P001

HLAs IN CHILDREN WITH NEPHROTIC SYNDROME AT SKMC IN ABU DHABI –UAE

Z Abu Sharar, J Rajah, H Parsons

Objective of Study: Human leukocyte antigen has been frequently reported in children with nephrotic syndrome. Previous studies showed there is ethnic variation of HLA in children with nephrotic syndrome but it is well documented that there is high prevalence of HLA DR7 in different ethnic groups. Arab studies showed the same that DR7 is more predominant. There are two studies from Arabic countries one from Kuwait and one from Egypt ,to our knowledge no studies of the local UAE patients have been done. The aim of this study was to identify the HLA typing of children with nephrotic syndrome (minimal change disease-MCD) at SKMC in Abu – Dhabi – UAE, compare the result with Arab and other studies and to see if there are certain HLAs, which may predict the clinical course of nephrotic syndrome.
Method: Thirteen patients with nephrotic syndrome (MCD) were studied for HLA typing class 1(A,B,C) and class II(DR, DQ) . We compared the results with 25 healthy controls for HLA typing class I and II. We also compared HLA typing of frequent and infrequent relapses. We used the Fisher exact test to calculate the significance of association. All p-value were subjected to Benferroni correction .Relative risk (RR) and confidence interval(CI) were calculated for significant p-value.
Result: Only DR7 was found significantly increased in patients with nephrotic syndrome (MCD),when compared with healthy controls(p value= 0.00125, corrected < 0.003, RR= 3.846,CI=1.6-8.8) .No significant difference of HLA frequencies when compared patients with frequent and infrequent relapsing MCD nephrotic syndrome .
Conclusion: The increase frequency of DR7 in MCD in UAE is similar to other Arab and other ethnic groups, our study and others support a universal association of DR7 with MCD. Unlike other studies where certain HLA association were stronger in those patients with frequent relapses ,our study showed frequent relapses was not associated with certain HLAs, the lack of association of certain HLAs with frequency of relapses may need additional study as the sample size was limited.

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P002

MYCOPHENOLATE MOFETIL (MMF) IS EFFECTIVE IN STEROID-DEPENDENT OR FREQUENTLY RELAPSING NEPHROTIC CHILDREN

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Objectives of Study: Corticosteroids are the first line of therapy in children with nephrotic syndrome (NS). Approximately 60% of nephrotic children develop a relapse after the initial episode of illness, and about 50% of those become steroid dependent (SD) or frequent relapsers (FR). However, the morbid side effects of steroid therapy limit their long-term usage. Second line agents, such as cyclophosphamide and cyclosporine have proven to be effective in treatment (Rx) of patients with SDNS or FRNS, however, their toxic side effects limit their chronic use or repeated courses of therapy. MMF, an immunosuppressive agent used primarily in transplanted patients for acute rejection prophylaxis, has emerged as a new agent for Rx of a variety of glomerular diseases.
Purpose of the study: To evaluate our own experience and results with MMF in treating pediatric patients with SDNS and/or FRNS.
Methods: This is a retrospective study of patients with SDNS and/or FRNS treated with MMF for at least 3 months.
Results: MMF was used in 9 patients with SDNS (3), FRNS (2), and SDNS/FRNS (4), 7 were male and 2 were female patients. Mean age at time of diagnosis of NS was 3 (1.1 – 8.5) years, with mean age of 5.8 (2.9 – 10) years at start of MMF. Mean time from diagnosis of nephrotic syndrome to start of MMF Rx was 29.6 (10 – 81) months. Four patients failed therapy with: levamisole (1), or cyclophosphamide (3) prior to Rx with MMF. Renal biopsy was performed in 7 patients prior to starting MMF, all had mesangial proliferative glomerulonephritis, with no evidence of focal segmental glomerulosclerosis. Mean follow up after starting MMF therapy was 8.8 (4 – 20) months. Mean MMF dose was 948 (500 – 1087) mg/m^2/day. Only 1 patient failed Rx with MMF and was switched to cyclophosphamide. Before Rx with MMF, the mean relapse rate was 4.1 (1.5 – 6) relapses/patient/year. MMF resulted in improvement in 8 of 9 patients, with a reduction in the mean relapse rate to 1.3 (0 – 3) relapse/patient/year, p = 0.001. The relative risk of relapse without MMF Rx was 4.25, p = 0.0012. Six of the 9 patients were off steroids at last follow-up, and 3 were being tapered off steroids. None of the patients had significant adverse events or intolerance to MMF therapy.
Conclusion: We conclude that MMF is safe and effective for Rx of children with SDNS and/or FRNS. It significantly reduces the rate and risk of relapse in these patients. Due to its low toxicity profile and lack of nephrotoxicity, MMF should be considered in Rx of children with SDNS and/or FRNS to avoid long-term toxic effects of steroids and other traditional agents.

