

DNA immunoadsorption for childhood-onset systemic lupus erythematosus

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ABSTRACT

We present a retrospective review of DNA immunoadsorption (DNA-IA) therapy on clinical symptoms as well as indicators in pediatric cases with systemic lupus erythematosus (SLE), and follow up the short-term curative effects. 16 SLE cases were treated by DNA-IA for 3 times every other day. We observed the changes on clinical manifestations and immunological indicators, in order to compare the alteration of these indicators including clinical manifestations, Systemic Lupus Erythematosus Disease Active Index (SLEDAI) scores, 24 h-urinary protein excretion, autoantibodies, serum IgG and complement C3. 13 cases were followed up regularly, within 3 months after DNA-IA therapy, 12 cases of clinical manifestations improved (92.3%). SLEDAI scores in 10 cases decreased from (16.20 ± 12.54) to less than 5 (76.9%), 8 cases of ANA, anti-DNA antibodies were negative (61.5%), 13 cases with IgG level in serum recovered to normal (10.39 ± 4.38) g/L, C3 level rose to normal (1.06 ± 0.23) g/L. 3 to 6 months after IA, clinical manifestations and laboratory examinations in all cases got maximum improved. 9 months after IA, SLEDAI score in 2 cases (15.4%) rose to more than 5, anti-DNA antibody in 2 cases (15.4%) became positive, and 1 case (7.7%) with serum C3 decreased again. 2 cases died from multiple organs dysfunction within 3 to 6 months after IA. No serious complications were found during DNA-IA. We recommend that DNA immunoadsorption is a safe and effective therapy for active childhood-onset SLE, which could improve clinical symptoms, eliminate ANA and anti-DNA antibodies. Combining with corticosteroids and immunosuppressive drugs, DNA-IA could significantly reduce the activity of disease and protect vital organs function in the short term.

Keywords: DNA Immunoadsorption (DNA-IA); Systemic Lupus Erythematosus (SLE); Childhood-Onset; Autoantibody

1. INTRODUCTION

SLE (systemic lupus erythematosus, SLE) is a common rheumatic disease in children. A large number of auto-antibodies, mainly antinuclear antibody in serum, play a critical role in the course of disease. As a kind of blood purification technology, deoxyribonucleic acid immunoadsorption (DNA-IA) therapy is developing very fast in recent decade, which can specifically remove the antinuclear antibody and immune complex in patient's blood. IgG category anti-ds-DNA antibody is the main auto-antibody which causes SLE pathological changes [1]. Terman [2], GH Stummvoll [3] and L. P. Kihm [4] confirmed that DNA immunoadsorption could reduce anti-ss-DNA antibodies and related immune complexes in the serum. Kong *et al.* [5,6] treated SLE with calf thymus DNA gel adsorption column, which made the plasma anti-ds-DNA antibody decreased by 80%. Nevertheless, patients with SLE have more serious system damage in children than those in adults [7], and there were few reports about the application of DNA Immunoadsorption in childhood onset SLE treatment.

For these reasons, this kind of early with DNA-IA treatment would play a crucial role in reducing vital organs injury and improving clinical symptoms and prognosis for pediatric SLE patients.

2. MATERIALS AND METHODS

2.1. Materials

Sixteen children hospitalized during July 2009 to January 2011 were analyzed. All of them were diagnosed as SLE according to ACR diagnostic criterion which was revised in 1982 [8]. Among 16 patients, sex ratio was 2:14 (male:female), age ranged from 7 to 15 years old with average age (11.38 ± 2.01) .

2.2. Methods

2.2.1. DNA IA Operation Method

Every patient received hemoperfusion treatment three times every other day by using DNA280 immunoadsorp-

tion column (produced by Jafron Biomedical Co., Ltd.).

