

**MINISTRY OF EDUCATION
AND TRAINING**

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**HAIPHONG UNIVERSITY OF
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**EFFICACY OF THE COMBINATION OF
INTRAVENOUS CYCLOPHOSPHAMIDE AND
PREDNISOLONE AND CHANGES IN SEVERAL
IMMUNE PARAMETERS IN TREATMENT OF
CHILDHOOD IDIOPATHIC NEPHROTIC SYNDROME**

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PhD. DISSERTATION SUMMARY

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The dissertation will be assessed and defended in front of the university-level Judging Council at Haiphong University of Medicine and Pharmacy

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INTRODUCTION

Idiopathic nephrotic syndrome (INS) is a quite common seen glomerulonephritis in pediatric population. Steroid - dependent and steroid - resistant INS are often clinically severe and frequently recurrent with 35-50% of patients developing end - stage renal disease (ESRD) after 5-10 years. The treatment still faces many difficulties, having detrimental effects on health and quality of life of these children and their families. In the context that in Vietnam, a developing country, not only are immunosuppressive drugs expensive and unavailable but also are not currently on the list of drugs covered by health insurance especially in Hai Phong and other provinces. The combination of intravenous cyclophosphamide (IC) and prednisolone used as a treatment therapy in a diverse range of clinical trial studies has been shown seemingly to be effective. On the other hand, some previous studies reported the important role of the immune system in the initiation or maintenance of idiopathic nephrotic syndrome. However, the pathophysiology of the disease has remained unknown. How do the humoral immunity and cellular immunity change during the period of illness and after treatment with intravenous cyclophosphamide? What are the changes after treatment with IC? Is IC effective indeed in treating patients with steroid - dependent and steroid - resistant INS? Therefore, we conducted this study with three objectives are as follows:

1. *To describe the epidemiologically clinical and biological characteristics of steroid - dependent and steroid - resistant INS in children at the Hai Phong Children's Hospital.*
2. *To evaluate the efficacy of the combination of intravenous cyclophosphamide and prednisolone in treatment of steroid - dependent and steroid - resistant INS in children.*
3. *To describe changes in several immune parameters before and after treatment in these patients.*

NEW CONTRIBUTIONS OF THE DESSERTATION

This is the first dissertation in Vietnam which studies on the efficacy of the combination of intravenous cyclophosphamid and prednisolone in treatment of steroid - dependent and steroid - resistant INS in children and has obtained certain results.

The obtained results may not only contribute to an better understanding of immunological changes in INS treated with medication and explain the pathogenesis of the disease but also be a basis for the application of immunosuppressive, immunomodulatory and

immunomodulatory therapy in the treatment of INS. It also contributes to better diagnosis, follow-up and prognosis of the disease. The dissertation also provides an addition to a better understanding of some immune characteristics of pediatric idiopathic nephrotic syndrome in Vietnam particularly and all over the world generally.

STRUCTURE OF THE DESSERTATION

The main part of the dissertation is 129 pages in length, including the main chapters as follows: Introduction: 3 pages, Chapter 1- Literature overview: 28 pages, Chapter 2 - Subjects and methods: 18 pages, Chapter 3 - Results: 43 pages, Chapter 4 - Discussion: 34 pages, Conclusion: 2 pages, Recommendations: 1 page. The dissertation has 192 references, of which 27 are in Vietnamese and 165 are in English. The dissertation has 39 tables, 9 figures and 1 diagram.

Chapter 1. LITERATURE OVERVIEW

1.1. Overview of idiopathic nephrotic syndrome (INS), steroid - dependent and steroid - resistant INS

1.1.1. Definition: According to KDIGO (Kidney Diseases Improve Global Outcome) in 2012, diagnostic criteria for INS are as follows: edema, nephrotic-range proteinuria $\geq 50\text{mg/kg/24 hours}$, serum albumin level $\leq 25\text{ g/l}$ (hypoalbuminemia), total serum protein level $\leq 56\text{ g/l}$ (hypoproteinemia), serum cholesterol level $\geq 5.5\text{ mmol/l}$ (or $\geq 220\text{ mg\%}$) (hypercholesterolemia).

1.1.2. Characteristics and situation of study on steroid - dependent and steroid - resistant INS

Studies on clinical and laboratory characteristics of steroid - dependent and steroid - resistant INS have reported that in the phase of illness there is a typically clinical setting in both types of INS, namely edema, hypoproteinemia, hypoalbuminemia and hypercholesterolemia. Steroid - dependent and steroid - resistant INS are frequently relapsed with more severe clinical symptoms such as generalized edema that may be accompanied by cardiovascular collapse, dyspnea, abdominal pain, hypertension, hematuria, fluid retention in multiple organs, fatigue, loss of appetite, venous or arterial thromboembolism and pulmonary embolism. The clinical symptoms and signs may vary and include a number of episodes of mild to severe infections such as respiratory tract infections, cellulitis, chicken pox, urinary tract infections and primary peritonitis. Especially, steroid - resistant INS can lead to a much higher rate of renal failure, hypertension and hematuria than other types. However, the clinical symptoms and signs of INS often vary and are

affected by many different factors, so there still has been inconsistency among numerous studies.

Initially established in 1965, the International Study of Kidney Disease in Children (ISKDC) conducted a series of multicenter studies with the involvement of numerous clinical researchers from North America, Europe and Asia. They announced a number of definitions, clinical relations review and recommendations for the diagnosis and treatment of INS in children which have been existed up to now. In 2012, KDIGO published a whole set of recommendations for the diagnosis and treatment of steroid - dependent and steroid - resistant INS.

In Vietnam, there have been a variety of studies on steroid - dependent and steroid - resistant INS in children. Nguyen Ngoc Sang (1999) carried out a study on the efficacy of treatment of steroid - resistant INS with methylprednisolon in his PhD dissertation. In 2009, Tran Thanh Thuy and Vu Huy Tru studied on the characteristics of steroid-resistant INS with partial and localized glomerular fibrosis in children at Children's Hospital 1. Duong Thuy Nga (2011) studied on the results of treatment of steroid-resistant INS at the Kidney - Urology Department of National Children's Hospital in her master thesis. Nguyen Van Sang (2013) conducted a study on the efficacy of oral cyclophosphamide in the treatment of childhood steroid - dependent and steroid - resistant INS in specialized level II thesis. In 2014, there was a study carried out by Tran Huu Minh Quan and Huynh Thoai Loan on the characteristics of steroid-resistant INS at Children Hospital I. In 2019, Nguyen Duc Quang, Vu Huy Tru and colleagues conducted a study to evaluate the efficacy of treatment and long-term flow-up of pediatric patients with steroid-resistant INS. Thus, there have been no studies in Vietnam that have evaluated the efficacy of a combination of intravenous cyclophosphamide and prednisolone in treating pediatric patients with steroid - dependent and steroid - resistant INS.