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P003

EXPERIENCE WITH LEVAMISOLE IN FR/SD CHILDHOOD NEPHROTIC SYNDROME IN A LARGE SAUDI CENTER

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Objectives of the study: To assess the efficacy and safety of the use of an immunomodulator drug; levamisole (L) in the treatment of frequently relapsing, steroid dependent (FR/SD) idiopathic childhood nephrotic syndrome.
Methods: A controlled prospective study study which was started on 1st January, 2002 and completed on 31st December, 2003. There were two groups: a treatment group who received L (2.5mg/Kg) on alternate days for 1 year and a control group who received low dose Prednisolone only (<0.5mg/Kg) on alternate days for 1 year.
Results: There were a total of 56 patients (32 in the treatment group and 24 in the control group). The demographic data were quite similar in both groups. The male to female ratio was 1.66/1 in both groups. The mean age upon initial diagnosis was 3.3 years in the L group versus 4.3 years in the control group. The mean duration from diagnosis to the start of the second line treatment was 3.2 years in the L group versus 2.8 years in the control group. The relapse rate and the total cumulative dose of prednisolone during the year prior to the second line therapy was compared to that during the year following the institution of second line therapy in 56 patients. The mean relapse rate was reduced more significantly in the L group. It was reduced by 0.29 versus 0.11 relapse/patient/month in control group (P=0.0001). The mean cumulative dose of steroids was also reduced more significantly in the L group. It was reduced by 293 versus 102mg/m^2/month in control group (P<0.0001). Therapy failure was seen in 3/32 (9.4%) in the L group versus 12/24 (50%) in the control group. In 20/32 (62.5%) patients using L, no relapses were seen at all in the follow up year post therapy versus 0% in the control group. No adverse effects of L were seen. The cost of therapy was estimated to be 25 US dollar per year for a 20Kg body weight child.
Conclusion: Levamisole is highly efficacious, safe and easily affordable therapy for idiopathic childhood FR/SD nephrotic syndrome.

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P004

SERUM IMMUNOGLOBULIN E IN CHILDREN WITH IDIOPATHIC NEPHROTIC SYNDROME

A Ninik, MS Noer

Immunoglobulin E (Ig E) is commonly associated with allergy. In glomerular diseases have been noted that there is an elevation of serum Ig E levels. Higher serum Ig E levels in children with nephrotic syndrome (NS) have been related to poor outcomes with frequent relapses or steroid resistant nephroticsyndrome (SRNS). However, some studies found that serum Ig E levels did not have correlation with the prognosis of NS.
Objectives of Study: The aim of this study was to investigate the relation ship between the serum Ig E levels and the outcomes of children with idiopathic nephrotic syndrome.
Methods: Prospective observational longitudinal study has been conducted on 23 children with idiopathic nephrotic syndrome (INS). Serum Ig E levels was measured before initiated the treatment of prednisone and the outcomes of the treatment were observed. The results were analysed using one way anove and logistic multiple regressi.
Results: The results of this study was as followed, 1 child was excluded from this study. Two children (9%) of 22 INS had steroid dependent nephrotic syndrome (SDNS), 1 (5%) had frequent relapses nephrotic syndrome (FRNS), 1 (5%) had SRNS and 18 (82%) had infrequent relapses nephrotic syndrome (IRNS). Twenty one (96%) of 22 children had elevation of serum Ig E levels with mean 2002.5 (55.2-9618 IU/l). Only one child with IRNS had normal serum Ig E level (55.2 IU/l). In the contrary, the highest serum Ig E level was also found in IRNS (9618 IU/l). There were no correlation between serum Ig E levels and the outcomes of the treatmen i.e; IRNS, SDNS, FRNS and SRNS (p>0.05).
Conclusion: This study supported the studies that there were no correlation between the serum Ig E levels and the outcomes of the treatment in children with idiopathic nephrotic syndrome.

