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ORIGINAL ARTICLE

Short- and long-term efficacy of levamisole as adjunctive therapy in childhood nephrotic syndrome

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Abstract Many children with steroid-dependent nephrotic syndrome (NS) have significant sequelae despite steroid-sparing therapies. Levamisole may reduce short-term relapse frequency (RF) with minimal side effects. Little data exist, however, as to its long-term effect. To assess both short- and long-term efficacy in NS, RF and cumulative annual steroid burden were quantified in ten consecutive children with steroid-dependent NS treated with levamisole. Data were analyzed for three time periods: 1 year prior to levamisole therapy (Pre-Lev), during 1 year of levamisole therapy (During-Lev), and the year after cessation of all levamisole therapy (Off-Lev). Median RF fell from 6.0 (4.0–9.0) relapses/patient per year Pre-Lev to 0.0 (0.0–4.0) During-Lev (p=0.002) with 6/10 patients having no relapse and 0.5 (0.0–8.0) Off-Lev (p=0.01) with 5/10 patients without relapse. Concurrently, cumulative annual steroid burden fell from 6,067 (1,660–8,691) mg/m^2 per year Pre-Lev to 2,920 (782–5,271) During-Lev (p=0.002) and 716 (0–3,637) Off-Lev (p=0.002). In 4/5 hypertensive children, blood pressure normalized During-Lev. Somatic indices also improved: height Z scores, which fell from 0.8 (−2.4 to 3.6) at diagnosis to −0.6 (−2.7 to 0.4) Pre-Lev (p=0.004), remained stable at −0.6 (−3.0 to 0.6) after 1 year of therapy and –0.5 (−2.6 to 0.2) Off-Lev. Height velocity improved from 3.0 (0.3–6.0) cm/year Pre-Lev to 3.7 (0.0–8.0) cm/year During-Lev and 5.4 (0.0–9.1) Off-Lev. We conclude that levamisole is an effective short- and long-term steroid-sparing agent in pediatric NS.

Keywords Children · Pediatric · Minimal-change disease · Steroids · Side effects · Growth · Height

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Introduction

Although most children with nephrotic syndrome (NS) are steroid sensitive, frequent relapses and steroid dependency are common [1], leading to prolonged or repeated exposures to steroids and ensuing serious sequelae. As a result, many children with NS come to be treated with alkylating agents such as cyclophosphamide or chlorambucil, antimetabolites such as azathioprine or mycophenolate mofetil, or calcineurin inhibitors such as cyclosporine or tacrolimus for their steroid-sparing effects. Although these agents do indeed reduce steroid burden, they all have potential serious adverse effects.

Levamisole, an antihelminthic immunomodulator, has been used for several decades to treat idiopathic childhood NS, especially steroid-dependent disease [2–8]. Although prior reports focused on levamisole as an effective adjunctive therapy in reducing short-term relapse frequency (RF), limited data exist as to its long-term efficacy. In a recent retrospective study, its steroid-sparing effects were suggested to last for months after its withdrawal [9]. Even more limited data exists as to the effect of levamisole on steroid-induced clinical complications, such as hypertension, or alterations in somatic measures, such as height and weight. In this report, we assess both the short- and long-term efficacy of levamisole therapy on NS relapse rates and annual steroid burden in a cadre of children with steroid-dependent nephrosis and also assess its effects on markers of steroid-induced clinical complications.

Materials and methods

Patients

We identified retrospectively ten consecutive children between 12 months and 18 years of age with idiopathic steroid-dependent NS treated with levamisole for evolving or established steroid sequelae at Children's Hospital Boston between 1999 and 2004. In accordance with criteria established by the International Study of Kidney Disease in Children (ISKDC), NS was defined as the association of proteinuria > 40 mg/m^2/h, hypoalbuminemia < 2.5 g/dL, edema, and hyperlipidemia. Recurrence of proteinuria = 2+ by dipstick of first morning urine void for at least 3 consecutive days and requiring initiation or augmentation of steroid therapy was considered a relapse. Steroid dependency was defined by two consecutive relapses while still on some sort of steroid regimen or within 2 weeks after their complete withdraw. Exclusion criteria included non-idiopathic NS such as membranous glomerulonephritis, membranoproliferative glomerulonephritis, immunoglobulin (Ig)A nephropathy, or NS related to a systemic disease.

