

Original Article

Immunological, Biochemical and Clinical Aspects of Childhood Nephrosis Before and After Different Immunomodulating Therapies

The Impact of Levamisole Therapy on the Immune Status of Nephrotic Children and on the Course of the Disease

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Abstract

Objectives: To study the immunological and clinical effects of long term levamisole therapy in nephrotic children.

Methods: The study included 24 children aged from 4-12 years (14 males and 10 females) who presented with nephrotic syndrome (8 with steroid-sensitive disease, 8 with steroid-dependent disease and 8 with steroid-resistant disease). The study also included 10 apparently healthy controls of matched age and sex. All patients and controls were subjected to the following immunologic studies: percentages of total T lymphocytes and their subsets including T-helper (CD₄) and T-suppressor (CD₈) cells, serum levels of interleukin-2 (IL-2), immunoglobulins (IgG, IgA, IgM and IgE), certain complement components (C₃ and C_{1q}) and circulating immune complexes (CICs) as well as lymphoblast transformation index (LTI). A follow up study of these parameters was done after 28 weeks therapy during which all the studied cases received daily prednisone for 4 weeks, followed by 24 weeks therapy with alternate day prednisone for the steroid-sensitive cases while both steroid-dependent and steroid-resistant cases received levamisole with concomitant prednisone therapy.

Results: Alterations in the studied parameters and the effect of different therapeutic regimens on these alterations are detailed.

Conclusions: We concluded that several immunologic alterations were found in childhood nephrotic syndrome. Data of the present study support the hypothesis that abnormal immunoregulation play a role in the pathogenesis of the disease. Also, the role of levamisole in the management of childhood nephrotic syndrome seems to be promising, and its restoration of normal lymphocyte number and function possibly helps to eliminate gradually the pathophysiological events leading to a nephrotic state. We advocate its use in cases with frequently relapsing disease, cases with steroid-dependent disease and/or those with steroid-resistant disease.

INTRODUCTION

Management of patients with idiopathic nephrotic syndrome (INS) is often complicated by drug toxicity. The most troublesome groups are those with either steroid-dependent or steroid-resistant disease, in whom standard alkylating

therapy (e.g. cyclophosphamide) is indicated. The long-term immunosuppression in these patients and the cumulative risk of further alkylating therapy are worrying. An alternative therapy with an immunostimulant would be an attractive option⁽¹⁾.

Levamisole is one such immunostimulant drug; this agent was originally developed as an antihelminthic but was subsequently shown to have immunomodulating properties^(2,3,4,5). There are several reports of successful treatment of INS with levamisole, with and without concomitant corticosteroid therapy^(1,4,5,6).

The present study was undertaken to assess the role of levamisole in the management of childhood nephrotic syndrome (NS).

SUBJECTS AND METHODS

The study included 24 children aged from 4-12 years (14 males and 10 females) who presented with N.S. (8 with steroid-sensitive disease, 8 with steroid-dependent disease and 8 with steroid-resistant disease), as well as 10 apparently healthy children of matched age and sex as controls. Cases were admitted to the Pediatric Department, Assiut University Hospital, during the period from August (1992) to March (1996).

The criteria for diagnosing NS were: edema, plasma albumin < 25 g/liter and proteinuria > 40 mg/m²/hr⁽⁷⁾. Patients with a history suggestive of collagen disease, malignant disease and/or chemotherapy as well as chronic diarrhea were excluded from the study. Also, none of the studied cases had clinically obvious infection at the time of the study.

Besides meticulous history and thorough clinical examination, all the cases and controls were subjected to renal sonography and also to the following investigations: complete blood picture, total plasma proteins and albumin/globulin ratio, serum

cholesterol, blood urea and serum creatinine, midstream urine culture and bacterial count, estimation of proteinuria/24 hours and its selectivity index⁽⁸⁾, urinary RBP⁽⁹⁾ and protein/creatinine ratio. Also, tests to study the immunologic state were done for cases and controls including: pan T-lymphocytes (CD₃) percentage and its subsets CD₄ and CD₈ as well as CD₄/CD₈ ratio by indirect immunofluorescence technique (Biotest, Gamma Trade); serum level of IL-2 by immunoenzymatic assay using kits supplied by Medgenix Diagnostic, Gamma Trade; serum levels of immunoglobulins IgG, IgA and IgM by single radial immunodiffusion technique⁽⁹⁾; serum level of IgE by ELISA technique using coated microtiter strips (Eurogenetics, Gamma Trade); serum levels of certain complement components C₃ and C_{1q}; serum level of circulating immune complexes according to Lin et al.⁽¹⁰⁾; and lymphoblast transformation index: the peripheral blood mononuclear cells were isolated by density centrifugation method⁽¹¹⁾.

