Immunogenicity of 23-Valent Pneumococcal Vaccine in Children with Systemic Lupus Erythematosus

Soheila Alyasin[1*], Marzieh Adab[2*], Asieh Hosseinpour[2], Reza Amin[1], Maryam Babaei[2]

[1]Allergy Research Center, Department of Pediatrics, Division of Clinical Immunology and Allergy, [2]Allergy Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

ABSTRACT

Background: Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease which is characterized by B-cell abnormality and auto-antibody generation. Since bacterial infections are the most important causes of mortality in these patients, pneumococcal vaccination is recommended for children with SLE. **Objective:** To investigate humoral immunity and specific-antibody formation in response to a 23-valent polysaccharide pneumococcal vaccination in SLE children and asthmatic control group. **Method:** The case and control groups consisted of 30 children with the mean age of 13 years who were matched by sex and age. Anti-pneumococcal antibody titers were determined using Enzyme-Linked Immunosorbent Assay (ELISA) before the vaccination with the 23-valent pneumococcal vaccine and 3 weeks later in both groups. Also the correlation between anti-pneumococcal antibody titer and different factors including age, sex, lupus activity, disease duration, medications, history of recurrent infections, and laboratory data were investigated. **Results**: Both groups showed significant increases in anti-pneumococcal antibody level after vaccination ($p \leq 0.001$). The increase in antibody level were almost the same in both groups ($p \geq 0.05$) such that 77.7% of SLE children and 86.2% of control children showed at least 2-fold increase in anti-pneumococcal antibody titer following immunization. Significant correlations were seen between the level of post-immunization anti-pneumococcal antibody with the age of children with SLE ($p=0.02$) and their age of disease onset ($p=0.02$). **Conclusion:** It is concluded that pneumococcal vaccination is generally immunogenic in children with SLE. However, a small group of patients show impaired response to the vaccine.

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Keywords: 23-Valent Pneumococcal Vaccine, Anti-Pneumococcal Antibody, Children, Immunogenicity, Systemic Lupus Erythematosus (SLE)

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*Corresponding authors: Dr. Soheila Alyasin and Marzieh Adab, Department of Pediatrics, Division of immunology and allergy, Shiraz University of Medical Sciences, Namazee Hospital, Shiraz, Iran, e-mail: alyasins@sums.ac.ir, marziehadab@yahoo.com

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is an autoimmune, chronic, episodic and multi-systemic disease (1). One of its features is abnormal activation of B-Cell lymphocyte and abnormal production of plasma cells and memory cells; abnormal memory cells have specificity for auto-antigens and abnormal plasma cells produce different pathogenic auto-antibodies (1). Therefore, the disease can be characterized by B-lymphocyte hyperactivity and auto-antibody production against cellular components including anti-dsDNA, anti-Ro, anti-La, anti-Sm, anti-RNP and ANA which can result in multi-organ damages (1).

Although the underlying causes of B-cell overactivity in SLE are unclear, it is obvious that abnormal lymphocyte function, production of auto-antibodies and cytokines, and B-cell and plasma cell auto-reactivity play a role in its pathogenesis (1,2). Also genetic predisposition, environmental triggering like exposure to ultraviolet radiation, and other factors including sex, hormonal factors, medications, viral infections, steroidal hormones, congenital deficiencies of C1q, C2, and C4, presence of some specific regions on chromosomenumber 1, HLA-B8, HLA-DR2, and HLA-DR3 play roles in the development of SLE (2,3). To evaluate the humoral immune system and antibody production by B-cells, different methods are being used including the measurement of serum IgA, isohemagglutinin titer and specific antibody level after tetanus toxoid vaccine or pneumococcal vaccine (4,5).

The most important causes of mortality and morbidity in patients with SLE are infections (6). Induction or exacerbation of SLE can be caused by infections which are responsible for 30 to 50 percent of mortality and morbidity in SLE patient (6). Additionally incidence of infections in these patients is 2-fold more than that of normal population (6,7). This increased risk of infection is not only due to immunologic reaction of the disease but also it can be attributed to immunosuppressive therapy and other intrinsic or extrinsic factors (7). Infections in SLE patient can be caused by all types of bacterial, viral, fungal and opportunistic organisms, but bacterial infections are the most common cause of mortality and morbidity among them (6,7). Therefore, immunization with vaccines such as pneumococcal vaccine is recommended in the SLE patients to prevent infections (7,8). However, the safety and efficacy of immunization is controversial in SLE patients (9-13), and possible exacerbation, onset and flare of SLE following immunization are reported in few studies (14,15).

The immunogenicity of vaccines in SLE patients has been a matter of controversy for many years, and reports indicate that antibody response to immunization with foreign antigens in SLE patients is variable. So far, specific antibody response to protein antigens following immunization with tetanus toxoid in SLE patients has been studied in several clinical trials (13,16,17); while some researchers have reported poor responses (13), normal responses are reported by others (16). Also in the case of polysaccharide antigens, studies on immunogenicity of pneumococcal vaccines in adult SLE patients have revealed that while the vaccine is immunogenic and safe in majority of patients (9,17-19), impaired response to this vaccine have also been reported (10,20).