Treatment

Levamisole therapy

All children received a 12-month course of oral levamisole at a dose of 2.5 mg/kg 3 alternate days a week, generally on Monday-Wednesday-Friday. During relapses or any augmentation of steroid therapy, levamisole therapy was not discontinued. If patients were tapered completely off steroids, levamisole was maintained as monotherapy at the same dose and dose frequency.

Steroid therapy

Before levamisole initiation, children were treated with prednisone according to an established local protocol. At the time of the initial diagnosis, prednisone 60 mg/m^2 per day to a maximal dose of 80 mg daily was started for 4 weeks. If remission had not been attained by 4 weeks, therapy was continued at the same daily dose for up to 8 weeks. If remission occurred by 4 weeks of daily steroids or during the ensuing 4 weeks, then children were tapered to 40 mg/m^2 on alternate days for an additional 4 weeks and then tapered completely off steroids over the next 4–8 weeks. Subsequent relapses were treated with oral prednisone at 60 mg/m^2 per day until patients reported 3 days free of proteinuria. Prednisone was then transitioned immediately to alternate-day therapy and the dose weaned over 4–8 weeks thereafter. When steroid dependency occurred, children were left on a maintenance dose of 0.1–0.5 mg/kg every other day for a period of up to 6 months, and then further tapering was reassessed. After the introduction of levamisole, when steroid therapy was necessary, it was provided in a routine identical to pre-levamisole therapy.

Evaluation of treatment efficacy and safety

Treatment efficacy was evaluated by its effect on RF, cumulative annual steroid burden, blood pressure, and measures of somatic growth. To assess the safety of levamisole therapy, data were abstracted from clinical and laboratory examinations done on patients at 2, 4, and 8 weeks postinitiation of levamisole and then at least every 4 months during therapy. RF, steroid burden, and growth velocity data were collected and analyzed for three separate time periods for each patient: 1 year prior to initiation of levamisole (Pre-Lev), the year of levamisole therapy (During-Lev, short-term effect), and the year following cessation of levamisole (Off-Lev, long-term effect). Somatic indices and laboratory values were compared between different time points: at time of diagnosis (at Dx), Pre-Lev, after 1 year of levamisole therapy (End-Lev), and Off-Lev.

Statistical analysis

Results are expressed as median (with minimum–maximum range) for quantitative data and as ratio for gender. Two-by-two comparisons of quantitative data between Dx/Pre-Lev, Pre-Lev/During-Lev, Pre-Lev/Off-Lev, and Pre-Lev/End-Lev were performed using the Wilcoxon's signed-rank test for paired samples. Two-by-two comparisons of quantitative data between children who attained long-term sustained remission and children who continued to relapse were done by the Wilcoxon's rank-sum test for independent samples. Fisher's exact test for independent samples was used to compare gender distributions between children who attained long-term sustained remission and children who continued to relapse. Statistical significance was set at $p<0.05$.

Results

Demographics and clinical characteristics

Demographic information and data regarding relapse rate and steroid burden is found in Table 1. Six out of the ten children were boys (60%). Median age at diagnosis was 4.2 (1.4–12.8) years, whereas the median duration of NS was 6.4 (2.1–10.3) years. Among the four children who had a biopsy-proven diagnosis, three had minimal-change disease and one had steroid-sensitive focal and segmental glomerulosclerosis.