Follow-up study for these immunologic parameters was done after 28 weeks of therapy for all the studied cases. Laboratory monitoring was performed weekly by full blood count, complete urinalysis and 24 hour urinary protein concentration for 4 weeks, then 2-weekly for the duration of therapy; manifestations of drug toxicity were recorded. Also monitoring was performed monthly for blood urea and serum creatinine. Plasma proteins and serum cholesterol were assessed at the end of treatment.

After stopping therapy, the studied

cases were followed up for 2 years, during which relapses and intercurrent infections were recorded.

Treatment Protocols:

Therapy was deferred for up to 2 weeks for cases with the first attack because of the possibility of spontaneous remission within the first 8-15 days, and also to diagnose and treat infections. Treatment for relapsers was deferred for up to 5 days but they were not allowed to become severely edematous. Prompt treatment was given for children with a past history of severe relapses, especially when complicated with hypovolemia⁽⁷⁾. All the cases were treated with prednisone at a dosage of 60 mg/m²/24 hr. (maximum daily dose 60 mg) divided into three or four doses over the day. Response was defined as the point at which urine became free of proteins. Five days after the urine had become free of proteins, the dose of prednisone was changed to 60 mg/m² (maximum dose of 60 mg) taken every other day as a single dose with breakfast. This alternate day regiment was continued for 24 weeks.

Levamisole was used regularly in a dose of 2.5 mg/kg on alternate days for 24 weeks with concomitant alternate day corticosteroid therapy for both steroid-dependent and steroid-resistant cases.

Definitions:

1. Remission: Urinary protein excretion < 4 mg/hr/m² or negative protein on a dipstick for 3 consecutive days⁽⁷⁾.
2. Steroid dependence: Two consecutive relapses occurring during corticosteroid treatment or within 14 days of its cessation⁽⁷⁾ or relapse shortly after

switching to alternate day therapy⁽¹²⁾.

3. Steroid resistance: failure to achieve response in spite of 4 weeks prednisone 60 mg/m²/day⁽¹²⁾.

RESULTS

The results of the present study showed that several immunologic alterations were found in childhood N.S. Also, it showed that addition of levamisole to the corticosteroids had favorable results when used for treatment of either steroid dependent or steroid resistant cases. Levamisole restored to normal all the studied immunological parameters, both in vivo and in vitro, while the reverse had occurred after treatment of the steroid sensitive cases with corticosteroids only as some of the studied immunological parameters worsened. Data are shown in Tables 1-4.

The immunoregulatory effect of levamisole correlated with the clinical course of the disease. The follow-up of the studied cases for 2 years after stopping therapy revealed that steroid sensitive cases showed significantly higher frequency of relapses (2.75 ± 0.43) than either steroid dependent cases (1.63 ± 0.48) or steroid resistant cases (1.88 ± 0.33) $p < 0.001$ for each. It is noteworthy that rates of relapsers among the last two groups were significantly higher (75% and 62.5% respectively) than that among steroid sensitive cases (12.5%) $p < 0.02$ and $p < 0.05$ respectively. None of the 16 patients treated with levamisole with concomitant prednisone had serious side effects. Neutrophils decreased below $4 \times 10^9/L$ in only one patient after 6 months of treatment. After levamisole withdrawal

Table (4): The studied immunological parameters after therapy in relation to the therapeutic regimen

