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Original article

Clinical significance of anti-Scl-70 antibody estimation in pediatric patients with systemic lupus erythematosus.

***Background**: Some patients with scleroderma have overlap features with systemic lupus erythematosus (SLE). Anti-Scl-70 antibody was reported in as many as 35% of patients with scleroderma and signifies an increased risk for the development of pulmonary fibrosis and pulmonary hypertension. This antibody was detected in 25% of adult patients with SLE.*
***Objective**: We hypothesized that a particular subset of lupus patients might be at an increased risk of certain complications if anti-Scl-70 antibody was elevated in their sera.*
***Methods**: Serum anti-Scl-70 antibody levels were assayed by ELISA from 34 pediatric patients with SLE and from 24 healthy controls. Patients were also subjected to clinical evaluation for system involvement and for disease activity by systemic lupus erythematosus disease activity index (SLEDAI). The ESR, serum anti DNA antibody, serum complement 3, creatinine clearance and 24 hours urinary protein excretion were assessed and renal biopsy for histopathology was performed in selected cases.*
***Results**: Anti Scl-70 antibody was elevated (> 25 U/ml) in 6 SLE patients (18%), borderline (15-25 U/ml) in 17 patients (50%) and normal (< 15 U/ml) in 11 of them (32%). The patient group had significant elevation of serum anti-Scl-70 antibody levels (mean [SD] = 32.5 [8.8] U/ml) as compared to the controls' (mean [SD]= 9.3 [4.1] U/ml; p< 0.001). Patients with clinical lupus nephritis had significant higher levels of this autoantibody as compared to those without clinically evident renal involvement. Also, pulmonary hypertension was found significantly related to the high serum levels of anti-Scl-70 antibody. A significant positive correlation could link anti-Scl-70 levels to the ESR values and SLEDAI scores. Anti-Scl-70 levels were neither affected by the presence of neuropsychiatric involvement nor by intake of cytotoxic drugs.*
***Conclusion**: Anti-Scl-70 antibody is present in a significant subset of patients with SLE. For this subset, it offers a good correlate of disease activity and suggests an increased risk for pulmonary hypertension and renal involvement.*

Key words: SLE; anti-Scl-70; pulmonary hypertension; lupus nephritis; SLEDAI.

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INTRODUCTION

Systemic lupus erythematosus (SLE), a rheumatic disease of unknown cause, is characterized by autoantibodies directed against self antigens and resulting in inflammatory damage to target organs[1]. Scleroderma is a connective tissue disease that causes fibrosis and vascular abnormalities. Some patients with scleroderma have overlap features with other autoimmune conditions as systemic lupus erythematosus. Antibodies that occur in scleroderma that were thought to be specific are present in other connective tissue diseases. For instance, anti-Scl-70 antibody is reported in as many as 35% of patients with scleroderma and signifies an increased risk for the development of pulmonary fibrosis and pulmonary hypertension. This antibody can be present in 25% of patients with SLE [2].

We sought to investigate whether a subset of pediatric lupus patients would be at increased risk for certain complications if the anti-Scl-70 antibody was elevated in their sera. If those patients could be identified, they may be offered careful monitoring and treatment at an early stage.

METHODS

This case control cross-sectional study took place in the Pediatric Allergy and Immunology Unit of the

Children's Hospital of Ain Shams University, Cairo, Egypt.

Study population:

The study comprised 34 children and adolescents with SLE and 24 age and sex matched healthy subjects for comparison. Subjects were enrolled consecutively as every SLE patient presented to the clinic during the study period. An informed consent was obtained from their parents or care-givers before enrollment. Their demographic data are as follows:

A) Patients:

The patients were 34 children and adolescents with SLE (32 females and 2 males). SLE was considered to be present if four of the 11 criteria of the American College of Rheumatology (ACR)[3] were fulfilled. Their ages ranged from 6.0 to 18.0 years with a mean value of 13.6 ± 3.0 years. The disease duration ranged from 1.0 to 10.0 years with a mean value of 5.2 ± 2.5 years. All patients were receiving corticosteroids in the form of prednisone (1-2 mg/Kg/day), either alone (n = 18), or in combination with other immunosuppressive drugs (n=16) in the form of intravenous cyclophosphamide (600 mg/m^2/month), or azathioprine (2 mg/Kg/day). Ten patients were receiving chloroquine (200 mg/day), while 15 patients were on ipobrufen (20-40mg/kg/day).

