Premature ovarian failure in systemic lupus erythematosus patients: is it related to cyclophosphamide treatment?

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Introduction
Systemic lupus erythematosus (SLE) is a chronic autoimmune systemic disease that mainly affects women during the childbearing period. Cyclophosphamide (CYC) is the drug of choice for severe SLE manifestations. However, many side effects had been reported. Premature ovarian failure (POF) is one of the serious complications that can occur in SLE patients.
Aim
The aim was to evaluate the prevalence of POF in female patients with SLE and whether it is related to CYC treatment or not.
Patients and methods
One hundred women with SLE satisfying the updated revised criteria for the classification of SLE were studied. The patients were allocated into two groups: CYC-treated group (n=55) and non-CYC-treated group (n=45). Patients were interviewed and demographic characteristics, clinical and serologic profiles, and menstrual histories were recorded. Disease activity was measured by the SLE disease activity index. Serum anti-Müllerian hormone was measured as a marker for ovarian reserve assessment in the two study groups.
Results
Ovarian failure occurred in 15 (27.3%) patients out of the 55 SLE patients treated with CYC. The cumulative CYC dose was significantly higher in patients with ovarian failure than in those without this condition (11.7 vs. 9.5 g; P=0.001). The cumulative dose of CYC and the older age at initiation were found to be associated more with POF.
Conclusion
In our population of female SLE patients, CYC-induced ovarian failure is a significant problem occurring in 27.3% of SLE patients receiving CYC. So, for SLE patients in whom the use of CYC is mandatory, a lower dosage and a shorter course of this agent should be considered. Co-treatment with gonadotropin-releasing hormone agonists might persevere the future fertility and ovarian function in young women. Ovarian banking before administration of CYC could be a possible solution in certain cases.

Keywords:
cyclophosphamide, intravenous cyclophosphamide, premature ovarian failure, systemic lupus erythematosus

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Introduction

Autoimmune diseases may selectively affect women in their reproductive years [1]. Systemic lupus erythematosus (SLE) is an autoimmune disease that affects most women of reproductive age [2]. It is characterized by deficiency of body's immune response that leads to the production of autoantibodies and failure of immune complex clearance [3]. Management of SLE is challenging due to the heterogeneous presentation and clinical manifestations of the disease [4].

Cyclophosphamide (CYC) is an alkylating agent that acts by transferring alkyl groups to biologically important cellular constituents [5]. CYC is a potent cytotoxic agent that has been reported to have important effects on the immune system, such as modulation of T-cell activation and inhibition of immunoglobulin production from B cells [6]. It remains the 'gold standard' treatment for severe organ-threatening SLE, especially renal and central nervous system lupus [7]. However, many side effects of long-term exposure to this drug had been reported including infection, bone marrow damage, malignancy, hemorrhagic cystitis, and premature ovarian failure (POF) [8].

POF is an ovarian defect characterized by the premature depletion of ovarian follicles before the age of 40 years [9]. POF is not merely an early menopause. Up to 50% of the patients with POF will have intermittent and unpredictable ovarian function which may persist for some years [10]. Moreover, POF is not only a problem of fertility, but also causes in the acceleration of arteriosclerosis and osteoporosis [11].

This study was therefore conducted to evaluate the prevalence of POF in female patients with SLE and whether it is related to CYC treatment or not.

Patients and methods

Patients

This was a cross-sectional study, conducted between May 2011 and April 2012. It included 100 female patients with SLE aged from 18 to 39 years; disease duration ranged from 1.5 to 12 years. The patients were diagnosed according to the American College of Rheumatology criteria [12]. All patients were either outpatients or inpatients of Rheumatology and Rehabilitation Department of El-Minia University Hospital, Egypt. The study was carried out with the approval of local ethics committee and in accordance with the national law and the Helsinki Declaration of 1975. Informed consent was obtained from all patients.

Exclusion criteria

Female patients who did not satisfy the American College of Rheumatology criteria, patients younger than 18 years or above 40 years, those with a family history of POF, SLE women with renal failure or on dialysis, patients with primary or secondary amenorrhea due to a known cause such as previous oophorectomy, pelvic irradiation, or hysterectomy. Current users of hormonal therapy and those in whom menopause had occurred before CYC treatment were also excluded from the study.

Data collection

The studied patients were allocated into two groups: CYC-treated group (n=55) and non-CYC-treated group (n=45). The following information was obtained from all patients: demographic data, age at onset of SLE, disease duration, autoantibody profile at presentation and clinical features during the course of the disease. For those who received CYC, age at the initiation of CYC and total cumulative doses received were calculated and recorded.