2.2.2. Observation Indicators before and after IA

1) Clinical manifestations: fever, rash, arthritis, mental status and other indicators were mainly observed; 2) The index of immunology: ANA, anti-single-stranded DNA (ss-DNA) antibody, anti-double-stranded DNA (ds-DNA) antibodies and other auto-antibodies (titer results of semi-quantified), serum immunoglobulin and complement C3; 3) Blood routine examination, urine routine examination, and liver-kidney function examination and so on.

2.2.3. Combination Treatment Measures

Each patient was treated with oral prednisone maintenance dose of 1 - 2 mg/(kg·d). After 3 times of IA, 14 patients were given methyl-prednisolone impact (intravenous methylprednisolone pulse therapy, IVMP) with dosage 15 - 30 mg/(kg·d) for 3 consecutive days, 8 cases were given cyclophosphamide impact (intravenous cyclophosphamide pulse therapy, IVCP) with dosage 10 - 12 mg/(kg·d), for 2 days consecutively, 1 time per month for 6 months, then they began consolidation treatment. Part of them took methotrexate or hydroxychloroquine as well.

2.2.4. Curative Effect Follow-Up

All patients were followed-up by telephone and outpatient visit, monitoring clinical manifestations, Systemic Lupus Erythematosus Disease Active Index (SLEDAI) scores (full score is 105), 24-hour urinary protein excretion and index of immunology at 1, 3, 6, 9 and 12 months respectively after the IA completion. Three of them were lost to follow-up after 3 times of IA.

2.2.5. Statistical Analysis

Compare laboratory parameters before and after IA. SPSS 18.0 statistical software was adopted for Wilcoxon signed rank test and paired test. $P < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Basic Information

Among 16 patients, sex ratio was 2:14 (male:female), age ranged from 7 to 15 with average age (11.38 ± 2.01). The first 14 cases in 1 month, subsequent 2 cases in 8 and 25 month were accepted IA therapy (**Table 1**).

Table 1. Basic information of 16 cases with SLE.

No.	Gender	Age	Course of disease	Significantly affected parts								Kidney biopsy	SLEDAI (before IA)
				Skin	Joint	Pleura	Blood	Heart	Nerve	Kidney	Vasculitis		
1	Female	15.9	25 months	+				+					4
2	Female	15	10 months							+			22
3	Female	10.7	8 months	+							+	II + III type	22
4	Female	9.6	0.5 month							+			47
5	Male	10.6	0.5 month								+	IV-G(A) type	12
6	Female	12.3	1 month	+	+						+	IV-G(A) type	24
7	Female	11.9	0.5 month				+						13
8	Female	12.2	1 month								+		4
9*	Female	11.1	1 month								+	IV-G(A/C) type	20
10	Male	12.1	1 month								+	IV-G(A/C) type	18
11	Female	10.2	0.5 month	+							+		10
12	Female	10.6	1 month	+									6
13	Female	10.8	1 month				+						9
14	Female	11.8	0.5 month					+			+	IV-G(A/C) type	18
15**	Female	7.4	0.5 month	+	+							+	15
16	Female	10	0.5 month								+	V + IV type	13

3.2. Short-Term Curative Effect after DNA Immunoabsorption

3.2.1. Clinical Symptoms

After 3 times of IA treatments, mental status & subjective symptom in all patients had improved. Facial rash of 6 cases reduced and color turned pale (100%), intermittent fever in 2 cases stopped (66.7%), joint swelling and pain of 2 cases got better in function improvement. Only 1 case did not improve in fever obviously.

3.2.2. Index of Laboratory before and after IA

Rechecked the indicators within 2 to 7 days after 3 times of IA treatments.

3.2.2.1. Effect of IA on Auto-Antibody

The changes of ANA, anti-ss-DNA antibody and anti-ds-DNA antibody were shown as **Figure 1**. ANA, anti-ss-DNA antibody and anti-ds-DNA antibody were tending to decline, and they respective compared before with after IA, all cases had significant difference (P values were 0.006, 0.006, 0.034 respectively). Other auto-antibodies before and after IA were shown in **Table 2**, among which only AHA (anti-histone antibody) and AnuA (anti-nucleosome antibody) got statistically significant differences (P values were 0.026, 0.026 respectively), the rest of the six antibodies had no statistical difference (P > 0.05).