Since we couldn't find any record on the evaluation of immunogenicity of 23-valent polysaccharide pneumococcal vaccine in the age group of children, herein specific antibody formation against polysaccharide antigens (humoral immunity function) in SLE children is evaluated. Also possible relationships between anti-pneumococcal antibody titer and different factors including lupus disease activity, duration of the

disease, medications, history of recurrent infections andlab data of patientsare investigated.

MATERIALS AND METHODS

Patients. In this study thirty children with SLE were participated as case group, and thirty age and sex matched asthmatic children were participated as control group.
Thirty SLE children with 2-18 years old, who were referred to Imam Reza immunology out-patient Clinic affiliated with SUMS and fulfilled 1997 revised criteria of American College of Rheumatology for SLE (21), were selected by convenient sampling method. Exclusion criteria were fever, pregnancy, recent immunization, history of previous pneumococcal vaccination, history of hypersensitivity reaction to vaccination, platelet level < 50,000, mixed connective tissue disorders and IVIG injection in last 3 months.
Thirty age and sex matched asthmatic controls, with the same exclusion criteria were selected from children who attended out-patient clinic of asthma and allergy of Imam Reza Clinic. The controls were selected from asthmatic patients because pneumococcal vaccine is recommended for this group (22,23) and as well they have no defects in immune system and specific antibody formation (the study variable) (24). The controls were selected from mild asthmatic patients, however the asthmatic patients with persistent or severe asthma, those with history of immunosuppressive therapy or PO corticosteroid consumption and those who had history of other immunodeficiency disorders were excluded from the control group.
At baseline a complete history was taken from both groups and all went through physical examinations. Specific questionnaires were prepared for patients and data such as: age, sex, duration of disease, drug and medication history, history of recurrent hospital admission and infection and history of lupus nephritis and proteinuria were recorded. Disease activity of SLE patients was scored using the Systemic Lupus Erythematous Disease Activity Index (SLEDAI) that consists of 24 items (25). Some Routine laboratory tests were done before vaccination and were recorded in questionnaires including: complete blood cell counts (CBC), complement components (C3 and C4), anti-double-strand DNA antibody (dsDNA), urinalysis, erythrocyte sedimentation rate (ESR), serum level of immunoglobulins (IgG, IgA, IgM), and C-reactive protein level (CRP).
Informed written consents were obtained from all participants or their parents and all the procedures used in this work were approved by the Ethics Committee of SUMS and is registered in Iranian Registry of Clinical Trials with the trial registration code of "IRCT 138811293361N1".
Vaccine. Before vaccination, 5 ml venous blood sample was taken from each individual in both case and control groups, and after centrifugation, serum samples were stored at -70 °C. Then each participant received 0.5 ml intradeltoid injection of 23-valent pneumococcal vaccine (pneumovax-23; Sanofi Pasteur MSD) which contained 25 μg of following capsular polysaccharide (Danish nomenclature): types 1–5, 6B, 7F, 8, 9N, 9V, 10A, 11A,12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F. After vaccinations patients were observed at the clinic for 30 minutes by an immunologist and adverse effects such as severe local swelling, redness, fever or weakness were recorded.

At least 3 weeks after vaccination, both groups were visited at the Immunology Clinic again. After history-taking and physical examination, another 5 ml venous blood sampling was done and after centrifugation, serum samples were stored at -70 °C.

Pneumococcal Polysaccharide Capsule-Specific Immunoglobulin Assays. Both pre-vaccination and post-vaccination serum samples of all participants were sent to Allergy Research Center of Shiraz (affiliated to SUMS) and anti-pneumococcal specific IgG titer was measured using ELISA. Antibody (IgG) response was analyzed with enzyme linked immunoassay kit specific for pneumococcal vaccine (VaccZyme™, pneumococcus IgG Binding Site, Birmingham, UK). The assay measuredantibody responses to pneumococcal vaccine incorporating 23 polysaccharides isolated from streptococcus pneumonia. The antibody response can be evaluated by the serological determination of their IgG anti-PCP (pneumococcal capsular polysaccharide) antibody level pre- and post-vaccination using this quantitative enzyme immunoassay (26-28).

Micro wells were pre-coated with the PCP antigen (1-5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, 33F – Danish nomenclature). Calibrators and controls were pre-adsorbed against capsular polysaccharide and samples were diluted in a diluent containing CPS (1:100). The calibrators, controls and diluted patient samples were added to the wells and antibodies recognizing the PCP antigen were bound during the first incubation at room temperature for 30 minutes.

After washing the wells to remove all unbound proteins, purified peroxidase labeled rabbit anti-human IgG (γ chain specific) conjugate was added. The conjugate binds to the captured human antibody and after second incubation, the excess unbound conjugate was removed by a further wash step. The bound conjugate was visualized with 3, 3', 5, 5' tetramethylbenzidine (TMB) substrate which gives a blue reaction product, the intensity of which is proportional to the concentration of antibody in the sample, and next incubation was done in dark. Phosphoric acid was added to each well to stop the reaction. Theyellow end point color, was read at 450nm.