Systemic lupus erythematosus activity

Current SLE disease activity was measured using the SLE disease activity index (SLEDAI) [13]. The SLEDAI is scored on 24 items observed during the preceding 10 days. Among the various instruments developed for assessing lupus activity, the SLEDAI was chosen for its validity and relatively low cost to complete [14].

Treatment regimens of cyclophosphamide

The CYC studied group who received the standard induction regimen, which was monthly intravenous cyclophosphamide (IV-CYC) at 750 mg/m^2 body surface area for 6 months before being included in the study, followed by a maintenance regimen of quarterly infusions for 2 years. The duration of the IV-CYC courses was adjusted according to clinical response and adverse effects. The dosage of CYC was also adjusted according to white blood cell count and the regimen was modified when there is leukopenia, infection, gastrointestinal tract intolerance, or when there was noncompliance with the treatment regimen.

Interventions

All SLE patients included in the study were subjected to the following.

Routine investigations

Simple urinalysis, 24-h urinary albumin, complete blood picture, first hour erythrocyte sedimentation rate (Westergren), serum aspartate aminotransferase and alanine aminotransferase, serum creatinine and blood urea, antinuclear antibody (ANA) by immunofluorescence technique, anti-ds DNA by enzyme-linked immunosorbent assay (ELISA), and anticardiolipin antibodies by ELISA were done for all patients.

Assessment of ovarian function

(1) Detailed menstrual history.
(2) Anti-Müllerian hormone assay (AMH).

Detailed menstrual history of participants included

Age of menarche, duration of menses, cycle length, and reliable last menstrual period (premorbid, preinduction, during induction, and during maintenance of CYC therapy). In the current study, those who had lack of menses for more than 3 months are categorized as having secondary amenorrhea.

Details of anti-Müllerian hormone assay

We used the Human Müllerian Inhibiting Substance/AMH, MIS/AMH ELISA Kit (MIS/AMH Elisa® Immnotech, Bechman-Coulter, California, USA), catalog no: E0228hEIAabTM. A calculated cutoff level of less than or equal to 1.26 ng/ml AMH was the laboratory diagnosis of women developing ovarian failure [15].

Statistical analysis

Data were analyzed by the Statistical Package for the Social Sciences (SPSS, version 21; SPSS Inc., Chicago, Illinois, USA). Differences in frequencies were analyzed by χ^2-test test and Fisher's exact test as appropriate. Student's *t*-test was used to compare parametric data while the χ^2-test was used when the data were nonparametric. Associations between interval, ordinal, and dichotomous variables were tested by Pearson's product moment correlation coefficients (*r*). Two-tailed tests were used throughout, with statistical significance set at the conventional 95% level.

Results

Demographic data and clinical features

The mean age of SLE patients (*n*=100) was 31.2±5.7 (rang: 18–39 years); the mean age of onset was 25.7 ±4.7 (range: 16.5–34.5 years); and the mean duration of illness was 5.5±2.4 (range: 1.5–12 years). None of the studied patients were smokers or alcohol drinkers.

At the time of the study, 55 patients were treated with IV-CYC. High-dose corticosteroid (prednisolone 1 mg/kg/day) was administered concomitantly in all CYC-treated patients and the dose was gradually tapered to a maintenance dose of 5–10 mg/day after 8–12 weeks. Antimalarial treatment was also administered to this entire group of patients. On the other group of SLE patients who never received CYC (*n*=45), antimalarial treatment was in use by 41 patients, steroids were in use by 19 patients, azathioprine was in use by 11 patients while mycophenolate mofetil was used in only five cases. The main indications of CYC therapy is summarized in Table 1.

Comparison between cyclophosphamide-treated patients and noncyclophosphamide-treated patients

SLE patients who were on CYC treatment were significantly younger, had an earlier disease onset, a lower disease activity index ($P<0.001$) than SLE patients who had never been treated with CYC, but the difference was not statistically different between the two groups in disease duration, number of swollen joints, or articular index (Table 2).

Moreover, no significant differences were noted in various clinical manifestations of SLE in both groups, except that the CYC-treated group had a statistically higher frequency of renal affection (Table 3).

By comparing the different laboratory parameters between the two groups of SLE patient; only titer of anti-dsDNA was statistically higher in that group treated with CYC than the other group of SLE patients, while other laboratory parameters did not statistically differ. Meanwhile, the level of AMH was significantly lower in the CYC-treated group when compared with the other group who did not receive the CYC therapy (Table 4).