3.2.2.2. The influence of IA on Immunoglobulin & Complement C3 Levels

Levels of serum IgG, IgA, IgM and Complement C3 were tending to decline as shown in **Table 3**, only a statistically significant differences in the level IgG, there were no statistically significant differences in the level of IgA, IgM and Complement C3 (P > 0.05).

3.2.2.3. The Influence of IA on Blood, Urine, Liver-Kidney Function Examination

There were no statistically significant difference between before and after IA in red blood cells, white blood cells and platelet numbers (P > 0.05). Proteinuria and hematuria did larger alternate and do not show the same changing tendency in urinalysis. There have no statistically significant differences in liver function and kidney function before and after IA (P > 0.05).

3.3. Short-Term Follow-Up after DNA Immunoabsorption

13 cases followed up regularly through outpatient service were divided into 3 groups based on vital organs involvement severity (the other 3 cases were lost to follow-up). Group A. involved 8 cases with lupus nephritis (LN), Group B included 2 cases with neuropsychiatric lupus

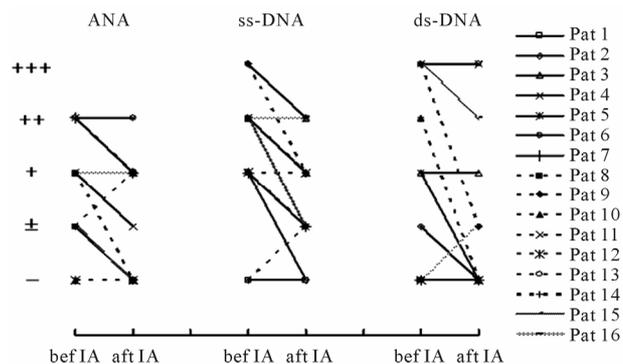


Figure 1. Changes of anti-nuclear antibody and anti-DNA antibodies before and after IA. After immunoabsorption treatment, ANA, anti-ss-DNA antibody and anti ds-DNA antibody were tending to decline (P values are 0.006, 0.006, 0.034 respectively). ANA or anti-DNA antibodies in some cases changed in the same way, which caused chart overlap.

Table 2. Wilcoxon signed rank test results for 8 auto-antibodies.

	Test Statistic							
	Sm	nRNP/Sm	SSA	Ro-52	SSB	AHA	AnuA	ARPA
Z	-0.184	-1.289	-0.816	-0.577	-1.000	-2.232	-2.232	-0.318
Asymp. Sig. (2-tailed)	0.854	0.197	0.414	0.564	0.317	0.026*	0.026*	0.750

Note: *P < 0.05.

Table 3. The influence of IA on immunoglobulin in 16 patients with SLE.

	IgG (g/L)	IgA (g/L)	IgM (g/L)	C3 (g/L)
Before IA	19.03 ± 5.97	2.58 ± 1.66	1.36 ± 0.72	0.42 ± 0.21
After IA	16.50 ± 6.67	2.05 ± 1.13	1.29 ± 0.79	0.36 ± 0.17
P value	0.030*	0.076	0.178	0.196

Note: *P < 0.05.

(NPSLE), and the other 3 cases without vital organs involvement were classified into Group C.

3.3.1. Clinical Manifestations

In group A, after IA, 5 cases have been completed recovered in edema, oliguria and hematuria, for 12 months. 2 cases show a short dropsy again one month after IA, and no repeated after symptomatic treatment in hospital for 6 months. Case No. 9 recurred in NPSLE with the mental symptoms, disorder of consciousness, and secondary epilepsy, she died of secondary respiratory infections 3 months later after IA.

Two cases of Group B improved gradually in cranial nerve function after 3 months of IA.

Two cases in Group C kept stable condition by follow-up for six months, and, without kidney and brain damaged. One of the 2 cases (case No. 15) with vasculitis showed edema in the beginning, and got high fever, abdominal pain followed at 6 months, CT scan and ventricle puncture showed brain stem bleeding, and died in 9 months after IA.