		CD ₃	CD ₄	CD ₈	CD ₄ / CD ₈	IL-2	Immunoglobulins IU/ml				Complement mg/dL		CICs	LTI
		%	%	%	ratio	IU/ml	IgG	IgA	IgM	IgE	C ₃	C _{1q}	µgEq/ml	
I- before therapy (n=8)	mean	62.62	40.06	20.13	1.71	0.99	123.7	105.5	195.2	225.2	112.0	5.58	8.11	36.35
	± SD	± 8.34	± 5.61	± 2.13	± 0.48	± 0.21	± 29.81	± 31.24	± 70.14	± 54.91	± 25.03	± 1.43	± 4.16	± 6.12
II- after therapy (n=8)	mean	75.34	49.26	20.07	2.48	1.46	134.9	106.3	180.2	233.6	95.16	5.23	7.95	79.03
	± SD	± 8.75	± 5.02	± 2.86	± 0.53	± 0.49	± 30.43	± 36.19	± 54.83	± 61.38	± 20.45	± 1.92	± 4.22	± 23.04
III- controls (n=10)	mean	72.5	47.71	21.11	2.21	1.41	135.1	104.5	183.9	278.9	94.11	4.56	7.46	67.09
	± SD	± 6.81	± 5.86	± 2.97	± 0.56	± 0.52	± 29.32	± 28.93	± 53.62	± 73.82	± 16.74	± 1.83	± 3.94	± 11.64
Significance of Diff	I vs II	***	****	N.S.	***	*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	*****
	I vs III	*	*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	*****
	II vs III	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

@ Cases received corticosteroid therapy

@@ Cases received levamisole with concomitant corticosteroids

N.S. Not significant

* p < 0.05

** p < 0.025

*** p < 0.02

**** p < 0.01

***** p < 0.005

***** p < 0.001

DISCUSSION

In agreement with our results, several studies revealed immunologic abnormalities in nephrotic patients, mainly, alterations in T lymphocyte numbers^(13,14) and function^(15,16,17), in addition to alterations in serum levels of immunoglobulins^(7,18,19), complement components^(7,19,20) and circulating immune complexes⁽²¹⁾. Some investigators considered these alterations a consequence of the nephrotic state^(15,22) while others reported that these alterations imply an involvement of the immune system in the pathogenesis of the NS^(23,24). Whether a cause or an effect, the immunological dysfunctions increase the risk of serious infections in these patients. On the other hand, it is remarkable that either immunosuppression with corticosteroids^(7,20,25) or immunostimulation with 1-tetramisole (levamisole)^(1,4,5,26) has a beneficial effect in patients with INS. The present study is a

trial to clarify this paradox by investigating the impact of levamisole on the immune status of nephrotic children and on the course of the disease. Levamisole - used as an antihelminthic - has immunoregulatory properties⁽⁵⁾. In therapeutic concentrations, levamisole restores to normal, both in vivo and in vitro, many of the effector functions of T-cells and phagocytes. It activates macrophages, promoting chemotaxis and phagocytosis, while enhancing the production of lymphokines⁽²⁾. Also, it induces maturation of T-cells. However, these effects are reproducible only when the immune system is depressed⁽³⁾. Prandota et al.⁽⁴⁾ found that levamisole had increased the number of circulating T-cells in patients with subnormal values; this finding suggests maturation of pre-T-cells to T-cells.

As regards the effect of levamisole therapy with concomitant prednisone on the studied immunological parameters in the

present study, both steroid-dependent and steroid-resistant cases showed significant elevation in CD₃ and CD₄ percentages, CD₄/CD₈ ratio, IgG and C_{1q} serum levels as well as LTI after therapy than before. Also, significant reduction in CD₈ percentage as well as IgM and C₃ serum levels were found in these two groups after therapy. Moreover, cases with steroid-dependent nephrotic syndrome (SDNS) showed significant reduction in IgE serum level after therapy than before.

Several studies tried to evaluate the immunoregulatory effect of levamisole in the management of primary nephrotic syndrome^(1,4,5,26). In a study by Mehta et al.⁽⁵⁾, fourteen patients were given levamisole therapy for a period of 20-24 weeks. Their results showed that blastogenesis index had increased, and serum IgG had increased to more than twice the pretreatment values. In another study, Drachman et al.⁽²⁶⁾ reported that LTI had increased to normal values in 7 cases with frequently relapsing nephrotic syndrome during levamisole therapy, and added that, in those cases, prednisolone dosage could be decreased significantly or discontinued altogether.

As regards the effect of levamisole on lymphocyte subpopulations, Prandota et al.⁽⁴⁾ reported that significant increment in CD₃ and CD₄ counts, as well as IgG serum level with normalization of IgM serum level were found after levamisole therapy for a period of 2.5-6 months in 14 children with steroid sensitive nephrotic syndrome (SSNS).

The immunostimulating effect of levamisole was also proved when it was used as adjuvant therapy in other disease

entities as chronic cholestatic liver diseases and metastatic murine pancreatic tumor⁽²⁷⁾ and enhanced lymphoblast transformation after levamisole therapy was proved in several studies^(28,29,30).

