and 30 apparently healthy subjects]. Full history taking and examination were carried out in Rheumatology and Rehabilitation Department. Laboratory investigations were conducted in Clinical Pathology Department, Faculty of Medicine, Zagazig University Hospitals. Antimullerian hormone level was measured in both patients and controls. **Results**: The study included 30 SLE female patients whose ages ranged from 19-50 years with a mean of 32.23 ± 8.846 years. The control group composed of 30 healthy female volunteers, whose ages ranged from 18 - 42 years and a mean of 29.03 ± 7.837 years. The mean of the disease duration was 6.87 ± 4.313 years. The study showed that the median of AMH level in SLE group was 2.7 [0.1-9.7] vs. 3.07 [0.3 – 20] in the control group, p value = 0.146 [P>0.05], therefore there was no statistically significant difference between both groups regarding AMH levels or between serum AMH and different drug use. **Conclusions:** AMH did not differ between SLE patients and controls & was not affected by the disease duration or activity. Moreover, immunosuppressive agents such as cyclophosphamide, azathioprine and mycophenolate mofetil did not significantly affect the AMH levels in SLE patients.

Keywords: Systemic lupus erythromatosus, Antimullerian hormone,Fertility.

INTRODUCTION

Systemic lupus erythromatosus [SLE] is an autoimmune disease that is characterized by autoantibody production, complement activation and and immune complex deposition leading to diverse clinical manifestations **[1]**. SLE mainly affects females in the reproductive age group, with dual negative effect on the fertility by the disease itself and by the medications used to treat it **[2]**. premature ovarian failure may occur due to autoimmune oophoritis **[3]**. The adverse effect of cyclophosphamide on the gonads [**4**] and high doses of corticosteroids,

have already been reported in the literature [**5**].

‘‘Ovarian reserve’’ is a term defined as the remaining functional capacity of the ovaries. It is used to reflect the number and the quality of the ovarian follicles **[6].** Antimullerian hormone [AMH] is one of the transforming growth factor-β [TGF-β] family, secreted by the granulosa cells of growing ovarian follicles, and its serum levels is considered to be a good marker of ovarian reserve **[7]**. AMH levels also do not change over the course of the menstrual cycle, which makes it more reliable than other measures of ovarian reserve **[8]**.

This study aimed to assess ovarian reserve in SLE patients by measuring AMH level compared with controls and to study the effect of different drugs on serum AMH.

METHODS

This study included 60 subjects, and was conducted in the Inpatient and Outpatient clinics of Rheumatology and Rehabilitation Department, Faculty of Medicine, Zagazig University Hospitals. AMH testing was done in the Clinical Pathology Departement's laboratories, Faculty of Medicine, Zagazig University Hospitals. Written informed consent was obtained from all participants and the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The work has been carried out in accordance with The Code of Ethics of the World Medical Association [Declaration of Helsinki] for studies involving humans.

Subjects were divided into two groups:

- Group I [SLE Group]: It included 30 female patients with SLE, fulfilled the SLICC revised criteria for classification [9]. Their ages ranged from 19–49 years. Exclusion criteria included: Patients with other autoimmune diseases and patients with previously diagnosed gynecological problems affecting fertility are excluded.
- Group II [Control Group]: It included 30 apparently healthy female volunteers. Their ages ranged from 18-49 years.

Analytical Methods

This was a case-control study. All patients were subjected to full history taking, thorough clinical examinationn, including measuring SLEDAI-2K score **[10]** and assessment of Systemic lupus International Collaborating Clinics/ American Collage of Rheumatology [SLICC/ACR] Damage Index for Systemic lupus erythromatosus **[11].** Laboratory investigations including CBC, ESR **[12]**, CRP **[13]**, liver & kidney function tests, protein/24 hour urine, ANA, Anti-ds DNA **[14]** were recorded. AMH testing **[15]** was done for both the patients and the controls. The AMH Gen II ELISA is an enzymatically amplified two-site immunoassay. In the assay, samples are incubated in microtitration wells, coated with anti-AMH antibody. Anti-AMH detection antibody labeled with biotin is added to each well. Finally streptavidin-horseradish peroxidase [HRP] is added to the wells.