1.2. Overview of cyclophosphamide in treatment of steroid - dependent and steroid - resistant INS

- Overview of cyclophosphamide in treatment of steroid – dependent INS

Currently, many immunosuppressive drugs that replace steroids such as cyclophosphamide, cyclosporin A, cellcept and rituximab can result in not only a decrease in the rate of relapse and various complications of the disease but longer remission period. They also help to reduce dose of medication and to give a contribution to sooner steroid

cessation. The KDIGO (2012) recommended that alkylated drugs such as cyclophosphamide were used to replace steroids in treatment of steroid – dependent INS and frequently relapsing nephrotic syndrome (FRNS).

For decades, cyclophosphamide has been shown to be effective in treating steroid - dependent and steroid - resistant INS. Intravenous cyclophosphamide (IC) was used for treatment in a number of clinical trial studies of various researchers such as Shah (2017), Kanigicherla (2018) and Berkane (2018) and was also shown to be effective. It helped to not only improve clinical signs and symptoms and laboratory parameters but also shorten the treatment time and remain a long-term remission in children with steroid - dependent and steroid - resistant INS. Ponticelli, through a Cochrane review, also reported that IC reduced the risk of recurrence after 6-12 months compared to prednisolone alone (RR 0.44, 95% CI 0.26–0.73). Based on the results of studies by Bircan (2003), Gulati (2001) and Prasad (2004), it is proved that IC was more effective and had fewer side effects than oral cyclophosphamide. However, more evidence from other high - quality clinical studies was needed. Therefore, more studies need to be conducted.

- *Overview of cyclophosphamide in treatment of steroid - resistant INS:*

Although there have been different perspectives on the efficacy of cyclophosphamide, some studies have been successful with pulse intravenous cyclophosphamide therapy in the treatment of steroid-resistant INS. Gulati (2000) reported that IC is a safe, effective and economical therapy for the treatment of steroid-resistant INS with a complete remission rate of 65%. Elhence R. et al (1994) studied on 13 patients with steroid-resistant INS with minimal change and compared the efficacy of oral and intravenous cyclophosphamide. The results showed that the remission rate in the group receiving intravenous cyclophosphamide (100%) was much higher than that in the remaining group receiving oral cyclophosphamide (25%). In another study by Cucer (2010) also found that IC was significantly better compared to oral administration (with remission rate of 31.3% in the intravenous group compared to 11.8% in oral group). Rennert et al. (1999) concluded that IC effectively resulted in long-term remission in 50% of patients with steroid-resistant INS and suffered from focal segmental glomerulosclerosis.

During treatment period with cyclophosphamide, a complete blood count should be performed every 1 to 2 weeks to monitor white

blood cell counts. When leukopenia occurs, a drop in dose or even discontinuation of cyclophosphamide should be considered. Other remarkable side effects that need to be considered are infections, hair loss, hemorrhagic cystitis, liver dysfunction, interstitial pneumonia, and syndrome of inappropriate antidiuretic hormone secretion.

1.3. The immunity in INS

There have been a large number of studies on the immunity in INS up to now. Studies have found that there are changes in the INS in terms of both humoral and cellular immunity.

- *Regarding humoral immunity:* Unlike post - infectious glomerulonephritis, in INS, immune complexes are rarely found circulating in the bloodstream or depositing in the glomeruli. The concentration of complements in general and C3 in particular are not decreased in INS according to comments of many authors. Evidence has shown that humoral immunity played little role in the formation of pathogenesis of INS although the majority of previous studies have found that there were changes in INS in terms of humoral immunity such as Giangiaco (1975), Gupta (1985), Banik (1994), and Pereira (2014).

- *Regarding cellular immunity:* Shalhoub (1974) was the first author who argued that INS was associated with functional abnormalities of T lymphocytes. Numerous damaged glomeruli in INS were related to the presence of released soluble factors and lymphokines circulating in the bloodstream due to abnormalities in primary cellular immunity. Subsequent studies contributed to the provement of the role of cellular immunity in the pathogenesis of INS. Ooi (1974), for example, found the cytotoxic factors of lymphocytes. Pereira (2014) pointed out that functional abnormalities of T lymphocytes lead to the production of cytokines that damage the glomerular basement membrane, resulting in the presence of proteinuria. This is explained by the cytokin immune response model and the response model of Th1 with Th2 in INS.

In today's world, thanks to the advances in modern immunology, the changes in various subtypes of T lymphocytes involved in the pathogenesis of INS have been found. Wang et al. (2009) further defined the role of T-lymphocyte subtypes which play protective roles as same as T $\gamma\delta$ cells. Matsumoto (1990), Fiser (1991), Sasdelli (1980) found that during the phase of illness, there was a decrease in TCD3 + lymphocyte count in INS. Fiser (1991), Hulton (1994), Herrod (1983), and Saxena (1992) also showed that during the phase of illness, there was a decrease in TCD4 + lymphocyte count.

Fiser (1991), Saxena (1992), Lama G (2002) and Wang Y (2001) showed that at the phase of illness, the number of TCD8 + lymphocytes increased.

In addition, several studies investigated the role of B lymphocytes in INS, but the number of studies was small. In addition, some studies have also shown the role of cytokines, chemokines and circulating factors in the pathophysiology of INS. Some cytokines and chemokines such as IL- 1, IL-6, IL-8 and CXCL8 are correlated with proteinuria range and have been suggested as factors that increase glomerular permeability, resulting in the presence of proteinuria in patients with INS or in experimental animal models. Some other mentioned factors are also related to the pathogenetic mechanism of INS such as vascular endothelial growth factors (VEGF), transcription factor called NF- κ B (nuclear factor kappa B) and soluble urokinase plasminogen activator receptor (su-PAR).

Chapter 2. SUBJECTS AND METHODS

2.1. Subject, time duration and location of the study

60 children who were diagnosed with INS and developed the disease from more than one year old, including 46 ones with steroid-dependent INS and 14 ones with steroid-resistant INS.

The study was conducted during a period of time since January 2015 to December 2018 at the Department of Nephrology - Hematology - Endocrinology in Hai Phong Children's Hospital.

2.1.1. Inclusion criteria

- The diagnostic criteria of INS are based on KDIGO (2012):
- Steroid - dependent idiopathic nephrotic syndrome (SDNS): achieve complete remission after 4 - 6 weeks of prednisolone with dose of 2mg/kg/day, but two or more consecutive relapses when having a decrease in dose, or within 14 days of ceasing corticosteroid therapy.
- Steroid-resistant idiopathic nephrotic syndrome (SRNS): Failure to achieve complete remission (edema and/or nephrotic-range proteinuria ≥ 50 mg/kg/24 hours):
 - + After 8 weeks of corticosteroid therapy: Oral prednisolone dose of 2mg/kg/day, or
 - + After 4 weeks of corticosteroid therapy: Oral prednisolone dose of 2mg/kg/day and the next 4 weeks with oral prednisolone dose of 1.5mg/kg/day every other day.