In the all 3 groups, 2 cases got skin rash shortly, 1 case had repeatedly activities and arthritis, the rest of the patients did not relapse, long-term rash, arthritis and other systems during this period.

3.3.2. SLEDAI Score

SLEDAI scores of 12 cases (12/13, 92.3%) showed a downward trend at 3 months after IA (**Figure 2**), the mean of SLEDAI scores were (15.58 ± 11.09) just after IA, (8.33 ± 6.20) 1 month after IA, (2.67 ± 3.85) 3 months after IA. including 10 cases' (76.9%) SLEDAI score decreased from (16.20 ± 12.54) of pre-adsorption to less-than 5 at 3 months after IA, and maintained 5 points at 6 months after IA (2.09 ± 3.05). In 9 months after IA, 5 of them were (1.80 ± 1.48). However, it began to have rising trend on 9 months after IA. 3 cases' SLEDAI score was (4.00 ± 3.46) at 12 months after IA.

3.3.3. 24-Hour Urine Protein Extraction

24-hour urinary protein excretion of 8 cases with LN after IA, 6 cases had gradual decline trend and remained normal after 6 months (**Figure 3**).

3.3.4. Auto-Antibodies

ANA and anti-DNA antibodies decreased gradually after IA, 8 cases (53.8%) became negative completely 3 months later, other auto-antibodies also decreased in different degrees.

3.3.5. Serum IgG and Complement C3

The serum IgG level of 13 was (17.75 ± 6.12) g/L just after IA, (12.57 ± 4.24) g/L at 1 month, (10.38 ± 4.38) g/L at 3 months after IA. IgG level of 11 cases was

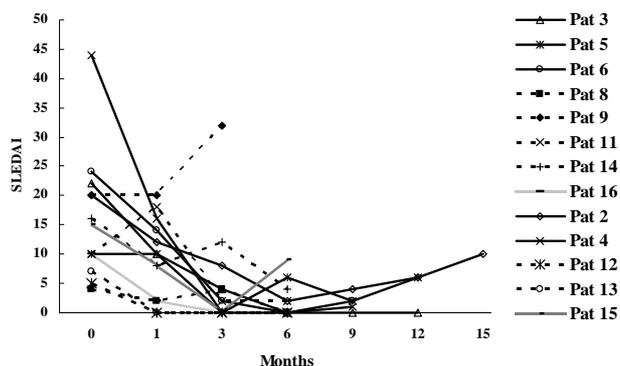


Figure 2. SLEDAI score changes of 13 regularly followed-up cases with SLE. 12 cases were on the downward trend in 0 - 3 months, maintained about 5 points in 3 - 9 months and began to rise 9 months later.

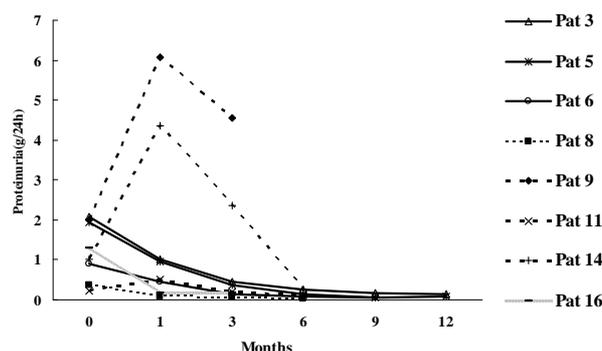


Figure 3. 24-hour proteinuria changes for 8 regularly followed-up cases with LN. 6 cases were on the downward trend and maintained normal level 6 months later.

(11.14 ± 5.47) g/L at 6 months, 5 case was (12.31 ± 4.88) g/L at 9 months, 3 cases was (18.51 ± 7.78) g/L at 12 months after IA (**Figure 4**).