Statistical Analysis

The data were coded, entered and processed on computer using SPSS [version 18]. The results were represented in tabular and diagrammatic forms then interpreted. Mean, standard deviation, range, frequency, and percentage were use as descriptive statistics. The following test was done: Student's t-test was used to assess the statistical significance of the difference between two population means. Mann Whitney U was used to assess the statistical significance of the difference between two population medians. Spearman’s correlation was used to correlate between AMH and different disease variables. P Value was considered significant as the following: $P > 0.05$: Non-significant. $P \leq 0.05$: Significant.

RESULTS

This study included 30 SLE female patients whose ages ranged from 19-50 years with a mean of 32.23 ± 8.846 years. The control group composed of 30 healthy female volunteers, whose ages ranged from 18 - 42 years with a mean of 29.03 ± 7.837 years. The mean of the disease duration was [6.87 ± 4.313] years. **Table [1]** shows the frequency distribution of clinical manifestations among

SLE patients where mucocutaneous [66.6%] and arthritis/arthralgia [66.6%] were the most frequent manifestations, followed by renal manifestations [36.6%] then hematological manifestations [33.3%], while neuropsychiatric [26.6%] and serositis [10%] were the least frequent ones . **Table [2]** shows no statistically significant difference amoung SLE and control groups regarding median values AMH [P>0.05], and the frequency distribution of abnormal values in SLE group was [6.7%] but it was [3.3%] in control group. **Table [3]** shows no statistically significant correlation between AMH level and other variables including age, BMI, disease duration, SLEDAI and Damage index [P>0.05]. **Table [4]** shows no significant difference between patients with SLE using or not using some medications [cyclophosphamide, azathioprine and mycophenolate mofotil] as regard to serum AMH levels. **Table [5]** shows no statistical significant correlation between serum level of AMH and the duration of usage of immunosuppressive therapies [cyclophosphamide, azathioprine and mycophenolate mofetil] and the cumulative doses among SLE patients.

Table 1. Clinical manifestations of studied SLE patients:

Variables	Number of Patients [n=30]	Percentage %
Mucocutaneous	20	66.67
Arthritis/Arthralgia	20	66.67
Serositis	3	10
Hematological	10	33.3
Renal	11	36.6
Vasculitis	4	13.33
Neuropsychiatric	3	10
Antiphospholipid	7	23.3

Table 2. Comparison between both groups regarding serum AMH levels:

	Group I [No= 30]	Group II [No= 30]	MW test	P. value
AMH *Median [Range]*	2.7 [0.1-9.7]	3.07 [0.3 – 20]	388.0	0.359
	SLE Group [No= 30]	**Control Group [No= 30]**	**X2**	**P**
Low AMH *No [%]*	2 [6.7%]	1[3.3%]	0.351	0.554
Normal AMH *No [%]*	28 [93.3%]	29 [96.7%]		

[Normal value:0.3-20 ng/mL]

Table 3. Correlation between Serum AMH and disease-related variables:

Variables	correlation with AMH	
	r	P
Age	0.443	0.07
BMI	0.390	0.08
Disease duration	0.031	0.140
SLEDAI score	0.16	0.68
Damage index	-0.254	0.094

BMI: Body Mass Index, SLEDAI Systemic Lupus Erythematosus Activity Index, AMH anti-mullerian hormone

Table 4. Comparison between AMH levels in relation to different drugs [Cyclophosphamide, Azathioprine and Mycophenolate Mofetil]:

	AMH *Median [Range]*	MW	P value
Azathiprine			
Non-Users [n=11] Users [n=19]	2.7 [0.1- 6.5] 2.7 [0.1- 9.7]	98	0.78
Mycophenolate Mofetil			
Non-Users [n=22] Users [n=8]	3.3 [0.1-9.7] 2.4 [0.6-3.5]	59	0.174
Cyclophosphamide			
Non-users [n=22] Users [n=8]	3.95 [0.25-9.7] 2.6 [0.1-6.5]	64.5	0.270

Table 5. Duration of immunosuppressive treatment and cumulative dose in studied SLE patients:

Variables	Mean ± SD	Correlation with AMH	
		r	P
Cyclophosphamide			
Duration [years] Cumulative dose [Grams]	1.36±0.71 8. 38 ±2.38	0.63 0.30	0.09 0.46
Azathioprine			
Duration [years] Cumulative dose [Grams]	4.6±2.4 3.2 ± 1.7	0.15 0.13	0.59 0.64
Mycophenolate mofeil			
Duration [years] Cumulative dose [Grams]	1.14±6.4 1120.2 ± 483.6	0.22 0.3	0.62 0.50