Table 1 Patient characteristics

Patient	Gender	Age (years)	NS duration (years)	Relapses			Steroid burden (mg/m^2 per year)		
				Pre-Lev	During-Lev	Off-Lev	Pre-Lev	During-Lev	Off-Lev
1	F	10.6	8.7	8	4	1	6633	3569	733
2	M	13.5	6.6	7	4	0	7466	4113	729
3	M	10.9	4.9	6	3	0	7732	3040	1219
4	F	4.2	2.8	6	4	8	8301	5271	3637
5	F	11.9	7.4	4	0	4	1660	782	1309
6	M	13.3	10.3	4	0	0	3680	1700	0
7	F	14.9	2.1	9	0	0	3716	939	0
8	M	10.4	6.1	6	0	0	3799	803	0
9	M	10.9	6.9	6	0	1	5500	2799	449
10	M	8.2	4.6	5	0	3	8691	3104	703

NS nephrotic syndrome, *Pre-Lev* year prior to levamisole therapy, *During-Lev* year on levamisole therapy, *Off-Lev* year after cessation of levamisole therapy, *F* female, *M* male

Pre-Lev, NS relapses were common, with a median rate of 6.0 (4.0–9.0) relapses/patient per year (Fig. 1). During-Lev, median RF fell to 0.0 (0.0–4.0) relapses/patient per year (p= 0.002), with six of the ten patients having no relapse. During-Lev, all children had marked diminution in annual steroid burden from a median of 6,067 (1,660–8,691) mg/m^2 per year Pre-Lev to 2,920 (782–5,271) mg/m^2 per year (p=0.002).

This effect was long lasting, as the median RF remained low Off-Lev, 0.5 (0.0–8.0) relapses/patient per year (p= 0.01), with five patients having no relapse. Annual steroid burden also continued to fall, to 716 (0–3,637) mg/m^2 per year (p=0.002), with three children receiving no steroids.

Children with sustained remission

In Table 2, the five children who attained long-term sustained remission are compared with the five children who continued to relapse, albeit at a reduced rate. Children with sustained remission on levamisole did not differ from children who relapsed.

Effects on steroid adverse events

Among the five children with hypertension Pre-Lev, blood pressure fell to normal within 1 year in three and in all but one child by the end of the year Off-Lev. Height Z scores

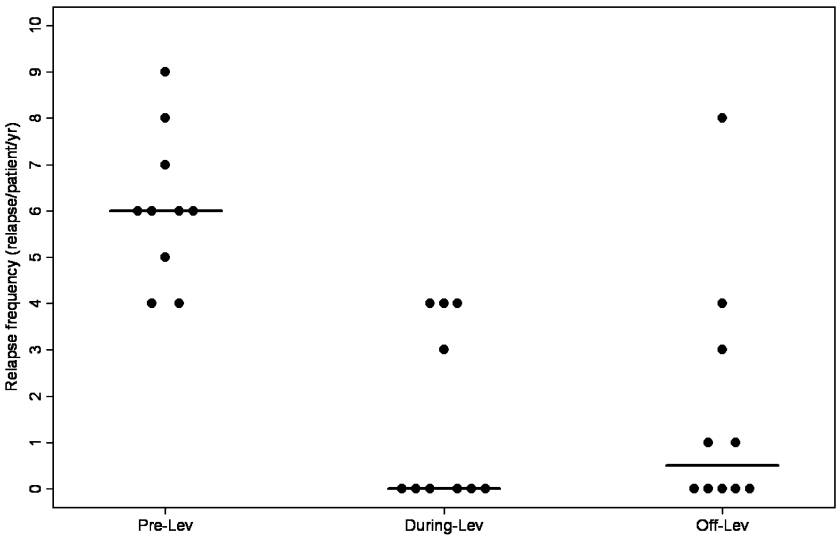

Fig. 1 Relapse frequency significantly decreased from the year prior to levamisole therapy (*Pre-Lev*) to the year during levamisole therapy (*During-Lev*) (p=0.002 by Wilcoxon's signed-rank test for paired samples) and remained stable the year after cessation of levamisole therapy (*Off-Lev*)

Table 2 Clinical characteristics of children with sustained remission during and following levamisole therapy and children who manifested frequent relapses