2.1.2. Exclusion criteria

- Nephrotic syndrome secondary to: Systemic lupus erythematosus, Schönlein - Henoch, diabetes, hepatitis B and HIV.
- Those who have withdrawal of treatment.
- Those who had been previously treated with other immunosuppressant drugs.
- Those who refuse to participate in the study.

2.2. Study methods

2.2.1. Study design: A prospective case series, an open-label trial

2.2.2. Sample size: All 60 children with steroid - dependent and steroid - resistant INS who met the inclusion criteria during the study period, convenience sampling.

2.2.3. Indicators and variables

2.2.3.1. Objective 1: *To describe the clinically epidemiological and biological characteristics of steroid - dependent and steroid - resistant INS in children.*

Age on admission, age at disease onset, duration of illness up to the time of study, gender, geography (urban/rural), number of recurrences up to the time of study, relevant medical history and family history. Perform hematological and biochemical blood tests, urine tests, radiological test in order to describe and compare clinical and laboratory characteristics of steroid - dependent and steroid - resistant INS in children.

2.2.3.2. Objective 2: *To evaluate the efficacy of the combination of intravenous cyclophosphamide and prednisolone in treatment of childhood steroid - dependent and steroid - resistant INS.*

- **Regimen for treatment of steroid - dependent and steroid - resistant INS** (based on the treatment regimen of National Hospital of Pediatrics): IC dose of 10mg/kg/time x 2 times/week, mixed with 100ml Glucose 5%, infusion for 1 hour. The total dose of cyclophosphamide should not exceed 150 mg/kg for the entire course of treatment. We use a brand name Endoxan (a bottle of 200 mg) of Baxter Health Care (ASIA) combined with oral prednisolone low dose of 0.5 mg/kg/24 hours.

- **Evaluate results after 4 weeks of treatment:** Assess the extent of remission before and after treatment of 2 studied groups, time duration to achieve remission, total cumulative dose of cyclophosphamide after treatment, changes in clinical symptoms and signs, changes in laboratory tests before and after treatment.

- **Long-term follow-up after hospital discharge:** Determine the rate of non-recurrence (cumulative remission) after 6 months, 12 months, 24 months and 48 months. Monitor long-term side effects of IC.

- **Identify some complications and side effects of drugs and the disease:** Respiratory and urinary tract infection, peritonitis, cellulitis, Cushing's syndrome, gastroduodenal ulcer, alopecia, hemorrhagic cystitis, erythropoiesis, leukopenia, platelets, drug allergy.

2.2.3.3. Objective 3: To describe changes in some immune parameters before and after treatment of steroid - dependent and steroid - resistant INS with the combination of intravenous cyclophosphamid and prednisolone.

Immunological tests, including humoral and cellular immunity, are performed twice: at the time of admission and time when patients are untreated by cyclophosphamide. The second time of testing was carried out when the patient is stable and is discharged after an average of 4 weeks of treatment.

- **To describe changes in humoral immunity:** quantify the serum Immunoglobulin level (Ig) (IgG, IgA, IgM) by the Nephelometry method on Abbott C4000 automatic biochemical machine to describe and compare the two groups before and after treatment .

- **To describe the changes in cellular immunity:** determine the number and percentage of TCD3, TCD4 + and TCD8 + lymphocytes. Compare the number and percentage of TCD3, TCD4 + and TCD8 + lymphocytes between two groups before and after treatment. Tests on cellular immunity are performed at the Department of Allergy Immunology, National Institute of Hematology and Blood Transfusion.

2.3. Tools for data collection and processing

The data is processed using the SPSS version 20.0 software.

2.4. Ethics in research

The study topic strictly follows the research ethics in Medicine. The research was permitted and approved by the Judging Council of Haiphong University of Medicine and Pharmacy and the Council of Ethics of Hai Phong Children's Hospital. We conducted the study with the consent of the parents and patrons of patients. They were explained, advised and committed to voluntarily participate in the study in papers. The patient's information was kept confidential and only served for scientific study.

Chapter 3. RESULTS

3.1. Regarding the clinically epidemiological and biological characteristics of steroid - dependent and steroid - resistant INS in children.

- The average admission age of the steroid-resistant group was 10 ± 4.2 years and that was 8.1 ± 4.3 years in steroid-dependent group.
- The average age at disease onset of the steroid-resistant group was 8.0 ± 4.4 years, higher than that of the steroid-dependent group which was 6.3 ± 4.0 years.
- The proportion of boys with INS was more higher than that of girls in both study groups.
- The urban/rural ratio in steroid-resistant group was 2.5, higher than that in steroid-dependent group which was 1.4, $p > 0.05$.
- The average duration of illness from the onset period of the disease to the study time was 1.8 ± 1.7 years. The average follow-up time was 2.9 ± 1.2 years.

Table 3.1. Patient distribution in terms of disease form

Clinical form	Group I	Group II	p (test χ^2)
	n(%)	n(%)	
Simple	34(73,9)	4(28,6)	<0,01
Non-simple	12(26,1)	10(71,4)	
Tổng	46(76,7)	14(23,3)	

Comments: Simple steroid-dependent INS accounted for 73.9% while 71.4% of steroid-resistant INS were non-simple. The difference was statistically significant, $p < 0.01$.

Table 3.2. Clinical manifestations on admission

Clinical manifestations	Group I (n = 46)	Group II (n = 14)	p (test χ^2)
	n(%)	n(%)	
Edema degree			>0,05
Severe	14(30,4)	7(50,0)	
Moderate	26(56,5)	5(35,7)	
Mild	6(13,0)	2(14,3)	
Fluid retention in multiple organs	8(17,4)	4(28,6)	>0,05
Hypertension	4(8,7)	7(50,0)	<0,01
Oliguria	19(41,3)	5(35,7)	>0,05

Comment: 100% of patients had edema on admission, mainly moderate and severe edema. Edema often accompanied by multilayer effusion and oliguria in both study groups, $p > 0.05$. The percentage of patients with hypertension in steroid-resistant group was much higher than that in steroid-dependent group, $p < 0.01$.