DISCUSSION

Recent studies of AMH levels have produced conflicting results regarding the reduction in ovarian reserve in women with SLE [**16**]. SLE activity may cause autoimmune reactions in the ovaries that may result in reduced ovarian reserve [**17**]. **Martins et al [18]** found AMH levels were decreased in 38.5% SLE female patients. Our study, in contrast, showed that the prevalence of low AMH [low ovarian reserve] was 6.7% **[18]**. In our study AMH levels were comparable between patients and controls [$P > 0.05$]. This agrees with **Di Mario et al [19]** who found AMH levels were comparable between patients and controls [4.2 ± 3.1 ng/ml vs. 5.0 ± 3.1 ng/ml, $p = 0.21$] **[19].** This agrees also with the study of

Gasparin et al [1] who aimed to investigate the ovarian reserve of patients with SLE by measuring AMH levels in 80 premenopausal SLE patients .They found mean AMH concentration was 22.79± 17.32 ng/ml in SLE patients and 21.41± 16.22 ng/ml in the control group and the difference between these values was insignificant [p = 0.71] **[1]**.

This study showed that, there were no statistically significant correlations between AMH and [SLEDAI score, activity score]. This is in agreement with **Di Mario et al [19]** who aimed to study the AMH serum levels in a cohort of SLE women patients in comparison with healthy controls and to assess whether the immunosuppresive medications or the disease activity may affect the ovarian reserve. They found that no association between AMH serum levels and SLEDAI score **[19]**.

Our study showed no significant difference between AMH value and damage index. **Di Mario et al [19]** also didn't find a significant difference in AMH level in patients with minor organ involvement. However lower AMH values in SLE patients with major organ involvement were detected in their study **[19]**.

There was no statistically significant difference in serum AMH levels in relation to different drugs [Cyc, AZA and MMF]. Moreover, this study showed that there was no significant correlation with the duration of use and cumulative doses of cyclophosphamide, azathioprine and MMF users. This disagrees with **Gasparin et al [1]** who found that AMH levels differed between patients who received cyclophosphamide compared to those using azathioprine and MMF **[1]**. Also, **Yang et al [20]** in his study on 45 SLE patients found that AMH level significantly decreased after 6 months of cyclophosphamide, but remained relatively the same after the same period of treatment with MMF and azathioprine **[20].** The association between AMH levels and exposure to cyclophosphamide and other immunosuppressants has also been previously studied in a Chinese cohort study of 216 patients with SLE,which found that 48 patients [22%] exposed to cyclophosphamide had lower mean AMH levels than the rest of the patients [1.58±2.92 versus 1.73±2.11 ng/ml], while exposure to other immunosuppressants [MMF 1.98 ± 2.5and AZA 1.62 ± 2.5] did not affect AMH levels [**19**]. But this agrees with **Velarde et al [16]** who did not find a correlation between AMH levels and the use of cyclophosphamide **[16]**. Moreover, the fact that multiple factors can contribute to changes in AMH levels must be considered; simply like smoking status **[21].** None of our cases were smokers as a cultural difference in our population compared to other populations.

This study showed that, there were statistically no significant correlations between AMH with BMI and age. **Gasparin et al [1] and Martins et al [18]** found no influence of BMI on the ovarian reserve of patients with SLE **[1, 18]**. Concurrently, some authors have found inverse associations between BMI and AMH levels **[22].** According to a recent study, the association between BMI and AMH may be affected by age, since both of these variables change over time **[7].** Moreover, **Di Mario et al [19]** found inverse correlation between current age and AMH serum concentration in SLE patients [$r = -0.43$; $p < 0.001$] **[19].**

CONCLUSIONS

We finally suggest that AMH, as a reflection of ovarian reserve, was not significantly different in our SLE patients. Moreover, it was not correlated with disease activity or damage indices. Immunosuppressive medications such as Cyc, AZA or MMF did not seem to significantly affect serum AMH levels in our SLE patients.

One of the limitations of our study was the lack of data regarding levels of AMH in SLE patients before administration of immunosuppressive drugs, to detect if it decreased from the baseline or remained

within the normal levels. Another limitation was the small sample size.

Declaration of interest
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.
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