Cyclophosphamide-induced premature ovarian failure

In SLE patients receiving CYC therapy, 15 (27.3%) patients had prolonged amenorrhea with documented ovarian failure, while none of the patients who did not receive CYC therapy had developed this complication.

By comparing the ovarian failure group (*n*=15) and the menstruating group (*n*=40) within the CYC-treated SLE patients (*n*=55), we found that the SLE patients who developed POF were significantly older, had a later disease onset, a more cumulative dose of CYC than the menstruating SLE patients group (Table 5).

In CYC-treated SLE patients, the presence of POF was significantly positively correlated with age of the SLE patient, activity of SLE, age at initiation of CYC treatment ($P<0.05$), and was highly correlated with the

Table 1 Main indications of cyclophosphamide in systemic lupus erythematosus treated patients

	SLE patients (*n*=55)	
	Number	%
Lupus nephritis	48	87.3
CNS cerebritis	4	7.3
Both lupus nephritis and cerebritis	2	3.6
Lupus vasculitis	1	1.8

CNS, central nervous system.

Table 2 Comparison between demographic features and systemic lupus erythematosus disease-related parameters in cyclophosphamide-treated systemic lupus erythematosus patients versus noncyclophosphamide-treated group

	CYC-treated group (*n*=55) (mean±SD)	Non-CYC-treated group (*n*=45) (mean±SD)	*P*
Age (years)	29.3±5.5	33.5±5.2	0.001**
Age at onset (years)	24.2±4.5	27.6±4.5	0.001*
Disease duration (years)	5.1±2.1	5.9±2.7	0.07
Articular index	16.1±14.4	20.1±12.8	0.15
Number of swollen joints	2.9±2.8	4.3±3.1	0.03
SLEDAI	11.6±6.9	25.9±11.2	0.001*

CYC, cyclophosphamide; SLEDAI, systemic lupus erythematosus disease activity index. *P<0.05, significant difference. **P<0.01, significant difference.

Table 3 Frequency of different clinical manifestations in cyclophosphamide-treated systemic lupus erythematosus patients versus noncyclophosphamide-treated group

	CYC-treated group (*n*=55)		Non-CYC-treated group (*n*=45)		χ^2	$P^{\#}$
	Frequency	%	Frequency	%		
Arthritis						
Present (*n*=70)	35	35	39	39	7.44	0.01*
Absent (*n*=26)	20	20	6	6		
Malar rash						
Present (*n*=76)	44	44	32	32	2.04	0.21
Absent (*n*=20)	11	11	13	13		
Photosensitivity						
Present (*n*=64)	34	34	29	29	0.02	1.00
Absent (*n*=32)	21	21	16	16		
Oral ulcers						
Present (*n*=60)	35	35	29	29	0.04	1.00
Absent (*n*=36)	20	20	16	16		
Renal disease						
Present	50	50	17	17	30.8	0.001**
Absent	5	5	28	28		
CNS cerebritis						
Present	6	6	1	1	1.4	0.37
Absent	49	49	44	44		

CNS, central nervous system; CYC, cyclophosphamide. #Fisher's exact test. *$P<0.05$, significant difference. **$P<0.01$, significant difference.

Table 4 Comparison between laboratory parameters, autoantibody profiles in cyclophosphamide-treated systemic lupus erythematosus patients versus noncyclophosphamide-treated group

	CYC-treated group (*n*=55)	Non-CYC-treated group (*n*=45)	*t*	*P*
Hb (g%)	10.3±1.6	10.1±1.5	0.73	0.46
WBCs (thousands? mm^3)	4.98±1.85	5.79±2.50	−1.8	0.07
Platelets (thousands/ mm^3)	271.69±82.42	281.11±76.08	−0.57	0.56
ESR (mmHg/ first hour	58.75±22.48	65.52±27.16	−1.34	0.18
AST (U/l)	20.08±13.32	25.43±16.15	−1.75	0.08
ALT (U/l)	32.58±16.53	31.11±6.87	0.58	0.56
Urea (mg/dl)	39.06±16.55	38.07±16.52	0.29	0.77
Creatinine (mg/dl)	0.76±0.22	0.80±0.18	−0.91	0.36
Anti-dsDNA (IU/ml)	187.6±58.4	104.8±28.7	8.5	0.001**
aCL antibodies	11.2±2.3	4.2±1.4	1.8	0.07
AMH (ng/ml)	1.7±0.6	2.1±1.0	−2.9	0.005**

aCL, anticardiolipin; ALT, alanine aminotransferase; AMH, anti-Müllerian hormone; anti-dsDNA, anti-double stranded DNA; AST, aspartate aminotransferase; CYC, cyclophosphamide; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; WBCs, white blood cells. **P<0.01, significant difference.

cumulative dose of CYC ($P<0.005$). On the other hand, there was no significant association between POF and the presence of anti-ds DNA or anticardiolipin antibodies (Table 6).