Table 3.3. Laboratory characteristics of patients on admission

Parameters	Group I (n = 46)	Group II (n = 14)	p
Proteinuria (mg/kg/24 hour)	126,41 (77,6-193,4)	183,9 (86,4-270,1)	>0,05
Serum protein level(g/l)	50,2 ± 8,7	49,5 ± 9,1	>0,05
Albumin (g/l)	23,2 ± 7,8	23,2 ± 8,3	>0,05
α1 globulin (g/l)	3,9 ± 1,3	3,9 ± 0,6	>0,05
α2 globulin (g/l)	19,3 ± 6,1	18,4 ± 7,2	>0,05
β globulin (g/l)	12,3 ± 3,8	11,4 ± 1,3	>0,05
γ globulin (g/l)	18,0 ± 2,6	18,4 ± 1,6	>0,05
Cholesterol (mmol/l)	9,5 ± 3,7	9,8 ± 3,5	>0,05
Triglycerid (mmol/l)	2,2(1,5-3,9)	2,5(2,0-4,3)	>0,05
HDL-C (mmol/l)	1,4(1,2-1,5)	1,4(1,2-1,5)	>0,05
LDL-C (mmol/l)	5,3 ± 2,3	6,9 ± 3,1	>0,05
Urea (mmol/l)	4,7(3,7-5,7)	4,2(3,9-6,4)	>0,05
Creatinine (μmol/l)	39,5(36,8-47,3)	41,0(37,3-53,0)	>0,05
Sodium (mmol/l)	138,3 ± 3,3	139,5 ± 2,9	>0,05
Potassium (mmol/l)	3,9 ± 0,4	3,8 ± 0,4	>0,05
Chloride (mmol/l)	105,1 ± 3,6	106,8 ± 3,3	>0,05
Total calcium (mmol/l)	2,0 ± 0,2	2,1 ± 0,3	>0,05
Ionized calcium	1,02 ± 0,09	1,03 ± 0,09	>0,05

Comments: In general, proteinuria was very much while serum total protein and albumin level were severely reduced. Serum α2 globulin concentration was high whereas that of serum α1 and γ globulin were at low range. Serum cholesterol, triglyceride and LDL-C level increased. Serum electrolytes, urea and creatinine level were within normal ranges. There was no difference in biochemical indices between the two study groups, $p > 0.05$.

3.2. Regarding the efficacy of the combination of intravenous cyclophosphamide and prednisolone in treatment of childhood INS

- Evaluate the effectiveness after 4 weeks of treatment:

The average duration of treatment in steroid-dependent group (12 days) was significantly shorter than that of steroid-resistant group (18 days), $p = 0.004$.

The total of average cumulative IC dose of steroid-dependent group (22.09 ± 12.31 mg/kg /period) was a half of that in the steroid-resistant group (47.53 ± 30.31 mg/ kg/period), $p < 0.01$.

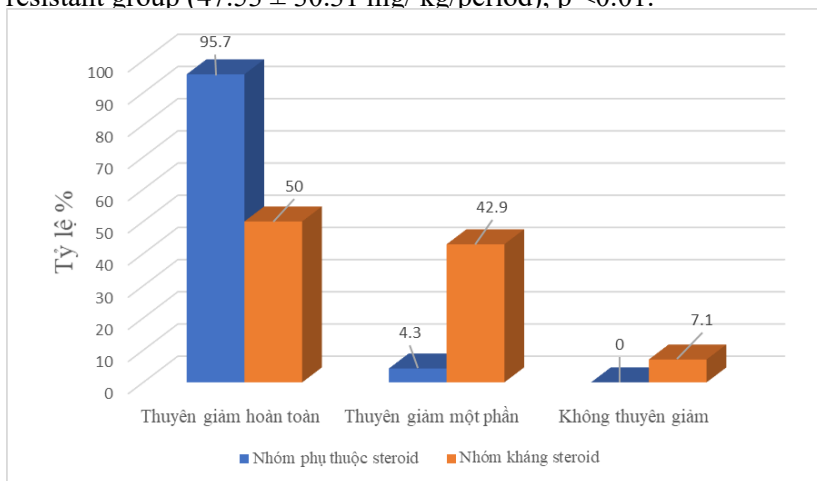


Figure 3.1. Comparison of the remission level after treatment in 2 groups ($n = 60$)

Comments: The majority of patients had complete remission. The steroid-dependent group achieved better remission than the steroid-resistant group after 4 weeks of treatment. The difference was statistically significant, $p < 0.001$.

- Evaluate changes in edema degree and clinical symptoms and signs:

Before treatment, 100% of patients had edema on admission, mainly moderate and severe edema. After treatment, no edema was observed and gradually returned to normal. After treatment, patients no longer manifested multilayer effusion. They had normal blood pressure and normal urine output.

- Evaluate changes in biochemical and urinary tests

Table 3.4. Changes in proteinuria range in 24 hours, serum protein and albumin level before and after treatment

Parameter	Group I	Group II	p
	(n = 46)	(n = 14)	
Proteinuria (mg/kg/24 giò)			
Before treatment	126,41 (77,6-193,4)	183,9 (86,4-270,1)	>0,05
After treatment	0(0-0)	0(0-38,1)	<0,001
p	<0,001	=0,001	
Serum protein level (g/l)			
Before treatment	50,4 ± 8,7	49,5 ± 9,1	>0,05
After treatment	57,8 ± 6,2	57,8 ± 8,7	>0,05
p	<0,001	<0,001	
Serum albumin level (g/l)			
Before treatment	23,4 ± 7,8	23,2 ± 8,3	>0,05
After treatment	30,4 ± 6,1	31,6 ± 6,5	>0,05
p	<0,001	0,002	

Comment: Before treatment, 24-hour proteinuria range rose while serum protein and albumin level decreased in both groups. After treatment, proteinuria level significantly decreased whereas serum protein and albumin level returned to normal range in both study groups.

- Results of long-term follow-up:

The average follow-up period was 2.94 ± 1.25 years, of which the least was 1 year and the longest was 4 years.

Table 3.5. The remission rate after long-term follow-up

Time after follow-up	Group I	Group II	p (test χ^2)
	(n = 46) n(%)	(n = 14) n(%)	
6 months	44(95,7)	13(92,9)	>0,05
12 months	40(87,0)	10(71,4)	>0,05
24 months	31(68,9)	8(57,1)	>0,05
48 months	30(65,2)	8(57,1)	>0,05

Comment: The cumulative remission rates after 6 months, 12 months, 24 months and 48 months were higher in the dependent -steroid group than that of the resistant -steroid group.

Table 3.6. Side effects of cyclophosphamide and prednisolone

Side effects	Group I	Group II	p
	(n = 46)	(n = 14)	
	n(%)	n(%)	
Cyclophosphamide			
Infection	19(41,3)	5(35,7)	>0,05
Alopecia	13(28,3)	8(57,1)	>0,05
Scalp tanning	6(13,0)	3(21,4)	>0,05
Fatigue, decreased appetite	5(10,9)	2(14,3)	>0,05
Leukopenia	0(0,0)	0(0,0)	>0,05
Hemorrhagic cystitis	0(0,0)	0(0,0)	>0,05
Prednisolone			
Hypertension	4(8,7)	7(50,0)	0,002
Gastritis	8(17,4)	1(7,1)	>0,05
Cushing's syndrome	10(21,7)	4(28,6)	>0,05

Comment: Common side effects and complications of intravenous cyclophosphamide and prednisolone were infections, followed by alopecia and Cushing's syndrome. Other less common side effects were hypertension and acute gastritis. There were no cases of leukopenia and hemorrhagic cystitis.