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P005

CLINICAL AND PATHOLOGICAL FEATURES OF EARLY ONSET NEPHROTIC SYNDROME IN TURKISH CHILDREN

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The causes of nephrotic syndrome (NS) occurring within the first year of life may vary according to the genetic and environmental characteristics of the population. It can be hypothesized that the spectrum of early onset NS in Turkish children may show some variations from the other series due to the wide heterogeneity of our population. For this purpose, we planned to evaluate the causes, courses and prognosis of early onset nephrotic syndrome in Turkish children.
From 1990 to 2003, 32 patients (19 F, 13 M) with NS younger than one year of age were evaluated. The mean age of the presentation was 3.2±1.4 months (3 days- 11 months). All patients had massive proteinuria, hypoalbuminemia and edema, and 5 of them had a positive family history for NS, there was the history of consanguineous marriage in 14 of the 32 families.. Renal biopsy was performed in all patients at the time of NS diagnosis. The mean follow-up time was 36.4 months (1 month- 8 years).
In 18 patients (8 F, 10 M), NS was observed in the first 3 months of life and they were classified as congenital NS. Renal biopsy revealed Finnish type NS in 11 cases (61%), diffuse mesangial sclerosis (DMS) in 5 (28%), focal segmental glomerulosclerosis (FSGS) in one and mesangio proliferative glomerulonephritis (MesPGN) in one case with congenital NS. All the patients with Finnish type NS were presented before 3 months of age. The remaining 14 patients (10 F, 4 M) presented with NS after the 3 months of age that were classified as infantile NS. The distribution of biopsy findings in this group was as follows; DMS in 5 cases (36%), minimal change NS in 5 (36%), FSGS in 2, MesPGN in 2 cases. Mutation of the WT1 gene was found in one of 5 children with isolated congenital NS with DMS.
End-stage renal disease was reached either concomitantly or within 4 months after the onset of NS in 6 of 10 patients with DMS. Two patients with Finnish type NS also reached to ESRD within the first year of life. Two of 10 DMS patients and 4 of 11 with Finnish type NS died due to the mainly septic complications during the study. Mortality rate was 18.8 % in Turkish children with early onset NS, while it was 33.3% in congenital NS group. There was no patient who was transplanted in the study group. FSGS patients responded to IV pulse steroid therapy and all were in remission at the last control, MCNS and MesPGN patients were also in remission after the oral steroid therapy.
As a conclusion, Finnish Type NS was the most frequent single cause of congenital NS in our population like the other series, and DMS was the second. DMS and MCNS were the main causes of Turkish infantile NS. We suggest that the spectrum of early onset NS is not vary from the other populations except the higher incidence of MCNS during infancy.

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P006

PARAOXONASE 192 POLYMORPHISM IN CHILDHOOD NEPHROTIC SYNDROME

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Objectives of study: Human paraoxonase I (PON) is a serum enzyme related with high density lipoprotein (HDL) which has a major role in preventing oxidative modification of low density lipoprotein (LDL). PON has two genetic polymorphisms both due to aminoacid substition, one involving glutamine (A genotype) and arginine (B genotype) at position 192 and the other leucine (L genotype) and methionine (M genotype) at position 55. Hyperlipidemia and increased lipid oxidation reactions in nephrotic syndrome may lead to glomerulosclerosis and progression of the glomerular disease. In this study we aimed to investigate lipid oxidation markers [malonydialdehyte (MDA), conjugated diene], serum PON activity and PON 192 polymorphism in children with steroid sensitive nephrotic syndrome (SSNS), focal segmental glomerulosclerosis (FSGS) and control subjects.
Methods: 25 children with SSNS diagnosed according to ISKDC criteria and 25 children with biopsy proven FSGS and 30 healty controls were included in the study. Lipid peroxidation products were measured in plasma –MDA- by the modified thiobarbituric acid assay method and conjugated diene levels were measured by Dormandy method. PON activity was measured by spectrophotometric assay of p-nitrophenol production following addition of paraoxon. Polymerase chain reaction, restriction fragment lenght polymorphism and agarose gel electophoresis techniques were used to determine PON 192 genotype.
Results: The mean age of the study population was 7.3 ± 3.5 years, 9.6 ± 5.3 years and 8.2 ± 3.1 years for SSNS, FSGS and controls respectively. Serum MDA and conjugated diene levels were both high in SSNS and FSGS patients in respect to control subjects. Serum PON activity was low in frequently relapsing SSNS and in FSGS patients whose duration of FSGS is more than 2 years. The results are shown on table 1. Distribution of PON 192 genotype (AA, AB, BB) in SSNS, FSGS patients and controls were similar. A significant correlation was detected between PON polymorphism and serum PON activity in FSGS and SSNS patients. PON polymorphism also showed significant correlations for the presence of hypertension, hematuria and early age of onset in SSNS patients.
Conclusion: Increased lipid oxidation reaction is present in SSNS during active phase of nephrotic syndrome and a persistent lipid oxidation reaction is present in FSGS patients. PON 192 polymorphism did not show any difference between SSNS, FSGS and control subjects. Low PON activity in frequently relapsing SSNS patients and in FSGS patients whose duration of FSGS is more than 2 years may have a role in pathogenesis of nephrotic syndrome.
Table 1: MDA, conjugated diene and serum PON activity results of the study group