Clinical characteristics	Children with sustained remission	Children with relapses	*P* value
Prelevamisole annual steroid burden (mg/m^2 per year)	3799 (3680–7732)	6633 (1660–8691)	0.55
Relapse frequency the year prior to levamisole (relapses/patient per year)	6.0 (4.0–9.0)	6.0 (4.0–8.0)	0.60
Relapse frequency on levamisole (relapses/patient per year)	0.0 (0.0–4.0)	0.0 (0.0–4.0)	1.00
Relapse frequency the year after withdrawal of levamisole (relapses/patient per year)	0.0 (0.0–0.0)	3.0 (1.0–8.0)	0.008
Age (years)	13.3 (10.4–14.9)	10.6 (4.2–11.9)	0.11
Duration of NS (years)	6.1 (2.1–10.3)	6.9 (2.8–8.7)	0.84
Gender ratio (M/F)	4.0	0.7	0.52

Statistical comparisons were performed using the Wilcoxon's rank-sum test for independent samples and the Fisher's exact test for the gender distribution
NS nephrotic syndrome, *F* female, *M* male

are depicted in Fig. 2. Median height Z score, which fell from 0.8 (−2.4 to 3.6) at diagnosis of NS to −0.6 (−2.7 to 0.4) Pre-Lev (p=0.004) remained stable at −0.6 (−3.0 to 0.6) End-Lev (p=0.32), and −0.5 (−2.6 to 0.2) Post-Lev (p= 0.45). Median height velocity improved from 3.0 (0.3–6.0) cm per year Pre-Lev to 3.7 (0.0–8.0) cm per year During-Lev (p=0.58) and 5.4 (0.0–9.1) Post-Lev (p= 0.19). Median weight Z scores fell from 0.4 (−0.9 to 4.1) Pre-Lev to 0.2 (−0.9 to 1.5) End-Lev (p=0.06) and 0.4 (−1.4 to 2.2) Post-Lev (p=0.06).

Adverse effects

None of the patients experienced any serious sequelae attributable to levamisole therapy. Pertinent laboratory data are shown in Table 3. Hematologic parameters fell slightly but remained well within normal limits During-Lev and rebounded End-Lev [median hematocrit 40.9% (35.3%–43.0%); median white blood cells 9.7/mm^3 (5.5–11.2)]. Hepatic transaminase and renal function tests were unchanged, as were estimated glomerular filtration rates by Schwartz formula. Serum albumin levels improved significantly During-Lev, from a median of 1.6 (0.8–3.8) g/dl Pre-Lev to 3.4 (1.1–4.3) Off-Lev (p=0.008). No rash or signs or symptoms of vasculitis occurred in any patient.

Discussion

Steroid-sparing therapy becomes an important component of the management of many children with NS. The short- and long-term toxicities of alkylating agents, traditional second-line therapy for steroid-sensitive disease, has led to the use of antimetabolites such as azathioprine and