B) Controls:

These were 22 females and 2 males with an age range of 6.0 – 18.0 years (mean ± SD = 13.9 ± 3.2 years). Exclusion criteria were the presence of personal or family history of rheumatological diseases, chronic illness, or weights or heights below the 5th or above the 95th percentiles for age [4].

Clinical Methods:

Evaluation was performed by detailed history through personal interview with patients and their parents besides the clinical examination and laboratory results obtained at the time of the study, aided by the medical records of the Ain Shams' Pediatric Allergy and Immunology Outpatient Clinic. Activity of SLE was assessed by the SLE Disease Activity Index (SLEDAI)[5]. The disease was considered active in 30 patients (SLEDAI > 8)[6]. Lupus nephritis was diagnosed in 20 patients by the presence of one or more of the following: protein in urine ≥ 0.2 gm/24 hrs, hematuria, pyuria, urinary casts and/or abnormal creatinine clearance. Renal biopsy was done for 15 of them and revealed World Health Organization (WHO) class II b (pure mesangiopathy) in 6 patients, class III (segmental and focal proliferative) in 3 patients, and class IV (diffuse proliferative) in 4 patients, while 2 patients suffered class V (diffuse membranous) lupus nephritis. Neuropsychiatric SLE was diagnosed (n=8) when a significant change in the baseline neurological and/or psychiatric function was identified by history and physical examination. Evidence of cutaneous vasculitis was recorded in 3 patients by the presence of any of the following: purpura, ulcers, subcutaneous nodules or livedo reticularis. System involvement was recorded according to the definitions of the American College of Rheumatology. [7-9]

Radiological Methods:

Plain X ray chest in the posteroanterior view and echocardiography (Esaote S.P.A. model 7250 Florence, Italy) was done for all patients in the Pediatric Radiology Lab of the Children's Hospital, Ain Shams University.

Laboratory Methods:

Blood sample collection

Five ml of blood were collected by venepuncture under complete aseptic conditions and were used as follows:

a) Two ml were added to EDTA as anticoagulant for ESR estimation.

b) Three ml were left to clot and the serum was obtained by flicking off after centrifugation for 15 minutes at 3000 rpm.

c) Part of the serum of each sample was transferred to a clean polystyrene tube which was stored at –20^{O}C till time of assay of anti Scl-70 antibody.

d) The rest of the serum was used for assessment of serum creatinine, ANA, anti-DNA and serum complement 3 (C3).

Laboratory investigation details:

1) The erythrocyte sedimentation rate was measured by Westergren method after dilution of the EDTA blood sample with the standard 10^9mmol/L (32 g/L) tri-sodium citrate in a ratio of 4 parts blood to 1 part citrate. Readings were obtained at room temperature (18-25^{o}C) at 1 and 2 hours interval.

2) Serum ANA and anti-DNA were assessed by indirect immunofluorescent microscopy (IMMCO Diagnostics, USA).

3) Serum C3 was estimated by turbidimetry (Turbiquant C3, Behringwerke Diagnostics - Marburg, Germany).

4) Serum and urinary creatinine and urinary proteins/24hs were measured by Synchron CX7 autoanalyzer (Beckman Instuments, Brea, California, USA).