	SSNS		FSGS		Control
MDA (µmole/L)	6.4 ± 3.8		7.6 ± 2.1		5.2 ± 2.1
Conjugated diene (nmolehydroperoxide/ml)	12.8 ± 8.3		13 ± 4.7		10.1 ± 2.1
PON activity (U/ml)	Attack number		Duration of FSGS		
	< = 3	> 3	< 2 years	> 2 years	164.8 ± 71.1
	215.8±145	109.1±71.1	256.7±108.9	93.6±44	

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P007

THE G686A POLYMORPHISM OF NPHS2 GENE IN POLISH CHILDREN WITH IDIOPATHIC NEPHROTIC SYNDROME (INS) – PRELIMINARY REPORT

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Mutations in the NPHS2 gene encoding podocin represent a frequent cause of steroid-resistant nephrotic syndrome (SRNS), occurring in approximately 20 to 30% of sporadic cases of SRNS.
The aim of our study was to evaluate the association between G686A transition in the NPHS2 gene and both the predisposition to idiopathic nephrotic syndrome and the prognosis in INS children. The study group consisted of 163 children with INS and 111 healthy children as controls. Genomic DNA from peripheral blood leukocytes was amplified by PCR method with primers flanking the polymorphic region. The A allele was identified by a loss of *Cla*I restriction site, while G allele (wild type) by a gain of *Cla*I restriction site. The homozygous genotype with two mutated A alleles has not been observed neither in a study nor in a control group.
No significant difference in genotype frequency has been observed between both groups (85.8% GG homozygotes and 14.2% AG heterozygotes in INS group versus 79.3% GG homozygotes and 20.7% AG heterozygotes in control group, n.s.). In addition, no association among NPHS2 genotypes and gender, age of INS onset, type of response to steroid treatment, INS recurrences, serum concentrations of creatinine and cholesterol as well as values of blood pressure was observed in INS group.
The results of our preliminary study suggest the lack of association between the G686a polymorphism of the NPHS2 gene podocin and both the predisposition to INS and the prognosis in Polish children with idiopathic nephrotic syndrome.

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P008

THE C(-31)T POLYMORPHISM OF INTERLEUKIN-1 BETA (IL-1β) GENE IN CHILDREN WITH IDIOPATHIC NEPHROTIC SYNDROME

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The C(-31)T polymorphism of the gene encoding interleukin-1β (IL-1β) has been associated with an enhanced IL-1β production. On the other hand, the response to steroid treatment significantly predicts the prognosis in children with idiopathic nephrotic syndrome (INS). Therefore, it raises the question whether C(-31)T polymorphism of IL-1β gene may be associated either with the susceptibility to INS or with the clinical course of INS. The study group consisted of 91 children with INS and 91 healthy children as controls. Genomic DNA from peripheral blood leukocytes was amplified by PCR method with primers flanking the polymorphic region. The T(-31) allele was identified by gain of *Alu*I restriction site. No significant difference in genotype frequency has been observed between both groups. In addition, no association between IL-1β genotypes and gender, age of INS onset, INS recurrences, serum concentrations of creatinine and cholesterol as well as values of blood pressure was observed in INS group. The results of our preliminary study suggest the lack of association between the C(-31)T polymorphism of the IL-1β gene and both the predisposition to INS and the prognosis in Polish children with idiopathic nephrotic syndrome.

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P009

SPLENIC GIANT EPITHELIAL CYST IN A 12 YEARS OLD CHILD – THE EXTREMELY RARE CAUSE OF PROTEINURIA - A CASE REPORT.

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Splenic cyst is a relatively rare entity, but the increasing use of ultrasound makes this diagnosis more frequent. A 12-year-old girl was admitted to the Clinic due to proteinuria and giant tumor in the left upper quadrant of the abdominal cavity, which had been accidentally found on routine physical examination. The history revealed periodic left upper quadrant discomfort or even pain, occurring for last 3-4 years. Parents denied abdominal injury in a child. A palpable tumor in the left upper quadrant and central abdomen extending 10 cm below the costal margin was found on physical examination. With the exception of decreased platelet count (104-132 G/L) other laboratory tests: SR, RBC, WBC, reticulocyte count, liver tests, BUN and blood ions were normal. Urinalysis revealed proteinuria (120-250 mg%); 24-hour proteinuria was 11-34 mg/kg/24h -whereas serum protein and cholesterol levels. Abdominal ultrasound revealed the presence of giant cyst (16.3 x 14.7 x 13.6 cm) that compressed renal vessels (distended left renal vein) and pushed down the left kidney. The size of both kidneys were normal. Abdominal CT scan revealed the single splenic cyst measuring 11 x 17 cm in transverse section and 13 cm in length. The stomach, pancreas and intestines were pushed to the right side. The girl underwent hemisplenectomy. Histopathological specimens showed features typical of congenital epidermoid (epithelial) cysts with no signs of atypia. On day 7 following surgery proteinuria and thrombocytopenia subsided. Control abdominal ultrasound did not show distended left renal vein seen on previous examination. The authors would like to underline the extremely rare coincidence of a childhood splenic giant epithelial cyst and proteinuria resulting from renal vessels compression by the cysts and its disappearance after elective cystectomy.