The optical density (OD) of each well was read at 450 nm by a microplate reader, within 30 minute of stopping the reaction. The calibration curve was achieved by plotting the anti-PCP IgG antibody concentration on the log scale against the OD on the linear scale for each calibrator. The levels of the anti-PCP IgG antibody in the diluted samples were read directly from the calibration curve and the results are reported in milligram per liter.

In this study immune responses are presumed as 2-fold increase in antibody titer and participants with more than 2-fold increase in antibody titer following immunization are considered as good responders; also typical range of the kit (31-90 mg/L) is presumed as normal range of antibody.

Statistical Analysis. Normality of continuous variables was evaluated by one-sample Kolmogorov-Smirnov test, and if they were normal, they were compared between groups using parametric tests (independent-samples t-test), and if they were not normal, comparing was done using non-parametric tests (Mann-Whitney test). Comparing of categorized (qualitative) variables was done using Chi-square test and Fisher's exact test. Comparing of variables before and after vaccination was done using paired-samples t-test. Correlation between two normal variables was investigated using Pearson Correlation Coefficient Test; and Spearman Correlation Coefficient Test was used for analyzing correlation between two non-normal variables. Statistical analyses were done using SPSS for windows, version 18. P values less than 0.05 were presumed as significant with two tailed assumption.

RESULTS

Patients and Controls Characteristics. Demographic and clinical characteristics of the participants as well as their lab data are shown in Table 1.

Table 1. Patients' characteristics

Characteristics	SLE Patients	Control Subjects
Number	30	30
Age, mean years (range)[a]	13.2 (3-18)	12.8 (4-18)
Sex, n (% female)[b]	23 (76.7)	23 (76.7)
Disease duration, mean years (range)	3.2 (0.25-15)	
Age of onset, mean years (range)	9.9 (0.5-16)	
Hx ofrecurrent infection, n (%)	8 (26.7)	
Hx of lupus nephritis, n (%)	8 (26.7)	
Active proteinuria[c], n (%)	4 (13.3)	
WBC, mean /μL (range)	7653 (3700-19900)	
Hb, mean gr/dl (range)	12.4 (8.5-14.9)	
Platelets, mean $*10^3$/μL (range)	268.37 (105-570)	
Anti-dsDNA, mean u/ml (range)	89.6 (1.6-516)	
C3, mean g/L (range)	1.16 (0.53-2.89)	
C4, mean g/L (range)	0.22 (0.05-0.49)	
ESR, mean mm/hr (range)	18.1 (3-70)	
CRP, mean mg/L (range)	1.85 (0-11.3)	
IgG, mean g/L (range)	12.8 (6.7-26.08)	
IgA, mean g/L (range)	2.4 (0.6-9.2)	
IgM, mean g/L (range)	1.6 (0.26-5.1)	
Active proteinuria, n (%) of patients	4 (13.3)	
Active SLE disease, n (%) of patients	11 (36.7)	
SLEDAI, mean (range)	6.6 (0-22)	

a. P= 0.69 for comparison of case and control groups.
b. P=1 for comparison of case and control groups.
c. Proteinuria at the time of immunization

Both study groupsconsisted of 30 members and both were dominantly female. The age of both groups were matched (p=0.69). Only a small number of patients (8.3%) had abnormal levels of immunoglobulins at the time of immunization; however, about half (n=16) of the patients (53.3%) had high levels of anti-dsDNA and 10 (33.3%) of them had complement deficiency.

Table 2. Patients' medications.

Medication received	n (%) of patients	Duration of consumption, mean years (range)	Dosage, mean (range)
Hydroxychloroquine	25 (83)	2.8 (0.1-12)	
Prednisolone	30 (100)	3.07 (0.1-10)	5.8 (1.25-15) mg/day
Prednisolone ≤ 10 mg/day	27 (90)		
Prednisolone > 10 mg/day	3 (10)		
Azathioprine	3 (10)	2.6 (1.5-5)	
Methotrexate	5 (16.7)	0.67 (0.05-3)	6 (2.5-20) mg/week
Cyclophosphamide	5 (16.7)	0.16 (0.1-0.25)	
Cellcept	3 (10)	1.5 (0.1-2.5)	
Others (e.g. Rituximab)	4 (13.3)		

Patients' medications are shown in Table 2. Prednisolone was the main treatment in all of the patients and only 3 of them (10%) were on oral prednisolone more than 10 mg/day. The majority of patients were receiving hydroxychloroquine and a small numbers of patients were receiving other medications such as methotrexate, cyclophosphamide, rituximab, azathioprine and cellcept.

Table 3. Change in Anti-pneumococcal antibody level following immunization.