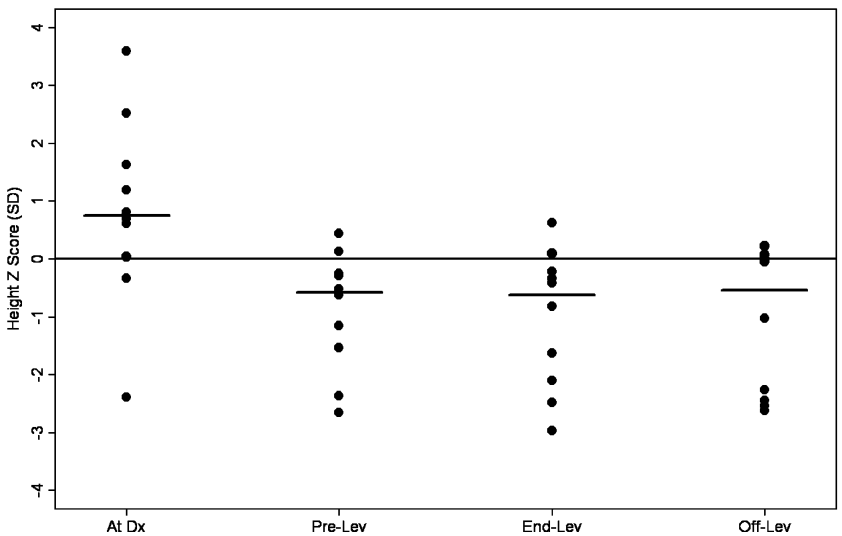

Fig. 2 Median height Z scores decreased by 1.4 standard deviations from diagnosis of nephrotic syndrome (*At Dx*) to the introduction of levamisole (*Pre-Lev*) (p=0.004) and remained stable at the end of the 12-month course of levamisole (*End-Lev*) (p=0.32) until 1 year after its discontinuation (*Off-Lev*) (p=0.45); p values were calculated using the Wilcoxon's signed-rank test for paired samples

Table 3 Laboratory surveillance

Laboratory tests	Pre-Lev Median (min-max)	End-Lev Median (min-max)	*P* value
Hb (g/dl)	15.0 (13.3–17.4)	14.0 (12.5–15.5)	0.002
HCT (%)	42.5 (38.9–50.3)	38.3 (36.1–46.7)	0.01
WBC (/mm3)	10.2 (6.9–15.7)	8.2 (4.2–11.4)	0.014
AST (IU/l)	20.0 (14.0–31.0)	20.5 (13.0–31.0)	0.96
ALT (IU/l)	18.5 (7.0–55.0)	17.5 (4.0–32.0)	0.68
Creatinine (mg/dl)	0.5 (0.2–0.7)	0.6 (0.2–1.2)	0.38
GFR (ml/min/1.73 m^2)	162 (121–257)	152 (75–282)	0.70
Albumin (g/dl)	1.6 (0.8–3.8)	3.4 (1.1–4.3)	0.008

Hb hemoglobin, *HCT* hematocrit, *WBC* white blood cells, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GFR* glomerular filtration rate

P values were calculated using the Wilcoxon's signed-rank test for paired data to compare laboratory tests before introduction of levamisole therapy (Pre-Lev) to results after 1 year of levamisole therapy (End-Lev)

mycophenolate mofetil, as well as more potent immunosuppressants such as the calcineurin inhibitors cyclosporine and tacrolimus. Although these agents may be effective steroid-sparing agents, all have potential serious systemic toxicities [10].

Especially outside of North America, the antihelminthic immunostimulant levamisole has been used as adjunctive therapy in steroid-dependent NS for over 25 years [2] and has been shown to be effective in maintaining remission in both case reports and a few randomized trials [3–8, 10, 11]. Limited data exist, however, as to the long-term efficacy of levamisole therapy after its cessation. A recent retrospective study [9] evaluated frequently relapsing steroid-dependent NS in 13 children treated with 2 mg/kg per day of levamisole for a mean duration of 17 months. Among the steroid-dependent children, 6/13 relapsed while on levamisole, but only 2/13 after its cessation, suggesting that levamisole may have a long-lived effect.

In this report, children with frequently relapsing NS of up to 10 years' duration demonstrated a significant reduction in average RF from a median of over six relapses a year Pre-Lev to a median of 0 relapses annually During-Lev and 0.5 relapses per year Off-Lev. In fact, a majority of children were relapse free During-Lev and half remained in remission for Post-Lev, leading to a marked diminution in steroid burden and potential for steroid toxicity.

Although all children treated with levamisole had been exposed to repeated steroid courses and there was no statistical differences in clinical parameters between the group who stayed in remission after Post-Lev and the group who had relapses, those children with the longest-lived response to levamisole tended to have lower initial steroid burdens, and they also tended to have had a longer duration of NS. This trend may suggest that the natural course of their disease had been improving somewhat, making them more responsive to the added immunomodulatory effects of levamisole. Such differences between long-term and short-term responders would be better elucidated by a larger cohort of children.

One child with steroid-sensitive focal segmental glomerulosclerosis (FSGS) improved During-Lev, with fewer relapses and decreased steroid burden. Off-Lev, the child once more become frequently relapsing and ultimately steroid resistant with progression to to end-stage renal disease. Her response while steroid sensitive suggests, however, that levamisole may also be steroid sparing in some children with FSGS, although larger numbers of such children would also need to be studied to best assess this effect.