As opposed to this, data of the present study showed significant reduction in CD₃ and CD₄ percentages as well as CD₄/CD₈ ratio in steroid sensitive nephrotic patients after corticosteroid therapy than before. This agrees with Yokoyama et al.⁽³¹⁾. The immunosuppressive action of glucocorticoids is mediated through perturbation of leucocyte traffic with a dramatic lytic effect on lymphocytes⁽³²⁾. Axelrod⁽³³⁾ and Winkelstein⁽³⁴⁾ reported that steroids cause a striking reduction in the number of circulating lymphocytes as a result of sequestration of recirculating lymphoid cells into lymphoid tissues, including the bone marrow. They added that, the total numbers of circulating T cells are markedly decreased, and among their subsets, numbers of CD₄ cells are reduced to a greater extent than are numbers of CD₈ cells.

As regards the significant decrease in IL-2 serum level which was found in the present study in cases with SSNS after corticosteroid therapy than before, Arya et al.⁽³⁵⁾ reported that glucocorticoids have a direct effect on lymphokine production and selectively inhibit IL-2 production. This inhibition is not only due to the blockage of IL-2 expression at the transcriptional level, but it is also a result of decreased IL-2 messenger RNA stability⁽³⁶⁾. Also, Axelrod⁽³³⁾ and Winkelstein⁽³⁴⁾ reported that the decrease in IL-2 serum level after corticosteroid therapy is most probably an

indirect result of suppressed IL-1 production by monocytes. In addition, the decreased CD₄ number after corticosteroid therapy may play a role, as it is considered the major source of IL-2⁽³⁷⁾.

In the present study, patients with SSNS showed significant reduction in IgM and IgE serum levels and significant elevation in IgG serum level after corticosteroid therapy than before. This is in agreement with previous studies^(38,39) and may be due to the anti-inflammatory effect of glucocorticoids which has been attributed to the induction of a set of proteins called lipocortins, which inhibit phospholipase A₂⁽⁴⁰⁾, as well as to direct inhibition of the expression of inflammatory mediators such as arachidonic acid metabolites and IL-1⁽⁴¹⁾. Suppression of the inflammation leads to correction of the glomerular basement membrane permeability with prevention of the passage of low molecular weight proteins such as albumin and IgG into urine. As a result, serum levels of albumin and IgG will increase. Also, with the increase in serum albumin level, the rate of hepatic synthesis of proteins with the concomitant synthesis of immunoglobulins will decrease. This may lead to the decrease in IgM and IgE serum levels. Furthermore, correction of the nephrotic state leads to correction of the patient's ability to switch their immunoglobulin synthesis from IgM to IgG⁽³⁴⁾.

Regarding C₃ and C_{1q}, it was reported that serum levels of the various classical and alternative pathways complement components are deranged during relapse but return to normal during remission⁽⁷⁾. This is in agreement with the results of the present

study. It is likely that most of the abnormalities of the complement system are secondary to the nephrotic state, particularly the urinary loss of low molecular weight proteins⁽⁴²⁾, as well as the increased hepatic protein synthesis consequent to the hypoalbuminemia⁽³⁹⁾.

In agreement with Broyer et al.⁽¹⁹⁾ and Tina et al.⁽⁴²⁾, our cases with SSNS showed significantly lower serum level of CICs after corticosteroid therapy than before. The pathogenicity of these immune complexes in minimal change nephrotic syndrome (MCNS) is doubtful, as renal histology does not show features of immune complex disease. Several antigens in these complexes have been identified suggesting that autoantibody production occurs in MCNS^(42,43).

Data of the present study showed that the LTI decreased significantly after corticosteroid therapy. It was reported that in the case of SSNS, steroid therapy has two opposing effects on the lymphocyte proliferative capacity. First, is its ability to decrease the suppressor lymphokine soluble immune response substance (SIRS) which has been described in blood and urine of patients with SSNS but not steroid-resistant nephrotic syndrome (SRNS), and is capable of decreasing immune responses *in vitro*⁽⁷⁾. Schnaper⁽¹⁵⁾ and Broyer et al.⁽¹⁹⁾ reported that SIRS concentration in blood and urine rises during relapse but decreases a few days before remission occurs. The second effect is the ability of steroid therapy to alter T lymphocyte activities. It was reported that steroid therapy inhibits the *in vitro* lymphoproliferative responses, in part from an impairment in the synthesis and secretion

of IL-2 which is essential for the clonal expansion of activated lymphocytes⁽⁴⁴⁾. Also, steroid therapy inhibits the production of IL-1 by monocytes as well as the response of T-cells to IL-1⁽⁴⁵⁾. Furthermore, corticosteroids can block the progression of phytohemagglutinin-stimulated lymphocytes through the mitotic cycle. They inhibit the entry of cells into the pre-DNA synthetic phase and arrest the progression of activated lymphocytes from the pre-DNA synthetic phase to the DNA synthesis phase⁽⁴⁴⁾.

