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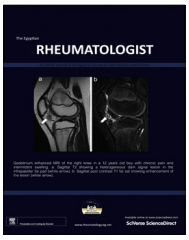

ORIGINAL ARTICLE

Does anti-DNA positivity increase the incidence of secondary antiphospholipid syndrome in lupus patients?

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KEYWORDS

SLE;
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Abstract *Aim of the work:* To detect the incidence of secondary antiphospholipid syndrome (APS) among Systemic lupus erythematosus (SLE) patients with positive anti-DNA antibodies.

Patients and methods: We studied 342 SLE patients; Group I: anti-DNA positive SLE patients ($n = 208$) and Group II: anti-DNA negative SLE patients ($n = 134$), with a female to male ratio of 9.39:1 and a mean age of 27.49 ± 7.94 years and disease duration of 5.74 ± 3.97 years. Full history taking, thorough clinical examination, laboratory and relevant radiological investigations were performed. Disease activity was assessed using systemic lupus erythematosus disease activity index (SLEDAI). Anti-dsDNA tests were carried out by indirect Immunofluorescence (IF) technique. Anti cardiolipin antibodies (IgG and IgM) and Anti-β_2 glycoprotein-I antibody of IgG and/or IgM isotype were detected by ELISA.

Results: The clinical manifestations, disease activity and laboratory investigations of the SLE patients varied according to the anti-DNA antibodies. Thirty-six patients (17.3%) had secondary APS in those with positive anti-DNA antibodies while only16 (11.9%) had secondary APS in those with negative anti-DNA antibodies, with no significant differences between both groups.

Conclusion: Apparent higher incidence of secondary APS was detected in anti-DNA positive SLE patients. The non significant differences between both groups may suggest that anti-DNA

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positivity cannot be considered as the only predictor of secondary APS and further studies may be needed to detect other factors which may increase the incidence of APS in SLE patients.

1. Introduction

Antiphospholipid syndrome (APS) comprises clinical features such as arterial or venous thromboses and the detection of so-called antiphospholipid antibodies (aPL) as anticardiolipin antibodies (aCL) or lupus anticoagulant (LA) [1]. Also patients with systemic lupus erythematosus (SLE) may form antibodies to a phospholipid-beta-2 glycoprotein I complex. Beta-2-glycoprotein I normally has an anticoagulant effect that is diminished by this antibody formation. This may explain why antiphospholipid antibodies are implicated in the etiology of the arterial and venous thromboses [2]. APS may be the most commonly acquired hypercoagulable state, occurring in up to 2% of the general population [1]. It is found in 20–35% of patients with SLE. The clinical course of the secondary syndrome is independent of the activity and severity of lupus, but the presence of APS worsens the prognosis of patients with lupus [3]. Antiphospholipid antibodies, like antinuclear antibodies (ANA), may be present prior to the diagnosis of SLE [2].

Antibodies to DNA (anti-DNA) occur in 50–70% of patients with SLE and are almost specific for this disease. They are very rarely found in healthy people, in patients with other diseases, or in the relatives of patients with SLE [4]. They are considered a serological hallmark of SLE, which is a prototypic autoimmune disease characterized by the production of antibodies to components of the cell nucleus (ANA) in association with diverse clinical manifestations [5]. Among these ANAs, anti-DNA antibodies serve as markers for diagnosis and prognosis and play an important role in immunopathogenesis via the formation of immune complexes [6]. A number of studies have shown that levels of anti-double stranded (dsDNA) antibodies tend to rise during flares of disease activity in SLE, particularly in lupus nephritis [7] and [8], even in some cases increases in their titer have been used as a guide to treat lupus patients with conventional therapy before flares are clinically apparent [9,10]. Moreover, a decrease in anti-dsDNA antibody titers has been associated with a clinical response to rituximab [11].

As lupus autoantibodies are correlated with the occurrence of specific clinical symptoms [12], according to the autoantibody profile of SLE patients, different sub-groups of SLE can be identified [13]. The aim of this study is to investigate if the presence of anti-DNA in Egyptian SLE patients is associated with a significant increase in the incidence of secondary antiphospholipid syndrome.

2. Patients and methods

The study, descriptive and cross-sectional, included 342 Egyptian SLE patients fulfilling the updated ACR revised criteria for the classification of SLE [14].