Anti-Pneumococcal Antibody	SLE Patients	Control Subjects	P Value
Pre.vaccination antibody (Ab), mean mg/L (range)	68.8 (5.79-326.59)	71.88 (6.13-269.74)	0.8
Post.vaccinationAb, mean mg/L (range)	244.7 (27.58-687.3)	341.6 (22.7-964.6)	0.068
Fold of increase in Ab, mean (range)	7.01 (1.13-33.4)	9.6 (1.3-47.4)	0.49
Increase in antibody, mean mg/L (range)	175.8 (7.8-649.3)	269.7 (13.05-820.5)	0.09
> 2-fold increase in Ab, n (%) of patients	21 (77.8)	24 (86.2)	0.4
> 3-fold increase in Ab, n (%) of patients	17 (63)	21 (72.4)	0.45
> 4-fold increase in Ab, n (%) of patients	15 (55.6)	19 (65.5)	0.44

Antigen Specific Antibody. As is represented in Table 3 both groups similarly showed significant increases in anti-pneumococcal antibody level, with mean fold of 7.01 in SLE and 9.6 in control group. Also as is shown in Figure 1 significant increases in anti-pneumococcal antibody were seen following immunization ($p<0.001$) in both groups.

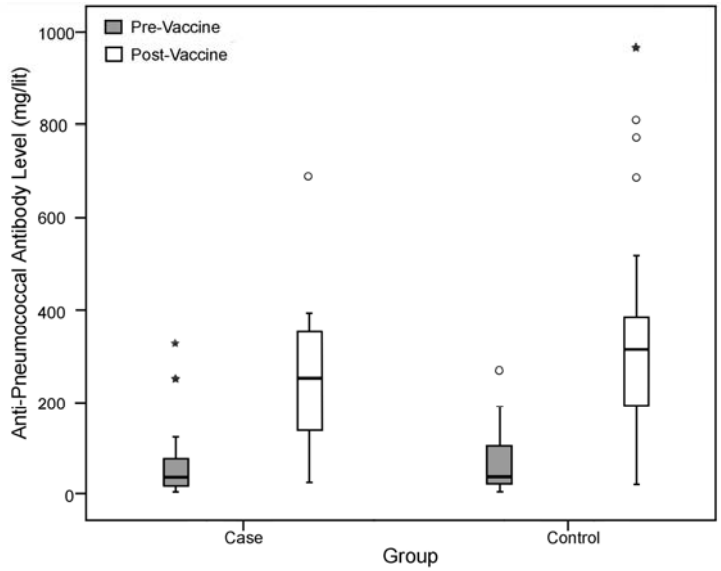

Figure 1. Anti-pneumococcal antibody level in case and control group.

Our results showed that anti-pneumococcal antibody titer following immunization does not differ significantly between case and control group according to different definitions of normal ranges and typical range of the kit. Also presuming at least 2-fold or 4-fold increase in antibody level as an immune response to 23-valent pneumococcal vaccine, majority of SLE and control children showed immune responses (Table 4).

Table 4. Responses to pneumococcal vaccine in SLE and control groups according to different definitions of immune response.

Reference	Definition of Immune Response	SLE patients, % of patients	Control subjects, % of subjects	P value
	More than 2-fold	77.8	86.2	0.4
	More than 3-fold	63	72.4	0.45
	More than 4-fold	55.6	65.5	0.44
Wernette et al. (27)	2-4 fold	77.8	86.2	0.4
VaccZyme™ anti-PCP IgG ELISA kit	kit range (31-90mg/L)	96.3	96.6	1.00
Schauer et al. (26)	More than 3-fold	63	72.4	0.44
Aghamohammadi et al. (29)	Increase in Post.titer ≥ 129 or ≥ 2-fold	81.5	86.2	0.72
Karimi et al. (30)	Pre.titer ≥ 200 or post ≥ 4-fold	66.7	69	0.85
Balmer et al. (28)	Titer ≥ 50 and ≥ 4-fold	55.6	65.5	0.44
Vendrell et al. (31)	More than lower limit of the 2-tailed 90% probability interval of post IgG of controls =268.76 mg/L	37	55.2	0.17

Effect of Age on Antibody Response. There was no significant difference between the mean age of good and poor responders (Table 5).

Table 5. Difference in clinical and laboratory parameters in patients with poor response and good response*.

Clinical and lab parameters	Poor Responders	Good Responders	P value
Age, mean years ± SD	12.9 ± 3.2	12.7 ± 4.4	0.9
Age of SLE onset, mean years ± SD	10.2 ± 3.6	10 ± 4.4	0.9
Disease duration, years	2.7 ± 1.4	2.8 ± 2	0.9
WBC, mean /µL ± SD	5800 ± 2046	8523 ± 4235	0.1
Hb, mean gr/dl ± SD	12.7 ± 1.3	12.4 ± 1.5	0.6
Platelets, mean /µL ± SD	330666 ± 130776	249190 ± 80753	0.07†
Anti-dsDNA, mean u/ml ± SD	112 ± 132.7	92 ± 139.5	0.7
C3, mean g/L ± SD	1.07 ± 0.28	1.2 ± 0.5	0.5
C4, mean g/L ± SD	0.18 ± 0.11	0.23 ± 0.11	0.3
ESR mean mm/hr ± SD	25.5 ± 13.6	13.5 ± 11.9	0.04+
CRP mean mg/L ± SD	1 ± 3.1	0.9 ± 3.1	0.9
IgG mean g/L ± SD	17.1 ± 8.6	11.9 ± 4.1	0.3
IgA mean g/L ± SD	2.1 ± 0.98	2.2 ± 1.8	0.9
IgM mean g/L ± SD	1.2 ± 0.48	1.7 ± 1.1	0.3
SLEDAI, mean ± SD	9 ± 2.7	5.8 ± 5.5	0.07†