In the present study, the follow-up of the studied cases for 2 years revealed that cases receiving prednisone only (steroid sensitive cases) showed significantly higher frequency of relapses than cases receiving levamisole with concomitant prednisone (both steroid dependent and steroid resistant cases). This is in agreement with Meregalli et al.⁽⁴⁶⁾. It is noteworthy that, in our study, rates of relapsers - obtained from the history data - among steroid dependent and steroid resistant cases were significantly higher than that among steroid sensitive group.

Neuhaus et al.⁽⁴⁷⁾ reported that most children with SSNS relapse after their initial treatment. About half of the children experience frequent relapses and most of these become steroid dependent, a few continuing with active disease in adult life. They added that levamisole proved to be effective as the initial form of alternative treatment in half of the patients with SSNS. In a study by Mehta et al.⁽⁵⁾ levamisole therapy led to prolongation of the duration of remission in patients with frequently relapsing INS. Also, in another study by the British Association for Pediatric Nephrology (BAFPN)⁽¹⁾, they

have confirmed a statistically significant steroid sparing effect during therapy with levamisole in their double-blind placebo-controlled study conducted in patients with a high degree of steroid dependency. Furthermore, Xu et al.⁽⁶⁾ found that the longer the course of levamisole therapy, the lower the recurrence rate. Additionally, in a randomized controlled trial, Dayal et al.⁽⁴⁸⁾ found that levamisole therapy during remission led to prolongation of remission maintenance. On the other hand, the present study showed that the beneficial effects of retreatment with levamisole in recurrent cases were much more rapid in onset and longer in duration than on initial treatment. This is in agreement with previous reports^(6,48).

The follow-up results of the present study revealed that cases with SSNS showed significantly higher frequency of intercurrent attacks of infections than either steroid-dependent or steroid-resistant cases. Levamisole has been used to treat certain varieties of recurrent and chronic infections with beneficial results. It reduces the frequency, duration and severity of infections in patients with immune deficiency diseases⁽²⁾ even in patients with HIV⁽⁴⁹⁾. In NS, it was found that treatment with levamisole definitely reduced the number and severity of infections that often accompany or precede a relapse⁽⁵⁾ while steroid therapy increases the immunological dysfunctions in nephrotic patients leading to frequent attacks of serious infections^(19,44,45).

The most worrying side effect of levamisole therapy is neutropenia or rarely agranulocytosis. These changes are rever-

sible and seem to bear no definite relation to dose frequency or duration of therapy⁽¹⁾. In the present study, none of the 16 patients received levamisole had serious side effects. Neutrophils decreased below $4 \times 10^9/L$ in *only one patient after 6 months of treatment*. After levamisole withdrawal neutrophil level increased rapidly to the normal count within one week. This agrees with several studies^(1,5,26).

In summary, several immunologic alterations were found in nephrotic syndrome. It is, however, not yet clear if these alterations play a role in the pathogenesis of the disease or whether they represent secondary manifestations. Our results with levamisole tend to support the former possibility. Not only did levamisole correct these abnormalities but we also observed a correlation between the immunoregulatory effect of the drug and the clinical course of the disease. Therefore, the role of levamisole in the management of INS seems

to be promising, and its restoration of normal lymphocyte number and function possibly helps to eliminate gradually the pathophysiological events leading to a nephrotic state.

Since levamisole is a relatively non-toxic drug, it is worth considering as an alternative to further alkylating therapy or cyclosporin, during corticosteroids induced remission in whom additional medication, apart from corticosteroids is being considered. We advocate its use on a trial basis in cases with frequently relapsing disease, cases with steroid-dependent disease and in cases resistant to steroid therapy. Regular full blood count monitoring should be undertaken during therapy. More studies on the optimal dosage, regularity of administration and duration of levamisole therapy as well as the dosage and course of the concomitant corticosteroids will be needed.

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