Not surprisingly, in this cohort, decreased steroid burden During-Lev and Post-Lev led to a beneficial effect on somatic indices of growth, namely, amelioration of the deleterious effects of steroids on linear height and weight gain, as well as improvement in steroid-induced hypertension [12–16]. Steroid therapy has been known for many years to reduce height velocity [14] and cause growth retardation in children [12, 13, 15, 16]. Although all children in our study had normal height Z scores at diagnosis, all lost significant height during their years of steroid therapy, averaging an incremental –1.4 Z score loss by the initiation of levamisole. In comparison, During-Lev and Post-Lev, there was no further significant loss of height, and Z scores remained stable near –0.5 as a result of improved annualized height velocities to more normal levels.

This is the first report looking at the efficacy of levamisole in attenuating the negative effects of steroid therapy on growth. Limited published data systematically examine the effects of other steroid-sparing therapies on growth in nephrotic children. One report assessed growth-velocity measurements in 12 steroid-dependent nephrotic children before and after a course of cyclophosphamide or chlorambucil [17]. Mean height velocities doubled after therapy, but several clinically significant sequelae from the alkylating agents were reported (infection and alopecia), and nearly half the children suffered relapses nonetheless

following therapy. Alkylating agents are also associated with serious long-term side effects such as infertility and malignancy [10].

In contrast, levamisole has been reported to be generally well tolerated. Rare cases of cutaneous or disseminated vasculitis [18, 19], convulsions [20], and ataxia [21] have been reported, as have many common reports of mild anemia or elevation in liver enzymes [22]. In this series, no adverse sequelae of clinical concern were noted. Although hematologic parameters changed statistically, with a fall in hemoglobin and hematocrit levels and total white blood cell count, values all remained within normal limits. It is unclear whether these hematologic changes were related to myelosuppression from the levamisole or were a result of decreased steroid dosing in these children and subsequent fall in any steroid-induced leukocytosis or erythropoiesis. Most importantly, in addition to the paucity of adverse drug effects, relapse rates with levamisole seemed to be as favorably impacted as in children treated with more toxic steroid-sparing alternatives.

Thus, in our cohort of children with well established, frequently relapsing, steroid-dependent NS, levamisole is shown to be a safe and effective steroid-sparing agent, with long-lasting effect even 12 months after withdrawal. During-Lev and Post-Lev both relapse rates and steroid burden were significantly reduced. In some children, levamisole induced a prolonged remission, and in almost all of them, it was an effective long-term steroid-sparing agent.

Unfortunately, levamisole has become increasingly difficult to obtain in certain areas, as it is no longer a component of certain gastrointestinal chemotherapy regimens and there is decreased economic incentive for its production. Moreover, the lack of experience with its use by most North American pediatric nephrologists has caused there to be little recognition of its potential efficacy in this population of nephrotic children who are then, generally, exposed to more toxic alternatives. This report in a cohort of American children not only reinforces published data in European and Asian children demonstrating the favorable effects of levamisole on short-term relapse rates during therapy but also demonstrates important and to date underreported long-term efficacy with respect to relapse rate, steroid burden, blood pressure, and somatic indices of growth. It is imperative to continue to report that levamisole has a place in the armamentarium of children with steroid-sensitive nephrosis to demonstrate an ongoing need for its production, availability, and inclusion in any multicenter trials examining steroid-sparing agents in pediatric NS.