3.3. Regarding changes in some immune parameters before and after treatment

3.3.1. The changes in count of white blood cells, neutrophils and lymphocytes

Total 3.7. White blood cell count (G/L) in 2 study groups before and after treatment

Point of time	Group I ¹	Group II ²	Difference ¹⁻²	p
	TB ± SD (n = 46)	TB ± SD (n = 14)		
White blood cell count (G/L)				
Before treatment (T0)	12,06 ± 4,68	9,41 ± 3,16	2,16	>0,05
After treatment (T4)	13,98 ± 5,09	11,38 ± 3,03	2,57	>0,05
Changes (T4-T0)				
Average	1,91	1,98	0,07	>0,05
p	>0,05	>0,05		

T0: Time of admission, T4: Time of discharge (usually after 4 weeks); TB ± SD: Average ± SD.

Comment: After treatment, the average white blood cell count in both groups tended to increase compared to before treatment, p > 0.05.

Table 3.8. The neutrophil counts (G/L) in 2 groups before and after treatment

Point of time	Group I ¹	Group II ²	Difference ¹⁻² (G/L)	p
	TB ± SD (n = 46)	TB ± SD (n = 14)		
Before treatment (T0) [†]	6,58 ± 3,06	6,13 ± 2,79	0,88	>0,05
After treatment (T4) [†]	10,01 ± 5,58	8,07 ± 2,97	2,04	>0,05
Changes (T4-T0)				
Average	3,44	1,94	1,5	>0,05
P	<0,01	>0,05		

T0: Time of admission, T4: Time of discharge (usually after 4 weeks); TB ± SD: Average ± SD.

Comment: Compared to the time before treatment, the average count of neutrophils in both study groups increased after treatment and significantly increased in the steroid - dependent group (p < 0.01).

Table 3.9. The lymphocyte counts (G/L) in 2 groups before and after treatment

Point of time	Group I	Group II	p ¹
	(n = 46)	(n = 14)	
Before treatment (T0)	3,3 (2,06-5,92)	2,62 (1,36-4,03)	>0,05
After treatment (T4)	2,16 (1,78-3,46)	1,88 (1,16-3,51)	>0,05
Changes T4-T0			
Average	1,83	0,21	>0,05
p²	<0,01	>0,05	

p¹: Mann-Whitney U test, p²: Wilcoxon Signed Ranks test

T0: Time of admission, T4: Time of discharge (usually after 4 weeks).

Comments: After treatment with intravenous cyclophosphamide, median lymphocyte counts decreased in both study groups and significantly decreased in the steroid - dependent group (p < 0.01).

3.3.2. Changes in humoral immunity.

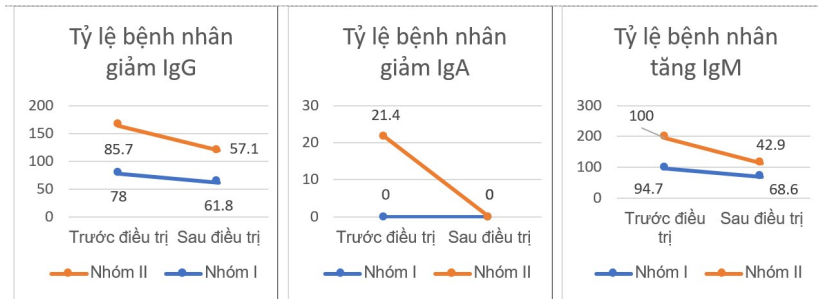


Figure 3.2. Changes in the proportion of patients with decreased serum IgG and IgA levels, and increased serum IgM level in 2 study groups before and after treatment

Comments: After treatment, there was a sharp drop in the proportion of patients with a decrease in serum IgG level and an increase in serum IgM level compared to the time before treatment in both study groups. This change is statistically significant ($p < 0.05$).

Table 3.10 Changes in median serum IgG, IgA, IgM levels before and after treatment

Parameters	Group I	Group II	P	
	(n = 46)	(n = 14)		
IgG	Before treatment	460,0 (319,5-827,0)	361,5 (319,0-770,25)	>0,05
	After treatment	743,0 (425,75-1070,75)	897,0 (355,25-1412,0)	>0,05
	P	<0,05	<0,05	
IgM	Before treatment	234,5 (175,25-273,5)	277,0 (174,25-332,0)	>0,05
	After treatment	156,0 (126,0-201,0)	134,5 (117,0-171,5)	>0,05
	P	<0,001	<0,01	
IgA	After treatment	171,0 (101,0-212,0)	108,5 (85,5-210,75)	>0,05
	After treatment	210,0 (177,0-296,0)	254,5 (164,75-314,75)	>0,05
	P	<0,001	<0,05	

Comments: Before treatment, serum IgA and IgG levels were severely decreased while serum IgM level increased. In contrast, after treatment serum IgA and IgG levels increased whereas serum IgM level decreased. Serum immunoglobulin level in patients tended to return significantly to normal range. The ratio of IgG/IgM level increased significantly compared to the time before treatment. There was no difference between the two study groups in median immunoglobulin concentrations, $p > 0.05$.

3.3.3. Changes in cellular immunity

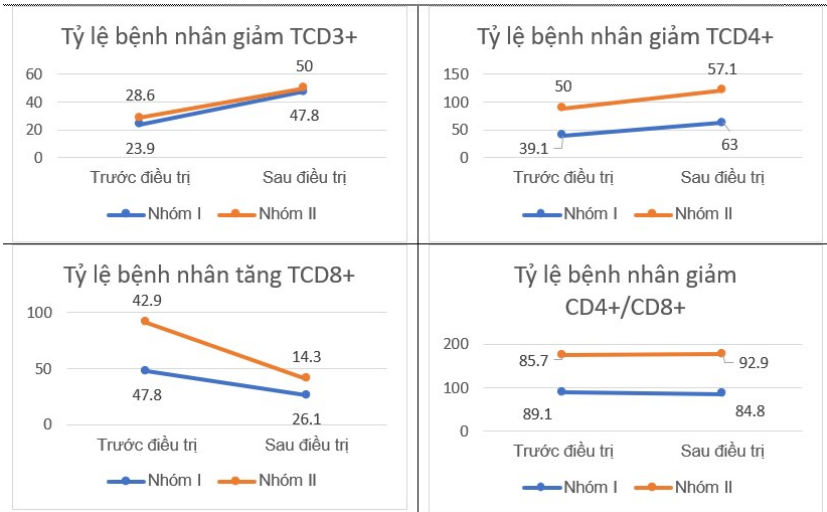


Figure 3.3. Changes in the proportion of patients with TCD3+, TCD4+, TCD8+ and CD4+/CD8+ reductions in the two study groups before and after treatment

Comments: The percentage of patients with TCD3+ and TCD4+ reduction increased after treatment in both study groups and significantly increased in the steroid - dependent group, $p < 0.05$. The percentage of patients with increases in TCD8+ counts decreased after treatment in both study groups and significantly decreased in the steroid-dependent group, $p < 0.05$.