*SD= Standard Deviation, Good responders = Patients with≥2-fold increase in antibody, Poor responders = Patients with≤2-fold increase in antibody.
†The difference is not significant but P-value is borderline and therefore further larger scale trials might be needed to reach a more exact relationship
+Significant difference

A significant correlation was seen between the age of participants and their post-immunization pneumococcal antibody level (p=0.02, r=0.3) (Figure 2) while no significant correlation was seen between pre-immunization pneumococcal antibody level or mean fold of increase in antibody and the age of the participants (p>0.05).

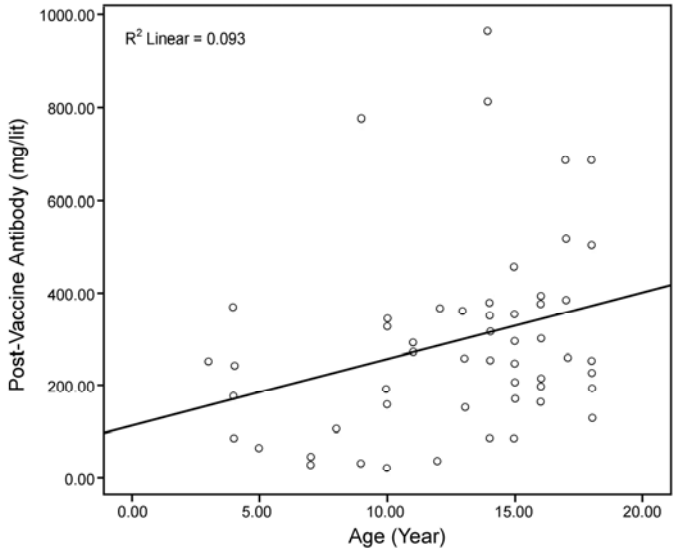

Figure 2. Correlation of age and post-immunization antibody level.

Results also showed that a significant correlation was seen between age of SLE onset and post-immunization antibody level (p=0.02, r=0.43) (Figure 3) while no relationship was seen between age of SLE onset and pre-immunization antibody level or mean fold of increase in antibody (p>0.05).

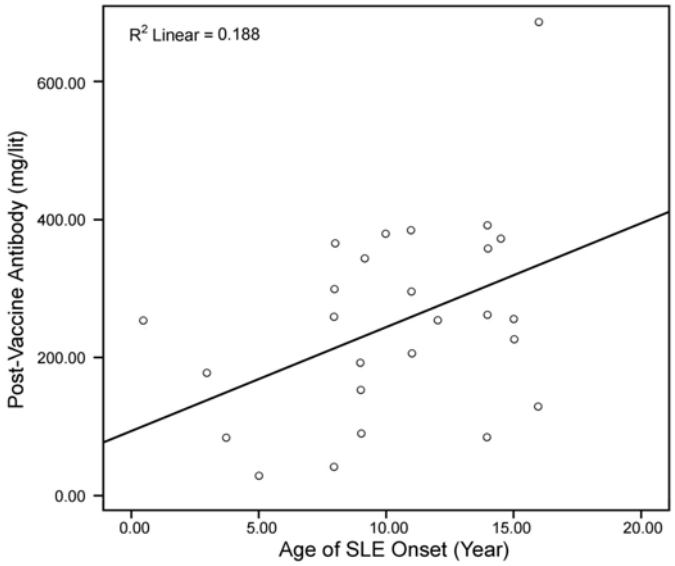

Figure 3. Correlation of age of SLE onset and post-immunization antibody level.

Effect of Disease Activity on Antibody Response. There was no significant relationship between the pre-immunization antibody, post-immunization pneumococcal antibody level or immune response and SLEDAI of patients (p>0.05).
Results showed that although the mean SLEDAI of good responders was less than that of poor responders, the difference was not significant (p=0.07) (Table 5).
As is clear from Table 6, the mean fold of increase in level of anti-pneumococcal antibody in patients with low SLEDAI was significantly higher than patients with active disease (p=0.012).

Table 6.Comparing antibody response in patients with active disease and inactive disease*.