References

1. Tarshish P, Tobin JN, Bernstein J, Edelmann CM Jr (1997) Prognostic significance of the early course of minimal change nephrotic syndrome: report of the International Study of Kidney Disease in Children. J Am Soc Nephrol 8:769–776
2. Tanphaichitr P, Tanphaichitr D, Sureeratanan J, Chatasingh S (1980) Treatment of nephrotic syndrome with levamisole. J Pediatr 96:490–493
3. British Association for Paediatric Nephrology (1991) Levamisole for corticosteroid-dependent nephrotic syndrome in childhood. Lancet 337:1555–1557
4. Niaudet P, Drachman R, Gagnadoux MF, Broyer M (1984) Treatment of idiopathic nephrotic syndrome with levamisole. Acta Paediatr Scand 73:637–641
5. Al-Saran K, Mirza K, Al-Ghanam G, Abdelkarim M (2006) Experience with levamisole in frequently relapsing, steroid-dependent nephrotic syndrome. Pediatr Nephrol 21:201–205
6. Dayal U, Dayal AK, Shastry JC, Raghupathy P (1994) Use of levamisole in maintaining remission in steroid-sensitive nephrotic syndrome in children. Nephron 66:408–412
7. Donia AF, Amer GM, Ahmed HA, Gazareen SH, Moustafa FE, Shoeib AA, Ismail AM, Khamis S, Sobh MA (2002) Levamisole: adjunctive therapy in steroid dependent minimal change nephrotic children. Pediatr Nephrol 17:355–358
8. Alsaran K, Grisaru S, Stephens D, Arbus G (2001) Levamisole vs. cyclophosphamide for frequently relapsing steroid-dependent nephrotic syndrome. Clin Nephrol 56:289–294
9. Sumegi V, Haszon I, Ivanyi B, Bereczki C, Papp F, Turi S (2004) Long-term effects of levamisole treatment in childhood nephrotic syndrome. Pediatr Nephrol 19:1354–1360
10. Durkan AM, Hodson EM, Willis NS, Craig JC (2001) Immunosuppressive agents in childhood nephrotic syndrome: a meta-analysis of randomized controlled trials. Kidney Int 59:1919–1927
11. Bagga A, Sharma A, Srivastava RN (1997) Levamisole therapy in corticosteroid-dependent nephrotic syndrome. Pediatr Nephrol 11:415–417
12. Donatti TL, Koch VH, Fujimura MD, Okay Y (2003) Growth in steroid-responsive nephrotic syndrome: a study of 85 pediatric patients. Pediatr Nephrol 18:789–795
13. Emma F, Sesto A, Rizzoni G (2003) Long-term linear growth of children with severe steroid-responsive nephrotic syndrome. Pediatr Nephrol 18:783–788
14. Chesney RW, Mazess RB, Rose P, Jax DK (1978) Effect of prednisone on growth and bone mineral content in childhood glomerular disease. Am J Dis Child 132:768–772
15. Blodgett FM, Burgin L, Iezzoni D, Gribetz D, Talbot NB (1956) Effects of prolonged cortisone therapy on the statural growth, skeletal maturation and metabolic status of children. N Engl J Med 254:636–641
16. Fleisher DS, Mc CW, Rapoport M (1960) The effects of intermittent doses of adrenocortical steroids on the statural growth of nephrotic children. J Pediatr 57:192–198
17. Padilla R, Brem AS (1989) Linear growth of children with nephrotic syndrome: effect of alkylating agents. Pediatrics 84:495–499
18. Barbano G, Ginevri F, Ghiggeri GM, Gusmano R (1999) Disseminated autoimmune disease during levamisole treatment of nephrotic syndrome. Pediatr Nephrol 13:602–603
19. Rongioletti F, Ghio L, Ginevri F, Bleidl D, Rinaldi S, Edefonti A, Gambini C, Rizzoni G, Rebora A (1999) Purpura of the ears: a distinctive vasculopathy with circulating autoantibodies complicating long-term treatment with levamisole in children. Br J Dermatol 140:948–951
20. Palcoux JB, Niaudet P, Goumy P (1994) Side effects of levamisole in children with nephrosis. Pediatr Nephrol 8:263–264
21. Dubey AK, Gupta RK, Sharma RK (2001) Levamisole induced ataxia. Indian Pediatr 38:417–419
22. Bulugahapitiya DT (1997) Liver toxicity in a nephrotic patient treated with levamisole. Arch Dis Child 76:289