Table 3.11. Changes in median TCD3, TCD4 and TCD8 counts before and after treatment

Characteristics of cellular immunity		Group I (n = 46)	Group II (n = 14)	p ^a
TCD3 (Cell/ul)	T0	2010,5 (1268,5-2975,0)	1788,0 (1019,3-2381,0)	>0,05
	T4	1448,5 (1007,75-2275,75)	1379,5 (795,3-1629,5)	>0,05
	T4-T0	455,35	579,79	>0,05
	p^b	>0,05	<0,05	
TCD4 (Cell/ul)	T0	830,5 (552,0-1407,5)	703,0 (494,5-1085,5)	>0,05
	T4	639,5 (481,0-1103,75)	619,5 (378,75-725,75)	>0,05
	T4-T0	178,67	256,93	>0,05
	p^b	>0,05	0,03	
TCD8 (Cell/ul)	T0	974,5 (581,5-1567,25)	952,0 (547,0-1208,25)	>0,05
	T4	720,0 (540,5-1150,5)	711,5 (421,75-882,5)	>0,05
	T4-T0	242,22	213,86	>0,05
	p^b	>0,05	>0,05	
TCD4+/ TCD8+	T0	0,96 ± 0,28	0,91 ± 0,26	>0,05
	T4	0,94 ± 0,32	0,80 ± 0,28	>0,05
	T4-T0	0,019	0,111*	

p^a: Mann-Whitney U test, p^b: Wilcoxon Signed Ranks test.

*: p < 0,05, test T

Comments: Before treatment, the TCD3+ count did not change significantly. TCD4+ count decreased and TCD8+ count increased. After treatment, the counts of TCD3+, TCD4+ and TCD8+ decreased in both 2 study groups. The average ratio of TCD4+/TCD8+ after treatment tended to decrease in both two study groups and significantly decreased in the steroid-resistant group (p < 0.05).

Chapter 4. DISCUSSION

4.1. Regarding of the clinically epidemiological and biological characteristics of steroid - dependent and steroid - resistant INS in children.

In terms of age and gender: The average admission age of the steroid-resistant group was 10 ± 4.2 years and that of the steroid-dependent group was 8.1 ± 4.3 years. In the steroid – resistant group, patients developed the disease at the average age of 8.0 ± 4.4 years (age of onset), higher than that of the steroid - dependent group which was 6.3 ± 4.0 years. The disease was seen more commonly in boys than girls. Our study's results were similar to other studies in Vietnam and abroad. Steroid - dependent and steroid - resistant INS were mainly seen in pre-school and school – aged children. The prevalence was always higher in boys than that in girls.

In terms of clinical symptoms and signs: Edema was always seen in INS and was the first sign causing a child to visit hospital. 100% of our patients had edema. Edema developed rapidly, and was white, soft, and concaved. Almost all patients had swelling in the whole body. Some patients could be accompanied by ascites or pleural effusion or scrotal swelling (in boys). Edema was frequently recurrent and accompanied by decreased urine output in 40% of patients but rarely anuria. The majority of patients in the steroid - dependent group had normal blood pressure is normal while 50% of patients in the steroid - resistant suffered from hypertension. Some other signs were tiredness, anorexia, cyanotic skin, and sometimes abdominal pain during the period of edema.

Proteinuria: According to international criteria, proteinuria was in nephrotic-range $\geq 50\text{mg/kg/24}$ hours. Our study results showed that the median 24-hour proteinuria of the steroid - dependent group was 126.4 mg/kg/24 hours and that was 183.9 mg/kg/24 hours in the steroid – resistant group. Thus, proteinuria was in very high range. This result was consistent with the comments of many other authors.

Serum total protein level and protein electrophoresis: Serum total protein levels of all patients were significantly decreased below the nephrotic - range, which was $50.2 \pm 8.7 \text{ g/l}$ in average in the steroid - dependent group and $49.5 \pm 9.1 \text{ g / l}$ in the steroid – resistant group (the normal range was $60 - 80\text{g/l}$). These patients also had severely decreased serum albumin level whereas α_2 globulin level was highly increased. Serum α_1 globulin and γ globulin levels

were slightly decreased in both two study groups. These results were consistent with the majority of studies on primary nephrotic syndrome in Vietnam and abroad. Mechanism of reduction and disturbance of components of serum protein was mainly due to massive loss of protein into the urine. It was believed that the decrease in serum protein level was associated with the increase in level of proteinuria. In other words, hypoproteinemia was inversely correlated with the increase in proteinuria level. The two signs namely nephrotic - range proteinuria and hypoproteinemia, especially hypoalbuminemia, were two mandatory criteria for diagnosis of nephrotic syndrome.

Serum cholesterol and triglyceride: Serum cholesterol, triglyceride and LDL-C levels increased (normal range of serum cholesterol was 3.1 - 5.9 mmol / l). This result was consistent with studies of numerous authors in the world. Disorder of lipid components was a manifestation of nephrotic syndrome and was directly related to decreased serum protein and albumin concentrations.

The majority of patients had normal serum urea and creatinine levels. Ionized calcium concentration usually decreased whilst serum sodium, potassium and chloride concentrations were in normal range. Normally calcium existed mainly in the form of binding to serum albumin. In idiopathic nephrotic syndrome, albumin decreases due to loss by urine so serum calcium concentration also decreases.

4.2. Regarding the efficacy of the combination of intravenous cyclophosphamide and prednisolone in treatment of childhood steroid - dependent and steroid - resistant INS.

In terms of efficacy after 4 weeks of treatment: After treatment, 95.7% of patients in the steroid-dependent group had complete remission and 4.3% had partial remission. In contrast, in the steroid-resistant group 50% of patients had complete remission, 42.9% had partial remission and 7.1% failed to achieve remission. Our results were higher than that of Nguyen Van Sang (2013) with 53.3% of patients had complete remission, 24% had partial remission and 22.7% failed to achieve remission. Although Nguyen Van Sang used oral CP for patients, we used intravenous cyclophosphamide. According to Ravi Elhence et al. (1994), the remission rate of patients receiving intravenous cyclophosphamide was much higher and the duration of remission was longer than that of patients

receiving oral cyclophosphamide. The total average cumulative dose of intravenous cyclophosphamide in the steroid-dependent group (22.09 ± 12.31 mg/kg/course) was a half of that of the steroid-resistant group (47.53 ± 30.31 mg/kg/course), $p < 0.01$. The average duration of treatment of the steroid-dependent group (12 days) was significantly shorter than that of the steroid-resistant group (18 days), $p = 0.004$.

Long-term effects: the remission rates after 6 months, 12 months, 24 months and 48 months of the steroid - dependent group were 95.7%, 87%, 68.9% and 65.2%, respectively which were higher than that of the steroid – resistant group (92.9%, 71.4%, 57.1% and 57.1%, respectively). A study conducted by Kumar et al (2017) on 50 patients with steroid-dependent INS showed that 43 of 50 patients responded to intravenous cyclophosphamide, in which 24 patients (accounted for 56%) achieved long-term remission and could had steroid cessation. 19 of total 24 patients achieved remission after 6 months and 9 of 24 patients did not experience relapses until the time of last follow-up with an average follow-up time of 2.1 years.