The patients were attending the Rheumatology department of Cairo University Hospitals during 2011. They were grouped according to anti-DNA positivity into Group I including anti-DNA positive patients and Group II the anti-DNA negative patients. All the patients had the disease duration more than 6 months at study entry. Full history taking, thorough clinical examination, laboratory and relevant radiological investigations were performed for all the patients. Disease activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). Antiphospholipid syndrome (APS) was diagnosed using Sapporo classification criteria [15].

The clinical manifestations are described based on the SLEDAI items. For all patients, serological examinations were made to discover the presence of:

(1) Anti-nuclear antibody and anti-ds DNA which were carried out by indirect Immunofluorescence (IIF) technique.
(2) Anti cardiolipin antibodies (IgG and IgM), Anti Ro (SSA), Anti La (SSB), and Anti-β_2 glycoprotein-I antibody of IgG and/or IgM isotype which were detected by ELISA.

The study has been approved by local ethics committee and it conforms to the standards currently applied in Cairo University Teaching Hospitals.

Statistics: The data were coded and entered using the statistical package SPSS version 15. The data were summarized using descriptive statistics: mean ± standard deviation (±SD), or frequencies (n) and percentages (%). Statistical differences between groups were tested using Chi Square test (v2) for qualitative variables, Independent sample t-test and ANOVA (analysis of variance) for quantitative normally distributed variables and nonparametric Mann–Whitney test and Kruskal–Wallis test for not normally distributed quantitative variables. Correlations were done to test for linear relations between variables. A probability value (p value) <0.05 was considered statistically significant.

3. Results

In the current study the ANA and anti DNA were positive in 329 (96.2%) and 208 (60.8%) of our patients respectively, Anti Ro was found to be positive in 149 patients (43.6%), Anti La in 53 patients (15.5%), while 56 patients (16.4%) showed positive antiphospholipid antibodies.

The SLE patients were divided according to the anti-DNA positivity into two groups; Group I: anti-DNA positive SLE patients (n = 208) and Group II: anti-DNA negative SLE patients (n = 134). The demographic features, disease activity and laboratory data of both groups are shown in Tables 1–4, respectively. There was no significant difference in these parameters according to DNA positivity except for SLE patients with nephritis in whom these parameters were significantly higher in anti-DNA positive patients (Group I) compared to those with negative anti-DNA (p = 0.000). Also,

Table 1 Demographic characteristics of the SLE patients (n = 342) with positive anti-DNA (Group I) and those with negative anti-DNA (Group II).

Feature	Group I (n = 208)	Group I (n = 134)
Sex male/female	172/36	118/16
Age (years) mean ± SD	26.71 ± 7.09	28.69 ± 9.01
Disease duration (Years) mean ± SD	5.58 ± 3.96	5.99 ± 3.99

there was no significant difference in the laboratory results according to anti-DNA antibody positivity, however the urine protein level was significantly higher in Group I (1.16 ± 1.18 g/24 h) compared to Group II SLE patients (0.69 ± 0.90 g/24 h) (p = 0.000). Particular interest was shown for the presence of secondary APS in SLE patients according to the presence of positive anti-DNA antibodies; 36 patients (17.3%) had secondary APS in those with positive anti-DNA antibodies while only 16 (11.9%) had secondary APS in those with negative anti-DNA antibodies. However, no significant differences were found between anti-DNA antibodies and the presence of secondary APS in both groups, p = NS (Table 5).

Table 4 Laboratory data of SLE patients with positive (Group I) and negative (Group II) anti-DNA antibodies.

Laboratory Investigation no. (%)	Group I (208)	Group II (134)
Hb (gm/dl)	11.16 ± 2.20	11.43 ± 1.82
WBC (dl)	8.10 ± 5.51	8.17 ± 6.21
PLT (dl)	280.42 ± 100.91	277.28 ± 83.86
ESR first (mm/h)	63.54 ± 56.36	52.89 ± 38.63
24 h Urinary protein (g)	1.16 ± 1.18	0.69 ± 0.90

Hb = Hemoglobin, WBC = White blood cell count, PLT = Platelet, ESR = Erythrocyte sedimentation rate.

Table 5 Comparison between presence of secondary antiphospholipid syndrome in both SLE groups with positive (Group I) and negative (Group II) anti-DNA antibodies.