Anti-pneumococcal Ab	Low SLEDAI	High SLEDAI	P Value
Pre-vaccination Ab, mean mg/L (range)	66.4 (5.79-326.6)	73.7 (13.87-247.7)	0.74
Post-vaccination Ab, mean mg/L (range)	279.1 (82.9-687.3)	175.8 (27.5-393.7)	0.08
Fold of increase in Ab, mean (range)	8.9 (1.1-33.4)	3.2 (1.25-9.7)	0.012
≥ 2-fold increase, % of patients	88.9	55.6	0.13

*. Low SLEDAI: SLEDAI≤8, High SLEDAI: SLEDAI>8

Table 7. Effect of different medications on antibody response*

Medications		Pre-vaccinAb,mean mg/L (range)	Post-vaccinAb, mean mg/L (range)	Fold of increase in Ab, mean (range)	Increase in Ab level, mean mg/L (range)	≥ 2-fold increase, % of patients
Hydroxychloroquin	+	76.9 (5.79-326.59)	232.1 (32.1-393.79)	6.7 (1.13-33.4)	155.1 (18.2-361.6)	77.3
	-	33.2 (19.7-56.1)	300.2 (27.5-687.3)	8.3 (1.4-18.06)	267 (7.8-649.3)	80
P value		0.56	0.78	0.44	0.44	1.00
Prednisolone	*>10 mg/day*	16.6 (7.4-25.9)	86.33 (82.9-89.7)	7.6 (3.2-12)	69.6 (57-82.2)	100
	<10 mg/day	73.06 (5.79-326.59)	257.4 (27.5-687.3)	6.9 (1.13-33.4)	184.3 (7.8-649.3)	76
P value		0.17	0.11	0.62	0.23	≈1.00
Azathioprine	+	104.4 (30.5-252.2)	311 (261-373.9)	6.6 (1.4-9.7)	206.6 (121.7-267.7)	66.7
	-	64.4 (5.79-326.59)	236.4 (27.5-687.3)	7.07 (1.13-33.4)	172 (7.8-649.3)	79.2
P value		0.63	0.21	0.85	0.39	0.54
Methotrexate	+	58.9 (19.7-110.06)	137.6 (27.5-207.4)	2.3 (1.4-3.7)	78.7 (7.8-130.9)	33.3
	-	70.1 (5.79-326.59)	258.1 (32.1-687.3)	7.6 (1.13-33.4)	188 (18.2-649.3)	83.3
P value		0.85	0.11	0.11	0.13	0.11
Cyclophosphamide	+	73.2 (7.48-247.7)	184 (27.6-393.8)	6.8 (1.4-12.3)	110.7 (7.8-206.8)	50
	-	68.1 (5.79-326.59)	255.3 (32.1-687.3)	7.05 (1.13-33.4)	187.1 (18.2-649.3)	82.6
P value		0.3	0.48	0.97	0.33	0.2
Others(e.g. Rituximab)	+	61.9 (19.7-110)	197.9 (27.5-358.8)	3.2 (1.4-6.39)	135.9 (7.8-302.69)	33.3
	-	69.7 (5.79-326.59)	250.5 (32.1-687.3)	7.49 (1.13-33.4)	180.8 (18.2-649.3)	83.3
P value		0.63	0.53	0.24	0.53	0.11

*. (+) indicates consumption of the medication.

Correlation of Other Demographic, Clinical and Laboratory Factors with Antibody Formation. There was no significant correlation between pre-immunization, post-immunization pneumococcal antibody level or immune response and duration of SLE disease ($p>0.05$) or the gender of patients. Also the mean duration of disease in good and poor responders showed no significant difference (Table 5). Regarding the effect of recurrent infections and lupus nephritis on antibody formation, it was seen that the mean level of anti-pneumococcal antibody as well as the level of immunoglobulins (IgG, IgM, IgA) in SLE children with history of recurrent infection and lupus nephritis was lower than patients without it; however, the difference was not statistically significant.

Concerning the effect of medication, it was seen that poor vaccine response was not significantly associated with use of any immunosuppressive agents including prednisolone, methotrexate, hydroxychloroquine, cyclophosphamide, azathioprine or other immunosuppressants (Table 7).

Although a trend toward decreased post-immunization antibody level and immune response in patients treated with different medications was seen in comparison with those patients who did not receive such treatments this was not statistically significant. It should be considered that all of the patients participated in the study were on prednisolone; therefore they were compared on the basis of their dosage of prednisolone; however, only 10% of patients were receiving prednisolone more than 10 mg/day.

Investigating the correlation between patients' routine lab data and antibody response showed that there is no significant association between post-immunization antibody or immune response and level of different laboratory parameters and it seems that poor vaccine response was not significantly associated with abnormalities in these parameters. However, a correlation was seen between pre-immunization pneumococcal antibody level and ESR of patients ($p=0.03$, $r=0.4$).

As it was mentioned, a small group of patients had impaired response to the vaccine, and therefore they might remain susceptible to infections despite vaccination. As is shown in Table 5, no strong relationship was seen between poor immune response and any clinical or laboratory parameters that could help to predict these poor responses before vaccination except ESR which can be presumed as the only predictor for poor response.

DISCUSSION

In this study it was observed that the majority of both SLE and control children showed adequate mean antibody responses which were similar to each other ($p\geq0.05$); however, a small group of patients had impaired response.