Changes in clinical signs: Before treatment, all patients had edema. The previous authors showed that all of cases had edema. After treatment, all 60 patients (100%) did not have edema, $p < 0.001$. Our results were higher than that of Nguyen Ngoc Sang et al (2014) who noted that after treatment edema went away in 90% of patients and 10% of patients had edema reduction. Edema was also a sign for diagnosing INS as well as a standard for follow-up.

Changes in laboratory tests: After treatment, proteinuria decreased and most of patients had no proteinuria. The proteinuria levels before and after treatment had significant differences ($p < 0.001$). Before treatment, all patients had decreased serum protein and albumin levels. After treatment, serum protein and albumin levels of both groups increased to normal ranges. This was similar to the results published by Nguyen Ngoc Sang (2014), Nguyen Van Sang (2013), Ravi Elhence (1994) and Gulati (2010).

Regarding side effects and complications: Common side effects of intravenous cyclophosphamide in combination with prednisolon were alopecia (35%), Cushing's syndrome (23.3%). Less common side effects were infections, hypertension, gastritis, fatigue, anorexia, darkening scalp and fingers, but these effects were all controlled. There were no cases with leukopenia or hemorrhagic cystitis. The percentage of patients with alopecia in our

study was higher than that of Nguyen Dinh Vu's results (30%) in 2002 and was lower than that of Nguyen Van Sang's results (48%) in 2013. This complication resulted from prolonged protein loss and previous steroid therapy. According to the literature in the world, alopecia was often transient and reversible. Cushing's syndrome was a complication of previous high doses of prednisolone used for a long time. Patients with steroid - dependent and steroid - resistant INS frequently had relapses. In each relapse course, patients had to receive an attack dose of prednisolone. The use of high doses of prednisolone for a long time resulted in Cushing's syndrome as a side effect in these patients. The manifestations of Cushing's syndrome in most patients were facial and abdominal obesity, excessive hair growth, mustache, acne, stretch marks, after treatment with intravenous cyclophosphamide combined with low doses of oral prednisolone for 3 to 6 months. These symptoms and signs were relieved. Most of patients manifested only mild Cushing's syndrome and had no severe complications. Infections found in our patients were mainly upper respiratory tract infection and dermatitis. Our study results were similar to Nguyen Dinh Vu (2002) with 33.3% and Nguyen Ngoc Sang (2014) with 37.3%. Most of infections occurred in upper respiratory tract, which can be explained that cyclophosphamide was an immunosuppressant and had effect on the lymphatic system activity. As a result, pediatric patients who received cyclophosphamide therapy were at high risk of having viral infection in the upper respiratory tract. Cyclophosphamide play an important role in inhibiting cell division by blocking DNA replication. However in our study there were no cases with leukopenia. The rate of leukopenia in the study of Nguyen Dinh Vu was 17% and that of Nguyen Van Sang was 16%.

4.3. Regarding changes in several immune parameters before and after treatment.

Changes in humoral immunity: Before treatment, both the average serum IgG and IgA levels considerably decreased compared to the normal range. In contrast, serum IgM levels went up. After treatment, the immunoglobulins levels gradually turned to normal values as there was a remarkable increase in serum IgG level but was still lower than normal range. The serum IgM level significantly declined but still was higher than normal range. Our results were similar to that of Bahbah (2015), Azat (2012), Gulati (2010), Feehally (1984) and Banik (1994) who reported that there were clear

changes in serum immunoglobulin concentration in nephrotic syndrome. The pathogenesis of changes in serum immunoglobulin level in patients with primary nephrotic syndrome has not been fully explained so far. Several mechanisms have been proposed to explain the phenomenon of decreased serum IgG level in primary nephrotic syndrome. This might be due to increased IgG catabolism, decreased IgG synthesis or changes in the distribution of IgG in plasma. According to Schnaper and Robson (1992), a decrease in serum IgG and IgA levels might be a result of partial loss to urine. However, this hypothesis was not enough to explain the phenomenon because both IgG and IgA had large molecular weights ($> 150,000$ daltons), making it difficult to penetrate the glomerular basement membrane. On the other hand, IgG and IgA levels decreased even when the patient had complete remission. According to Pondman (1984), the first immunoglobulin synthesized in B lymphocytes was the IgM, after which there was a process of layer transferring to synthesize IgA and IgG. This transfer process was dependent upon activated T lymphocytes. In primary nephrotic syndrome, there was an imbalance in subgroups of T lymphocytes, so the synthesis of immunoglobulin was disturbed, leading to an increase in IgM and a decline in IgG and IgA levels. Our results were also in line with the results of many authors who noted that the IgG/IgM ratio decreased at the onset stage of the disease and the considerably decreased in patients whose response to steroids was poor.

Changes in cellular immunity: Before treatment, in both groups, the counts of TCD3⁺ and TCD4⁺ decreased while that of TCD8⁺ increased. The ratio of TCD4⁺/ TCD8⁺ decreased compared to normal value. After treatment, the number of subgroups of T lymphocytes also decreased, but the count of TCD8⁺ was still higher than normal.

+ **TCD3:** The count of TCD3⁺ decreased on admission and also decreased after treatment with intravenous cyclophosphamide combined with prednisolone. Our research results were consistent with the comments of the majority of both domestic and foreign authors such as Matsumoto (1990), Fiser (1991) and Sasdelli (1980). They reported that at the onset stage of INS there was a decrease in the count of T lymphocytes compared to normal range. Other authors did not find a significant change in the count of TCD3⁺. Some other studies showed that the percentage of TCD3⁺ increased but these studies only calculated the percentage of TCD3⁺ without counting

the number of TCD3+. Feehally (1984) also concluded that intravenous cyclophosphamide cause the count of TCD3+ lymphocytes to decline.

+ **TCD4:** The count of TCD4+ decreased on admission and after treatment with the combination of intravenous cyclophosphamide and prednisolone. Similarly, the study results of many authors showed that the count of TCD4+ lymphocytes decreased during the period onset of idiopathic nephrotic syndrome such as Smuk (2010), Fiser (1991), Hulton (1994) and Saxena (1992). Some stuides of Cagnoli (1982) and Chatenoud (1981) found that the count of TCD4+ was in normal range. The study of Nguyen Ngoc Sang (1999) showed an increase in TCD4+ count on admission. We had not read any literature or textbooks explaining why the count of TCD4+ lymphocytes increased or decreased in idiopathic nephrotic syndrome, so further studies were needed to explain this change. Previous studies noted that the count of TCD4+ decreased within 1 to 3 months after treatment cessation.