5) Complete microscopic urine analysis for WBCs, RBCs, and casts was carried out.
6) Assessment of anti Scl-70 antibody by ELISA[10]: this was carried out by using a commercially available indirect solid phase enzyme immunoassay kit (Orgentec Diagnostika GmbH /Germany). The kit contains combined calibrators with IgG class anti-Scl-70 antibodies. The averaged optical density of each calibrator was plotted versus the concentration by Lin-Log Graph paper. The best fitting curve was drawn approximating the path of all calibrator points. The calibrator points were connected with straight line segments. The concentration of unknowns was estimated from the calibration curve by interpolation. The following ranges for interpretation of the results of anti-Scl-70 (U/ml) were used according to manufacturer's instructions [normal: < 15, borderline: 15-25 and elevated > 25 (U/ml)].

Statistical Analysis:
The results were statistically analyzed via a standard computer program (StatView). The quantitative variables were presented as mean, SD and range. Qualitative variables were described as numbers and %. Chi-Square test was used to compare qualitative variables. Unpaired t-test was used for inter-group analysis, and the correlation coefficient (r) for intra-group analysis. Stepwise regression analysis or multivariate analysis was done to find out the most significant independent predictors of anti-Scl-70 as a dependant variable. p values < 0.05 were considered significant.

RESULTS

Analysis of the SLE patients' data revealed that SLEDAI scores of the patients ranged from 4 to 56 with mean value of 31.6 ±15.9. The ESR values in the first hour ranged from 10 to 82 mm with a mean value of 36.7 ± 24.5 mm. The C3 values ranged from 24 to 101 mg/dl with mean value of 60.3 ± 20.9 mg/dl. The anti-DNA was positive in 29 (93%) patients. Lupus nephritis was detected in 20 patients (59%). Eight patients had CNS involvement (24%) and pulmonary hypertension was detected in 5 patients (15%). Characteristics of the patients and markers of disease activity are displayed in table (1).

Analysis of anti Scl-70 antibody results:
The anti-Scl-70 antibody serum levels ranged from 10 to 38 U/ml with a mean value of 32.5 ± 8.8 U/ml which is significantly elevated than the control mean (p< 0.001) (Table 2). The anti Scl-70 antibody was actually elevated (> 25 U/ml) in 6 patients (18%), borderline (15-25 U/ml) in 17 patients (50%) and normal (< 15 U/ml) in 11 (32%) of them (Figure 1) while it was normal in all subjects in the control group.

Table 1. Some clinical and laboratory data of the SLE patients.

Age (years)	
Range	6-18
mean ± SD	13.6 ± 3.0
Gender: male/female	2/32
Disease duration (years)	
mean ± SD	5.2 ± 2.5
SLEDAI scores	
Range	4-56
mean ± SD	31.6±15.9
Patients receiving cytotoxic drugs	
Number(%)	8 (40%)
Lupus nephritis	
Number (%)	20 (59%)
Pulmonary hypertension	5(15%)
Number (%)	
CNS disease	
Number (%)	8(24%)
Markers of disease activity:	
ESR (mm)	
Range	10-82
Mean ± SD	36.7±24.5
C3 level (mg/dl)	
Range	24 -101
Mean ± SD	60.3±20.9
Consumed{number(%)of patients}	15(45%)
Anti-DNA positivity	
{number(%)of patients}	29(93%)

Anti-DNA= anti-deoxyribonucleic acid; C3 = complement 3; CNS = central nervous system; ESR = erythrocyte sedimentation rate; SD = standard deviation; SLEDAI = systemic lupus erythematosus disease activity index.

Table 2. Anti Scl-70 antibodies in the cases and controls.

	Patients n=34	**Controls n=24**	**t**	**p**
	Mean±SD			
Anti-Scl-70 (U/ml)	32.5 ± 8.8	9.3 ± 4.1	9.4	< 0.001
p < 0.001 = highly significant				

Relation between anti Scl-70 and pulmonary hypertension:
Five SLE patients had pulmonary hypertension. Three (60%) of the patients with pulmonary hypertension had elevated levels of anti-Scl-70 antibody, while the remaining 2 (40%) had

borderline levels of this antibody. No pulmonary hypertension was detected among patients with normal levels of anti-Scl-70 antibody. Patients with elevated or borderline levels of anti Scl 70 had significantly higher occurrence of pulmonary hypertension than those with normal serum levels of this antibody (Figure 2).