The immunogenicity of vaccines in SLE patients has been a matter of controversy since many years ago. Several studies have reported significantly lower levels of mean antibody following vaccination in SLE patients than that in healthy subjects (10,13,20). However, some reports may be found on immunogenicity of pneumococcal or tetanus immunization in SLE patients or other chronic diseases in which the mean antibody levels in such patients are similar to those in controls (9,18,22,32,33). Battafarano *et al.* immunized 73 SLE patients with pneumococcal, tetanus toxoid, and *Haemophilus influenza* type B vaccine and the majority of patients developed with less than 4-fold

increase in antibody level after vaccination, but protective antibody levels,that was a more important clinical measure of immunization response than a 2-, 3-, or 4-fold increase in antibody level following immunization, were achieved in them despite immunosuppressive therapy or active disease (17). In our study, changes in mean antibody level from pre-vaccination to 3 weeks after 23-valent pneumococcal vaccination were 68.8 mg/L to 244.7 in SLE children and 71.88 mg/L to 341.6 in controls ($p<0.001$), that were similar in both groups ($p>0.05$). As we see both groups showed significant increases in anti-pneumococcal antibody level after vaccination, with mean fold of 7.01 in SLE group and 9.6 in control group and with the mean increase in specific antibody of 175.8 mg/L in SLE group and 269.7 mg/L in controls. Also it should be said that 77.8% of SLE children showed at least 2-fold, 63% showed at least 3-fold and 55.6% showed at least 4-fold antibody response after vaccination, that were similar to controls with 86.2% of at least 2-fold, 72.4% of at least 3-fold and 65.5% of at least 4-fold antibody response after vaccination. It should be mentioned that in interpretation of specific-pneumococcal antibody, patients who have high level of baseline antibody are less likely to have increase in antibody level after immunization (5), and this fact was seen in majority of our patients with less than 2-fold increase in antibody following immunization. The mean pre-vaccination antibody level in SLE patients with less than 2-fold and more than 2-fold increase in antibody was 180.02 mg/L and 36.13 mg/L respectively. These high pre-vaccine antibody levels may be acquired during life as a result of colonization or previous infection with streptococcus pneumonia or cross-reacting organisms.

It should be stated that the interpretation of specific-pneumococcal antibody is based on increased post-immunization over pre-immunization antibody concentrations (immune response), and on the final post-immunization antibody level regardless of pre-immunization level (antibody concentration) (5). The main problem in interpretation of immune response to polysaccharide capsule antigen is the lack of standardized or universal criteria for adequate antibody response to pneumococcal polysaccharide; also an exact protective value or normal range for pneumococcal IgG antibody is not well defined (4,19). However, a number of researches have been done to determine normal ranges or ranges that discriminate between normal and abnormal immunity (26). A number of studies (26,31) have been done according to ELISA method, which is also used in the present paper, and different normal ranges for whole pneumococcal IgG antibody have been defined (Table 4). Also in a trial that was reported by VaccZyme™ anti-PCP IgG ELISA kit, from 100 normal adult blood donor, majority of them had an anti-PCP IgG titer in the range of 31-90 mg/L. Because of these different definitions, we have shown our results according to different definitions of normal range for whole pneumococcal IgG antibody in Table 4 and it is seen that the majority of patients have achieved protective level to this vaccine and the immune responses in SLE patients are similar to that of controls, however, a substantial group in both groups are remained unprotected.

It is reported thatSLE patients show significant increases in mean antibody response at 1 month after pneumococcal vaccination similar to control subjects; also none of the demographic parameters (such as age, sex, duration of disease), measures of disease activity, evaluated clinical and laboratory measures, levels of B12, folate, or use of any immunosuppressive agents could predict poor response to pneumococcal vaccine (18). In the study by Jarrett *et al.* the decreased response to pneumococcal vaccine was not correlated with drug therapy at the time of immunization or other parameters such as

anergy state, renal function and serum immunoglobulin levels (10). It is also observed that in the patients receiving immunosuppressive therapy, either alone or in combination, antibody response was lower than that of other SLE patients, but the difference was not statistically significant (17). It was shown that there is a trend toward decreased antibody response with higher disease activity score measured by either the SLEDAI or the LACC; however, this did not reach statistical significance (17). Several studies have correlated lower antibody response in SLE, rheumatoid disease or high-risk patients than in normal controls to disease severity or immunosuppressive therapy (11,32). In contrast, several studies indicated that there were no significant correlation between antibody levels following immunization and steroid or immunosuppressive therapy (33). Several investigations on the effect of DMARDs on immune responses after immunization with the pneumococcal vaccine in patients with immunity disease showed that TNF antagonists (e.g. etanercept and infleximab) do not impair the mean antibody responses to pneumococcal vaccination (12,34); while patients treated with MTX had reduced responses (32,34).