+ **TCD8+:** Our study results showed that TCD8+ count increased on admission and decreased after treatment but was still high compared to normal values. This was similar to the judgment of many authors such as Fiser (1991), Saxena (1992) and Herrod (1983) who found that the percentage of TCD8+ cells was not different from normal values. While Nguyen Ngoc Sang (1999) found that at the onset period of the disease, the TCD8+ count decreased compared to normal range. According to Taube (1984), the successful treatment of primary nephrotic syndrome with intravenous cyclophosphamide along with prolonged decline in inhibitory T lymphocyte function (TCD8+) suggested that TCD8+ lymphocytes contributed to the pathogenesis of primary nephrotic syndrome.

+ **Regarding TCD4/TCD8 ratio:** Similar to the studies of Herrod (1983) and Lama (2002) which showed that there was changes in the ratio of TCD4+/TCD8 + in bloodstream of patients with primary nephrotic syndrome, with a decrease in TCD4+ count, an increase in TCD8+ count and natural killer (NK) cells count. As a result, there was a decline in the ratio of TCD4+/TCD8+ compared to normal values.

Clinical meaning: Testing of immunological parameters will be valuable to contribute to the diagnosis, especially to the follow-up and prognosis of primary nephrotic syndrome. Study results of many other authors also showed that there were changes in

immunity in nephrotic which was obviously seen in the period of illness and decreased in the remission period. Many immune parameters returned to normal values when patients had remission. reduction. However, the results of different studies on TCD3+, TCD4+ and TCD8+ counts in the period of illness of INS varied. Therefore, more and more studies were needed to clarify the changes in cellular immunity in INS in general and after treatment with immunosuppressive drugs in particular.

In summary, through this study we found that in idiopathic nephrotic syndrome there were changes in both humoral and cellular immunity. Humoral immunity disorder probably resulted from cellular immunity disorder.

SEVERAL LIMITATIONS OF THE DISSERTATION

Genetic tests have not been done in this study, especially in steroid – resistant form.

The duration of follow – up was short (2 years), so other long-term side effects of cyclophosphamide have not been assessed.

Kidney biopsy was not available for all patients because the family did not agree to perform this procedure. As a result, no histopathological diagnosis was made.

CONCLUSION

1. Regarding the clinically epidemiological and biological characteristics of steroid - dependent and steroid - resistant INS in children.

The age at disease onset was mainly at pre - school and school – aged period with an average of 6.7 ± 4.1 years. The disease was seen more common in boys than girls. In our study, nephrotic syndrome was mainly in the simple form. The steroid-resistant idiopathic nephrotic syndrome had a higher percentage of non-simple form (71.4%) than the steroid-dependent INS (26.1%). Clinical manifestations were mainly edema in multiple organs. The major changes in laboratory test results were nephrotic – range proteinuria and albuminuria, hypoproteinemia, hypoalbuminemia, hypercholesterolemia and hypertriglyceridemia.

2. Regarding the efficacy of the combination of intravenous cyclophosphamide and prednisolone in treatment of childhood idiopathic nephrotic syndrome.

Intravenous cyclophosphamide combined with prednisolone showed a noticeable efficacy after 4 weeks of treatment for both steroid - dependent and steroid - resistant INS and was more effective in the steroid-dependent form. The cumulative total dose of intravenous cyclophosphamide in the steroid-dependent group (22.09 ± 12.31 mg/kg/course) was significantly lower than that of the steroid-resistant group (47.53 ± 30.31 mg/kg/course), $p < 0.01$. The hospitalization period of patients with steroid - dependent INS (12.0 (10.0-15.0) days) was significantly shorter than that of the steroid-resistant group (18.0 (13.5-25.25) days.), $p < 0.01$. Intravenous cyclophosphamide improved clinical and laboratory characteristics after treatment compared to the time of admission, $p < 0.001$.

The rates of no relapse after 6 months, 12 months, 24 months and 48 months in the steroid - dependent group were higher than that of the steroid-resistant group.

Common side effects of intravenous cyclophosphamide in combination with prednisolone were alopecia (35%) and Cushing's syndrome (23.3%). Less common side effects were infections, hypertension, and gastritis but these could be controlled.

3. Regarding changes in several immune parameters before and after treatment of steroid - dependent and steroid - resistant INS with the combination of intravenous cyclophosphamide and prednisolone.

After treatment, the white cell and neutrophil counts increased while lymphocyte count decreased in both study groups.

Before treatment, serum IgG concentration considerably decreased and serum IgA concentration also decreased while serum IgM concentration increased. The ratio of IgG/IgM decreased. After treatment, serum IgG and IgA concentrations went up whereas serum IgM level declined, and the IgG/IgM ratio gradually increased and returned to normal.

Before treatment, in both two groups, the TCD3⁺ and TCD4⁺ cell counts decreased while the TCD8⁺ count increased. The TCD4⁺/TCD8⁺ ratio decreased. After treatment with intravenous cyclophosphamide combined with prednisolone, the TCD3⁺, TCD4⁺ and TCD8⁺ counts and the TCD4⁺/TCD8⁺ ratio in both two study groups decreased compared to the time of admission but the TCD8⁺ count was still higher than normal range.

RECOMMENDATIONS

1. In limited economic conditions, the choice of cyclophosphamide is appropriate and effective for the treatment of steroid - dependent and steroid - resistant idiopathic nephrotic syndrome in children.
2. In the context of current difficulties in Vietnam, the results of this study can be applied in HaiPhong Children's Hospital and other provincial hospitals to give a hand to diagnosis, treatment, follow-up and prognosis of patients with steroid - dependent and steroid - resistant idiopathic nephrotic syndrome.
3. When funding is available, further studies should be underwent on functional disorders of subgroups of T lymphocytes, B lymphocytes, cytokines and chemokines to better understand the pathogenesis of the disease.

**PUBLISHED SCIENTIFIC PAPERS
RELATED TO THE CURRENT DISSERTATION**

1. Nguyen Bui Binh and Nguyen Ngoc Sang (2016). Clinical, laboratory characteristics and results of treatment of intravenous cyclophosphamide therapy in steroid-dependent primary nephrotic syndrome in children. *Journal of Practical Medicine*, No. 1004, 358-362.
2. Nguyen Bui Binh, Nguyen Ngoc Sang and Nguyen Thi Quynh Huong (2019). Clinical, laboratory characteristics and results of treatment of steroid - dependent and steroid - resistant idiopathic nephrotic syndrome in children with the combination of intravenous cyclophosphamide and prednisolone. *Journal of Vietnamese Medicine*, vol. 484 Special issue, 130-139..
3. Nguyen Bui Binh, Nguyen Ngoc Sang and Nguyen Thi Quynh Huong (2019). Alteration of some immune parameters before and after treatment of steroid - dependent and steroid - resistant idiopathic nephrotic syndrome in children with the combination of intravenous cyclophosphamide and prednisolone. *Journal of Vietnamese Medicine*, vol 484, Special issue, 140-148.