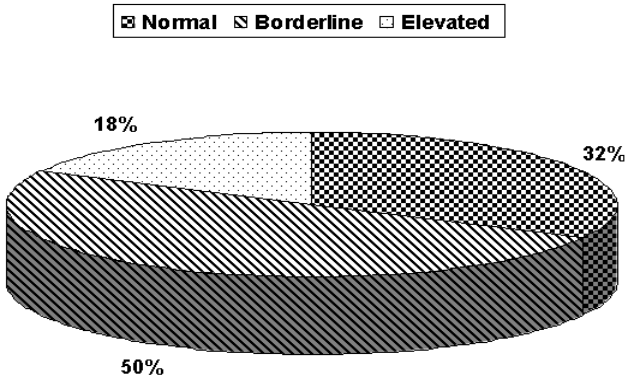

Figure 1. Anti-Scl-70 among patients with SLE.

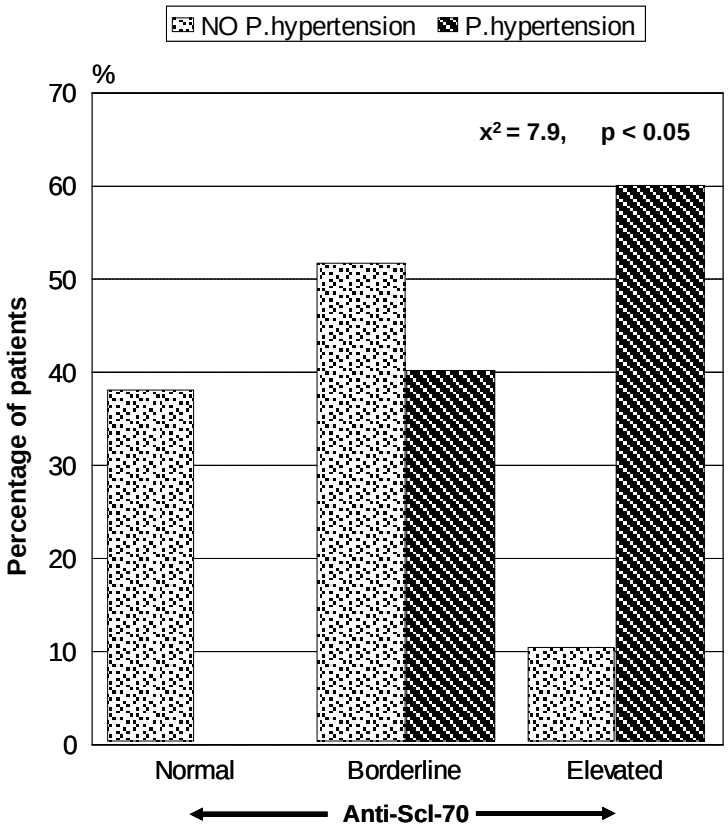

Figure 2. Relation between anti-Scl-70 and pulmonary hypertension.

Relation between anti Scl-70 and lupus nephritis:
Only two of lupus nephritis patients had normal serum levels of anti-Scl-70 (10%), while 13 (65%) and 5 (25%) of them had borderline and elevated levels of this antibody respectively (table 3).

Table 3. Relation between anti-Scl-70 and lupus nephritis

Anti-Scl-70	Lupus nephritis	
	No	**Yes**
Normal	9(64.3%)	2 (10%)
Borderline	4 (28.6%)	13 (65%)
Elevated	1 (7.1%)	5 (25%)
Significance	$x^2 = 11.2$	p< 0.001

p < 0.001 = highly significant

Analysis of the results according to the clinical subgroups of SLE patients:
No significant difference was found between patients with neuropsychiatric lupus and those without CNS disease as far as the serum levels of anti-Scl-70 antibody were concerned ($x^2 = 0.72$, p> 0.05). Also, the results of anti Scl-70 antibody did not vary with anti–DNA positivity ($x^2 = 2.5$; p> 0.05). Again, the results were comparable among patients who received steroids and patients on combined steroids and cytotoxic drugs ($x^2 = 0.81$; p > 0.05).