Group	SLE patients (n = 342) no. (%)	p Value
Group I (208)		
−ve APS	172 (82.7)	0.217
+ve APS	36 (17.3)	
Group II (134)		
−ve APS	118 (88.1)	
+ve APS	16 (11.9)	

APS = antiphospholipid syndrome.

4. Discussion

One of the most distinctive laboratory features of SLE is the presence of autoantibodies to nuclear antigens including dsDNA [16]. Antibodies to DNA are highly specific for SLE and were reported to be found in 40–85% of the SLE patients in one study [10] and in 50–70% in another [4]. This is in agreement with the present results as the percentage of anti-DNA positivity was 60.8%. The relation between anti-dsDNA with lupus nephritis is documented in many studies [4,17,18], this finding is in line with the significant statistical difference found in the current study between Group I (DNA positive) and Group II (DNA negative) regarding lupus nephritis (p = 0.000) and also the significant statistical difference regarding 24 h urinary protein between Group I and II (p = 0.000).

Antiphospholipid antibodies are detected in approximately 1/3 of the SLE patients. High anticardiolipin antibody titers, lupus anticoagulant and especially anti-beta2 glycoprotein I antibodies are important predictors of APS [19]. The aPL predict early damage in patients with SLE disease and add to its burden [20], including the renal involvement [21]. *Loizou* et al. [22] found that raised levels of aCL were associated with lupus nephritis (LN) but were unable to show an association with anti-b2-glycoprotein I and did not look for the presence of Lupus anticoagulant (LA). Moreover, they found that the presence of aCL in conjunction with raised levels of anti-dsDNA and anti-C1q antibodies is highly specific for LN [16]. *Alba* et al. found that only the presence of LA was a significantly independent factor for the development of nephritis.

Table 2 Disease activity index of the SLE patients with positive (Group I) and negative (Group II) anti-DNA antibodies.

p Value	Group II (n = 134)	Group I (n = 208)	Disease activity mean ± SD
0.361	18.99 ± 9.93	17.76 ± 9.12	SLEDAI

SLEDAI = systemic lupus erythematosus disease activity index.

Table 3 Clinical manifestations of SLE patients with positive (Group I) and negative (Group II) anti-DNA antibodies.

Clinical manifestations no. (%)	All patients (342)	Group I (208)	Group II (134)
Constitutional	194 (56.7)	118 (56.7)	76 (56.7)
Mucocutaneous	198 (57.9)	113 (54.3)	85 (63.4)
Arthritis	263 (76.9)	160 (76.9)	103(76.9)
Renal	196 (57.3)	165 (79.3)	31 (23.1)
Neuropsychiatric	125 (36.5)	70 (33.7)	55 (41.0)
Pericarditis	58 (14.23)	36 (17.3)	22 (16.4)
Pleurisy	181 (52.9)	107(51.4)	74 (55.2)

The presence of LA has been associated with certain clinical features, in particular, a predisposition to venous and arterial thrombotic vascular disorders in multiple organ systems. The thrombotic effects may also extend to the renal circulation, resulting in renal thrombotic microangiopathy or renal artery stenosis [23].

The link between Anti DNA positivity and lupus nephritis, and the association between the Anti DNA positivity and SLE severity encouraged us in this study to investigate if this positivity was associated with increased incidence of secondary APS in Egyptian SLE patients. Our results showed that APS was found in 15.2% of our patients, which is not far away from what was reported by Amigo and Khamashta 2000 [3], with a higher incidence in the DNA positive group (17.3%) compared to the negative group (11.9%), however the difference between the two groups was statistically non significant.

The higher incidence of secondary APS detected in anti-DNA positive SLE patients in this study is in line with what was noted regarding the impact of anti-dsDNA as a useful tool for the diagnosis and the prognosis of SLE [24], however the non significant difference between the two groups in the present study may suggest that although DNA may be associated with a higher incidence of secondary APS, it cannot be considered as the main predictor of APS in SLE patients.

In conclusion, an apparently higher incidence of secondary APS was detected in anti-DNA positive SLE patients. The non significant differences between both groups may suggest that Anti-DNA positivity cannot be considered as the only predictor of secondary APS and further studies may be needed to detect other factors which may increase the incidence of APS in SLE patients. Further studies including a higher number of patients will be needed to study impact of anti DNA and other lupus parameters as predictor of secondary APS in SLE.

Conflict of interest

None.

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