Discussion

SLE remains a therapeutic challenge. One of the most successful therapies for severe SLE has been administration of monthly IV-CYC for 6 months, followed by quarterly maintenance infusions for 2 years (traditional/NIH IVCY regimen) [8,16,17]. Although it is a very useful drug, it has some serious side effects. Prolonged amenorrhea due to POF leading to infertility is one of the serious side effects of CYC [18]. Reports of ovarian failure related to CYC have varied between 12 and 83% [19–23].

The mechanism by which CYC can induce ovarian failure had been discussed in the literature as it causes progressive and irreversible damage to oocytes in a dose-dependent manner, thereby reducing the number of oocytes in ovaries. With high doses and longer duration, the oocyte number is reduced drastically resulting in POF and those who do not develop POF are at risk of developing premature menopause in future due to reduced oocyte reserve [24].

Among the different biomarkers which assess ovarian function, AMH has emerged as a sensitive indicator of impaired ovarian function; it represents the more sensitive endocrine marker to assess the age-related decline of reproductive capacity [25].

The goal of this study was to evaluate the prevalence of POF in female patients with SLE and whether it is related to CYC treatment or not.

Table 5 Comparison between ovarian failure group and menstruating group within cyclophosphamide- treated systemic lupus erythematosus patients (*n*=55)

	Ovarian failure patients (*n*=15) (mean±SD)	Menstruating patients (*n*=40) (mean±SD)	Difference	
			t	*P*
Age (years)	32.3±3.6	28.3±5.6	2.4	0.01*
Age at onset of SLE (years)	26.1±3.5	23.5±4.6	2.1	0.04*
Disease duration (years)	6.1±2.0	4.8±2.7	2.0	0.05
Disease activity (SLEDAI)	15.7±8.5	10.4±5.9	2.4	0.02
Age at initiation of CYC	29.2±3.5	25.7±4.7	-2.8	0.01*
Total cumulative CYC dose (g)	11.7±1.3	9.5±2.5	3.8	0.001**
AMH levels (ng/ml)	0.6±0.3	1.8±0.3	14.8	0.001**

AMH, anti-Müllerian hormone; CYC, cyclophosphamide; SLEDAI, systemic lupus erythematosus disease activity index. *$P<0.05$, significant difference. **$P<0.01$, significant difference.

Table 6 Correlation between premature ovarian failure and different systemic lupus erythematosus disease manifestations

	Premature ovarian failure	
	r	*P*
Age	0.33	0.02*
Age at onset	0.25	0.07
Duration of disease	0.24	0.09
Age at start of CYC treatment	0.32	0.02*
Cumulative dose of CYC	0.37	0.006**
SLEDAI	0.31	0.02*
Corticosteroids	0.22	0.12
Azathioprine	0.20	0.15
DNA	0.15	0.29
Anticardiolipin	0.29	0.03*
AMH	–0.90	0.001**

AMH, anti-Müllerian hormone; CYC, cyclophosphamide; SLEDAI, systemic lupus erythematosus disease activity index. *P<0.05, significant difference. **P<0.01, significant difference.

Our study included 100 Egyptian SLE patients satisfying the updated revised criteria for the classification of SLE [12]. Diagnosis of ovarian failure was based on menstrual history and AMH assay.

In the present study, POF was developed in 15 (27.3%) of the SLE patients receiving CYC therapy. In accordance with our results, there was a prevalence of 26% in a study of 70 premenopausal female patients who were treated with both oral and intravenous form of CYC [26]. Another one showed a prevalence of 24% in a study of 17 women with lupus nephritis who were treated with IV-CYC [22].

A higher incidence had been reported by other studies. In an earlier investigation by Warne *et al.* [20], who studied 20 female patients who had received oral CYC for an average of 19 months for the treatment of glomerulonephritis, 11 (55%) patients out of the 20 developed amenorrhea, which persisted during a follow-up period of 5–31 months after discontinuation of CYC. Another study by McDermott and Powell [27] had reported that the incidence of ovarian failure in the premenopausal CYC-treated group was 54%.