Analysis of anti-Scl-70 levels in relation to other parameters:
The serum levels of anti-Scl-70 correlated positively to the SLEDAI scores of the patients (r = 0.56, p< 0.001) (Figure 3). A significant positive correlation was also found between the levels of anti-Scl-70 and ESR results (r = 0.35, p< 0.05). On the other hand, no significant correlation could be elicited between anti-Scl-70 and age, disease duration or serum levels of C3.

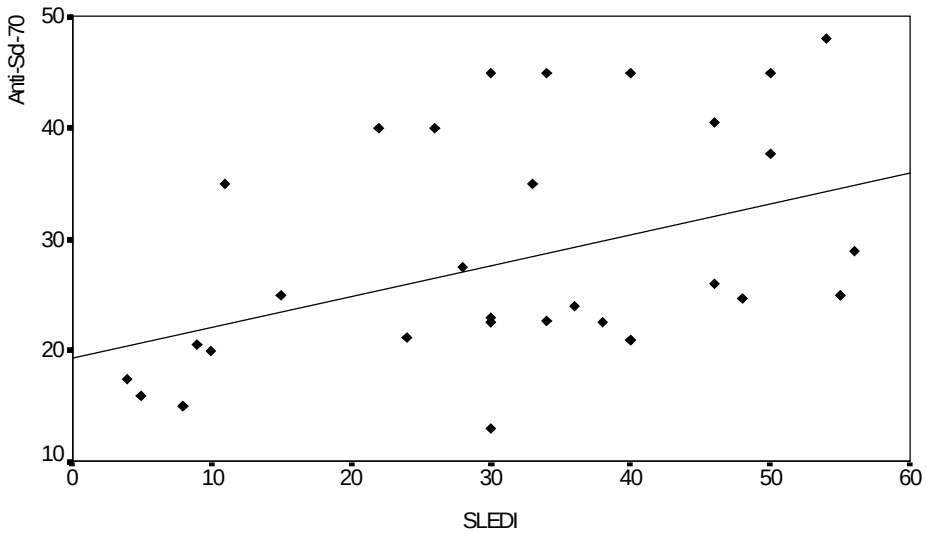

Figure 3. Correlation between serum anti-Scl-70 and SLEDAI scores of the patients.

DISCUSSION

The results revealed that serum anti-Scl-70 antibody levels were significantly elevated in 34 subjects with SLE (mean [SD] = 32.5 [8.8] U/ml) as compared to a group of age and sex matched healthy subjects (mean [SD] = 9.3 [4.1]) U/ml; p< 0.001). Our data on anti-Scl-70 estimation using the ELISA technique come in accordance with previous studies that detected anti-Scl-70 more frequently in SLE and rheumatoid arthritis as compared to healthy controls using the indirect immunofluorescence technique.[11]

In our series, anti-Scl-70 was elevated in 18% of 34 patients with SLE which is comparable to data from a hospital – based study in which anti-Scl-70 was elevated in 13% of 31 patients with SLE[12]. Another investigation revealed that 25% of

SLE patients were positive for anti-Scl-70 antibody.[13]

Some investigators considered anti Scl-70 as a predictive marker for possible future development of scleroderma in SLE patients.[14] They described one SLE patient with positive anti-Scl-70 from the onset of the disease, who developed scleroderma and pulmonary fibrosis after 12 years of follow up. Others concluded that anti-Scl-70 is possibly one of the encountered autoantibodies in patients with SLE without features of, or risk to develop scleroderma.[15] Such assumption needs long term wide scale studies to verify.