Serum complement C3 level of 13 was (0.38 ± 0.20) g/L at IA completion, it was (0.80 ± 0.28) g/L at 1 month and (1.06 ± 0.23) g/L at 3 months. C3 mean level of 11 cases was (1.05 ± 0.32) g/L at 6 months, of 5 cases was (0.92 ± 0.32) g/L at 9 months, and of 3 cases was (0.76 ± 0.34) g/L at 12 months after IA. It showed that complement C3 gradually increased to normal level from IA completion to 3 months later, and began to decline after 6 months of IA (**Figure 5**).

3.4. Adverse Reactions during DNA Immunoabsorption

2 cases were with transient chills at the beginning of IA treatment and were relieved after dexamethasone intravenous and promethazine muscle injections. 1 case showed palpitation, and was relieved after being given oxygen and slowed down blood flow in IA. No severe complications such as blood coagulation and thrombocytopenia were observed in all cases.

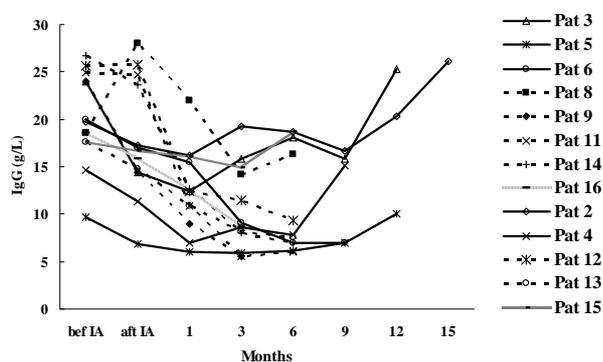


Figure 4. Changes in serum IgG of 13 regularly followed-up cases with SLE. Level of Serum IgG gradually decreased from pre-IA to the 3 months after IA and increased after 3 months of IA.

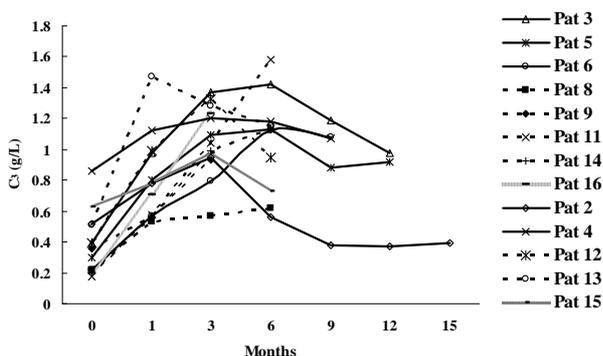


Figure 5. Changes in serum C3 of 13 regularly followed-up cases with SLE. Complement C3 gradually increased to normal from IA completion to 3 months later, and it began to decline after 6 months.

4. DISCUSSION

There were few reports about the application of DNA Immunoabsorption in pediatric SLE. In LIU Ying & WU Yubin [9]'s research, they found that pediatric patients' signs and symptoms were significantly improved 4 after DNA immunoabsorption treatment, ANA antibody titers decreased substantially after IA, anti-ds-DNA antibodies were removed completely, IgG significantly decreased after adsorption, serum complement was on upward trend, it had no significant effect on the liver and kidney function, myocardial enzyme and erythrocyte sedimentation rate (ESR). 4 cases had thrombocytopenia, but it had no long-term follow-up comparison for immunological parameters before and after IA.

This research included 16 SLE patients in active stage by IA treatment and had no significant adverse reaction, among which only 3 cases had slight anaphylactic reaction and were relieved by using glucocorticoid and antihistamine. It proved that this therapy is as safe as other routine blood purification methods. For the 16 SLE cases (among which 13 cases had no immunosuppression treatment before IA, and 13 cases follow-up regular), ANA,

anti-DNA antibody and IgG level decreased obviously in 2 - 7 days after IA, which were consistent with their clinical symptoms and signs improvement. The quick relief of this disease in short time proved IA validity. Complement C3 didn't go up because of rechecking in a short time. The auto-antibodies reduction of AHA, AnuA after IA were probably because the compositions of plasmosome include histone and DNA.