On the contrary, D'Cruz *et al.* [28] reported that none of his patients had developed any menstrual abnormalities with the intravenous CYC regimen. This was probably because the majority of his patients received no more than 2.5 g of CYC. In that study, weekly intravenous pulses of 500 mg was used for 3–5 weeks to achieve partial or complete remission for the treatment of lupus nephritis, followed by oral CYC 2 mg/kg/day or azathioprine 2 mg/kg/day. The authors commented that the risk of ovarian failure was markedly reduced with the use of this shorter regimen of CYC.

The main explanation of this variation of the prevalence rates might be due to the fact that most of the studies mentioned before were retrospective studies in which incomplete or unclear records may confound the results of this type of studies. Another important factor could be related to the discrepancy in the treatment protocols, differences in the route of administration of CYC whether oral form or intravenous form, and hence, the cumulative doses of CYC administered will differ.

Differences in the age of the patients recruited may play a role in this variation as the reserve of ovarian follicles appears to be an important key factor in the risk for ovarian failure related to CYC treatment [26]. In our study, POF was increased with increasing the age of the patients. Most studies [19,27] have consistently demonstrated a trend of increasing risk for ovarian failure with increasing age. Actually and under normal circumstances, the number of ovarian follicles decreases steadily with age until the last decade before menopause, when the number falls even more dramatically [29]. This explains our results that why patients who are younger at the start of CYC therapy are more resistant to CYC-induced ovarian damage.

In the present study, no one of our SLE patients who were never treated with CYC had POF. This could be explained by that the occurrence of ovarian failure in the CYC-treated group was due to the cytotoxic effect of CYC rather than due to the disease itself. Factors other than CYC which may influence the development of ovarian failure were also studied as none of our patients were smokers or alcohol drinkers, no association was found between POF and azathioprine or mycophenolate mofetil usage which is in agreement with the study by Silva *et al.* [30].

In the present study, POF was increased with a higher cumulative dose of CYC. Several studies [31–33] in the literature strongly support an association between the early occurrence of ovarian failure and the cumulative dose of CYC. Medeiros *et al.* [33] found his SLE patients treated with a cumulative CYC dose greater than 10 g had a 3.2 times higher risk of developing ovarian insufficiency than patients receiving a cumulative dose lower than 10 g. [33]. Mok *et al.* [26] found that the mean cumulative CYC dose was higher (28 g) in the group with ovarian failure, while it was 15 gm in the group without ovarian failure.Most of the studies mentioned before used serum follicle-stimulating hormone (FSH) as a marker of ovarian function as it has long been recognized and remains in very widespread clinical use. However, significant problems may arise with FSH measurement like marked intercycle variation and the need for blood sampling in the early follicular phase of the menstrual cycle [34]. On the contrary, AMH is a product of the granulosa cells of small growing follicles and its expression increases as soon as the follicle starts to grow and importantly falls to low levels at the early antral stage of development [35,36]. This means that serum AMH is much more stable over the menstrual cycle than other ovarian follicle hormones (inhibins A and B, estradiol) and FSH [37,38]; thus for practical purposes, blood sampling can be undertaken on any day of the cycle [34].

However, our study has some limitations. The first limitation of the study come from the point that some of the data related to the menstrual history like age at menarche, menstrual regularity, and duration of amenorrhea was relatively inaccurate as they relied upon participant recall. However, amenorrhea was not evaluated by only history as we depend mainly on serum AMH as a sensitive marker of POF. The second limitation was involved with some problems related to interpretation of AMH hormone levels as a marker of ovarian failure. This is because the test has not been in routine use for many years; the levels considered to be 'normal' are not yet clarified and agreed on by the experts. Longer follow-up is needed to reveal putative differences.

Conclusion

In conclusion, this study had characterized ovarian function in female patients with SLE assigned for CYC therapy using AMH as a sensitive marker. Our results showed that ovarian failure was a significant problem in this CYC-treated patient group, occurring in about 27.3% of the cases. In case of absence of CYC treatment, the prevalence of POF in SLE patients is consistent with the general population reports.

We recommend that for older patients who do not complete their families, in whom the use of CYC is warranted, a shorter course and lower dosage could be considered. Also, it is recommended for care givers for SLE patients to arrange for AMH assay before initiating CYC therapy as a better step toward counseling women at risk of ovarian failure. Modifying treatment protocols by suggesting azathioprine or mycophenolate mofetil as a cyclophosphamide CYC-sparing agent for long-term therapy. To preserve ovarian function, co-treatment with gonadotropin-releasing hormone analog may persevere the future fertility and ovarian function in women with severe lupus. Ovarian banking before administration of CYC should be considered in selected patients.

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Conflicts of interest

There are no conflicts of interest.

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