The lung is involved in 5-77% of children with SLE. Pulmonary involvement includes pleuritis, pneumonitis, pulmonary hemorrhage and pulmonary hypertension [16]. Anti-Scl-70 antibody (unlike anticentromere antibody) was found to identify systemic sclerosis patients at high risk to develop interstitial pulmonary disease secondary to vascular pathology [17] and pulmonary hypertension.[2] We report that pediatric SLE patients with pulmonary hypertension (15%) in our series had significant higher levels of anti-Scl-70 antibodies. Similar findings were reported in two previous studies on SLE adults [13,16]. A third investigation concluded that patients with SLE and positive anti-Scl-70 antibody often develop pulmonary interstitial fibrosis and peripheral vascular disease[18].

Lupus nephritis is one of the main clinical features in up to 80% of pediatric SLE patients and it usually determines the course and outcome of the disease [19]. Lupus nephritis was detected in 59% of our series. Patients with lupus nephritis had significantly higher levels of anti-Scl-70 antibody as compared to those without clinically evident renal involvement. Such finding is supported by a former report that SLE adults with positive anti Scl 70 antibody had significantly more frequent renal involvement than patients who were negative for this antibody [13].

Central nervous system disease in SLE is driven by cross-talk between the peripheral immune system and the brain's immune system, which leads to overproduction of brain cytokines and ultimate tissue injury[20]**.** In the current study, patients with neuropsychiatric lupus (24%) had comparable levels of anti-Scl-70 antibody to those without CNS disease. The only cited publication relevant to this observation was that of 3 cases with anti-Scl-70 antibody and central nervous system lupus without renal affection[21]**.** Wider scale studies are needed to investigate this relation.

A major problem in the management of SLE patients is to assess disease activity. Widely used serological markers are sometimes normal while the patient exhibits obvious disease related signs and symptoms. In the current study, anti-Scl-70 antibody was significantly correlated with disease activity as assessed by the SLEDAI score and ESR ($p<0.001$). This finding is limited by the relatively imprecise nature of retrospective clinical data collection used for calculation of the SLEDAI scores. However, our observation is supported by a previous report on the presence of a highly significant correlation between systemic lupus activity measure (SLAM) scores and serum levels of anti Scl-70 antibody in 128 SLE patients, as well as a significant correlation between serum levels of this autoantibody and ESR of the patients[13]. Also, a study which explored the value of the routine measurement of a panel of 8 nuclear autoantibodies in139 patients with SLE revealed that autoantibody levels of anti-ssDNA, ds-DNA and Scl 70 were the best individual correlates to SLAM scores of the patients [22].

The "gold standard" serologic test to measure disease activity in SLE is the anti-dsDNA antibody test, which has been used as a marker of disease activity by clinicians in SLE for over 35 years. Anti-dsDNA antibodies perform best in those with lupus nephritis, especially in the presence of a proliferative lesion (WHO class III or IV) in renal biopsy. In one recent meta-analysis, the overall predictive value of anti-dsDNA as a marker of disease activity was small[23]. In our series, patients with positive anti-dsDNA had comparable levels of anti-Scl-70 antibody to those with negative anti-dsDNA. This finding was unlike the previous reports of a significant correlation between levels of anti-Scl-70 and anti-dsDNA. [13] This discrepancy may be related to our smaller sample size (34 patients) compared to their series (134 patients) or could be age-related as the mentioned study included only adults. Also, they measured the anti-dsDNA levels quantitatively which offers a better correlate to disease activity. Another explanation is the smaller number of patients with lupus nephritis in our sample taking in consideration that anti-DNA testing performs best when the kidney is involved.

Complement may have a deleterious role in the pathogenesis of SLE. Patients with SLE present with decreased circulating complement level and complement deposition in inflamed tissues, suggestive of a harmful role of complement in the effector phase of disease [24]**.** In our series, we could not establish a link between levels of anti-Scl-70 and those of C3. We also, did not find an effect of different treatment regimens on the serum levels of

anti-Scl-70. The findings are probably limited by the sample size.

Taken together, anti-Scl-70 antibody may provide a good correlate of SLE activity and suggests an increased risk of pulmonary and renal involvement. SLE patients with positive anti-Scl-70 antibody should be monitored for development of pulmonary hypertension and lupus nephritis, so that early intervention can be instituted before irreversible damage takes place.

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