In our study no relationship was seen between different demographic parameters of patients such as sex, duration of disease, history of recurrent infection, history of lupus nephritis, active proteinuria or use of any immunosuppressive agents (including prednisolone, methotrexate, hydroxychloroquine, azathioprine,cellcept, cyclophosphamide and others such as rituximab) and the mean anti-pneumococcal antibody level (Table 7). However, it was seen that the mean level of post-vaccination antibody in patients with history of recurrent infection, lupus nephritis, active proteinuria or use of different immunosuppressive agents was lower than the patients without them, but the difference was not significant. Since the number of patients receiving azathioprine, cellcept, rituximab or high dose corticosteroid were small, the effect of these medications could not be determined exactly; therefore large scale studies may be needed in this regard. Generally, according to our results and previous studies it could be concluded that pneumococcal vaccination in patients with SLE before starting potent immunosuppressive agents or high dose corticosteroids seems to be ideal.

The only relationship that was seen in the study, was a significant correlation between age of patients and their post-immunization pneumococcal antibody level ($p=0.02$, $r=0.3$) (Figure 2); it seems that this relationship is mainly pertinent to SLE group (p.value=0.03, $r=0.4$). Another relationship was observed between age of SLE onset and post-immunization pneumococcal antibody level ($p=0.02$, $r=0.4$) (Figure 3). Up to now several studies have been done to show the effect of age on response to pneumococcal vaccine (26,32,35). In a trial by O'dell *et al.* it was shown that the age of the patient did not affect the response to pneumococcal vaccine (32) while in some other studies anti-PCP antibody titers increased steadily with age (26,35); however, in our study it could be concluded that the older the patient was the more post-immunization antibody level was. This finding could be due to various factors including: development of natural immunity during life, and probable acquisition of antibodies as a result of colonization or previous infections with streptococcus pneumonia or cross-reacting organisms. Also we could conclude that the earlier the SLE onset in children was the lower the post-immunization antibody level was; and this could be due to the fact that the younger children with SLE do not have a complete immune system, or due to early beginning of immunosuppressant agents; however, more researches might be needed in this field.

Regarding the effect of SLE disease activity on immune response there was no significant relationship between the pre-immunization antibody, post-immunization pneumococcal antibody level or immune response and SLEDAI of patients (P>0.05) in the present study. Although the mean SLEDAI of good responders was 5.8 ± 5.5 and in poor responders was 9 ± 2.7, but the difference is not significant with p=0.07 (it may become significant in other studies with large scale participants).

Comparing patients having SLEDAI less than 8 with those having active disease in our study, revealed that the mean pre-vaccination anti-pneumococcal antibody level in patients with inactive disease were lower than the patients with active disease, which may be due to more susceptibility to infection and more history of recurrent infections in patients with active disease in their past histories (Table 6). Also it was seen that the mean post-vaccination level of anti-pneumococcal antibody in patients with low SLEDAI were more than the patients with active disease (Table 6). Since the patients with more pre-vaccination level of antibody could not produce good post-vaccination antibody, the mean fold of increase in antibody response in patients with active disease (3.2 ± 2.7) is lower than the patients with high SLEDAI (8.9 ± 7.8) (p=0.012). Therefore, general antibody response is lower in patients with higher disease activity. It should be said that usually the patients with higher SLEDAI were receiving more immunosuppressive medications or having more history of recurrent infections and hospital admissions, and we could not exactly differentiate the cause of these poor antibody response in patients with active disease, whether it was due to higher SLEDAI or due to other factors that affected them.

Considering laboratory factors, our results show that there is no significant association between post-immunization antibody or immune response and level of different parameters in routine lab data. Presuming 2-fold increase in antibody following immunization as a good response, our results show that the mean level of different parameters in routine lab data (including WBC, Hb, Plt, C3, C4, CRP, IgG, IgA, IgM and anti-dsDNA) in good responders does not differ significantly with poor ones; therefore it seems that poor vaccine response was not significantly associated with abnormality in these parameters except ESR that showed a significant difference (p=0.04) (Table 5). However, in poor responders, complement level was lower and CRP, ESR, and anti-dsDNA was higher, but the only significant relationship was seen for ESR that could be considered as indicators for inflammation and disease activity in these patients.

In conclusion**,** vaccination of SLE children with 23-valent pneumococcal vaccine was immunogenic in majority of them; however, a substantial subgroup of them showed poor response to this vaccine and may remain susceptible for infections despite vaccination. Also it could be concluded that immunosuppressive therapy does not seem to interfere with development of consistent immunity to pneumococcal vaccine in children with SLE. However, immune response to vaccine appears to be most optimal in SLE patients with less disease activity and minimal immunosuppressive therapy. So similar to other high-risk patients, pneumococcal immunization is probably cost-effective in SLE patients because of increased risk of infection in them. Therefore, children with SLE, should receive immunization according to recommendations of the Centers for Disease Control and Prevention and the Immunization Practice Advisory Committee (8).

Large scale studies are needed to confirm the safety of this vaccine and determine the efficacy of that in SLE children and also other studies are needed to determine any

demographic, clinical or laboratory factors that could predict the patients with poor response to pneumococcal vaccine which may help clinicians to manage these poor responses better.

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