Recent follow-up results of this study showed SLE pediatric patient would be gradually relieved by combination treatment of glucocorticoid and immunosuppressive after IA. Clinical symptoms and signs, disease activity SLEDAI score, kidney damage of 24-hour urine protein extraction, autoantibody, immunoglobulin, complement C3 been improvement obviously within 3 to 6 months after IA. During this time, clinical manifestations and urinary protein of LN patients remained stable, neurological symptoms and signs of NPSLE patients disappeared completely. For the other patients without kidney or brain involvement in the beginning, their clinical manifestations remained stable, all immunological indicators continued to be relieved within 6 to 9 months after IA, but some immune parameters such as IgG, complement C3, anti-ds-DNA antibodies repeatedly after 9 months.

Willeke P., *et al.* [10] found that for SLE patients peripheral blood, mononuclear cells increased which was the expression of GM-CSF (Granulocyte macrophage colony stimulating factor), CD4+T cells increased which was expression of CD71, B cells increased which was expression of CD86, while immunoabsorption was to reduce anti-ds-DNA at the same time, these cells also reduced accordingly. In addition, Willeke P., *et al.* [11] also found that mononuclear cells decreased after IL-10 secretion in peripheral blood. The clinical manifestation improvements in fever, arthritis, serositis as well as damages to kidney and brain in this study might be related with the above-mentioned mechanisms. Immunological parameters such as anti-ds-DNA antibody, IgG, complement C3 were improved continuously to normal in 3 to 6 months after IA, which might be connected with the application of IA immune regulated [6], reticuloendothelial system function recovery [12] and high-dose glucocorticoid application. Immunological parameters relapsed in 6 to 9 months after IA might because of dosage reduction in glucocorticoid and other immunosuppressive drugs at remission induction stage, and remarkable decreased of self-antibodies after IA, might because of negative feedback, immune response stimulated by infection and so on.

Additionally, it should be pointed out that for 2 dead cases in this report, hospital duration of Case No. 9 was only 20 days, but anemia, hypertension and renal function damage were obvious, renal biopsy showed lupus nephritis P-G (A/C), and Case No. 9 was taken 1 time of intravenous mabthera after 1.5 months of IA, her periph-

eral blood lymphocytes classification CD19+ cell count decreased from 5.5% to zero after 5 mabthera 3 weeks later, and died in 3 months after IA., the cause of death might because of acute progressive nephritis, lupus encephalopathy and respiratory infection caused by secondary B cell deficiency. Case No.15 was reexamined regularly in out-patient department, EEG and brain image were normal during re-check, without kidney damaged, her clinical manifestation remained stable for 3 months after IA. However, after 6 months of IA, the patient got high fever, abdominal pain and convulsion, and died 2 days after convulsion, the cause probably was brain stem hemorrhage caused by cerebral vasculitis. We could make the conclusion that SLE need multi-target therapy from these two severe cases.

Our research shows that DNA immunoadsorption (DNA-IA) can effectively improve SLE patients' clinical symptoms and immunological parameters, IA also been used to relieve clinical manifestations, disease activity and laboratory parameters. DNA-IA might have synergistic effect with glucocorticoid in relieving SLE symptoms, protecting vital organs and improving long-term prognosis. Moreover, at induced remission stage, IA application would be useful in decrease dosage of traditional medicine. It might have a catalytic role in reducing toxicity caused by long-term use of corticosteroid and cytotoxic drugs.

5. CONCLUSION

DNA immunoadsorption is a safe and effective therapy for active SLE in childhood, which could improve clinical symptoms, eliminate ANA and anti-DNA antibodies. Combining with corticosteroids and immunosuppressive drugs, it could significantly reduce the activity of disease and protect vital organs function in the short term after DNA-IA therapy.

6. ACKNOWLEDGEMENTS

We declare that this study has been approved by our hospital's ethic committee, we informed all patients involved in the experiment and signed the agreements before clinical trial.

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