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P010

HODGKIN LYMPHOMA – THE EXTREMELY RARE CAUSE OF STEROID RESISTANT NEPHROTIC SYNDROME IN 17 YEAR OLD CHILD – A CASE REPORT

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The case of a 17-year-old girl with Hodgkin lymphoma (HL) manifested in form of nephrotic syndrome (NS) has been described.
She was admitted to the local hospital due to edema and proteinuria. The diagnosis of NS was made followed by administration of steroids. The girl did not respond to standard therapy thus was transferred to the Division of Nephrology and Dialysis, City Hospital. On admission: sever edema of lower extremities, swollen eyelids and effusions of the body cavities were found. Laboratory tests revealed: nephrotic proteinuria, decreased serum level of albumin, hypercholesterolemia. Continuation of prednisone therapy did not result in remission. Renal biopsy revealed: minimal change nephrotic syndrome (MCNS). Chest X-ray showed mediastinal widening. Based on histopathological studies of the mediastinal wedge biopsy of the anterior mediastinum the diagnosis of Hodgkin lymphoma (HL) NS (nodular sclerosis) type was made. The girl was transferred to the I[st] Department of Pediatrics. Laboratory studies revealed features of active nephrotic syndrome. Abdominal ultrasound and CT scan, bone marrow biopsy, bone trephine biopsy were normal. Chest CT scan confirmed the presence of anterior mediastinal mass. She was classified as stage III HL and treated with HD 97 protocol. Clinical and laboratory signs and symptoms of NS resolved within one week from the onset of therapy. Control chest CT scan showed no mediastinal mass. The patient is followed up at the Outpatient Hemato-Oncology Clinic.
The authors wanted to underline the rare association of HL and nephrotic syndrome, unresponsiveness of NS to prednisone therapy and early remission of nephrotic syndrome as the result of chemotherapy of HL.

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P011

EFFECT OF Ω – 3 FATTY ACIDES ON SERUM HOMOCYSTEINE IN CHILDHOOD STEROID-SENSITIVE NEPHROTIC SYNDROME

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Prothrombotic state is well known in nephrotic syndrome (NS). Many of investigators demonstrated that mild hyperhomocysteinemia is an independent risk factor for venous thromboembolism.The dietary intake of ω – 3 fatty acides (FA) alters the serum fatty acid profile, reduces plattelet agregation and improves blood rheology. The influence of ω – 3 FA on plasma homocysteine (Hcy) in hyperlipidemic human remains controversial.
Objectives of study is to investigate influence of ω – 3 FA on serum Hcy level in childhood steroid – sensitive nephrotic syndrome (SSNS).
Methods: Twenty five patients aged 6.6 ± 2.4 years (2.3 – 11.7 years) with SSNS were included in the study. 12 children were treated with prednisolone (control group) and 13 with prednisolone plus fish oil (6 mg/ day) for 12 weeks. Serum total Hcy level was investigated by a fluorescence polarisation immunoassay (Axis-Shield AS, Oslo, Norway) before and after treatment. No significant differences in age, serum lipides, fatty acid profile, serum Hcy concentration between groups before the treatment were observed.
Results: The mean fasting serum Hcy levels in SSNS children were 6.65 ± 2.43 μmol/l and statistically did not differ from the healthy children. Significant increase in serum Hcy concentrations from 6.96 to 8.86 μmol/l in fich oil group after treatment was found ($p < 0.05$) . There were statistical differences ($p < 0.02$) in serum Hcy levels between the gruops after the treatment.
Conclusion: several pathways and vitamin cofactors are involved in Hcy metabolism. A. Piolot et al. showed apparent interaction of ω – 3 FA and nitritic oxide on Hcy metabolism in health. To answer the question if ω – 3 FA and what particular dosage and treatment duration increase serum Hcy concentration in SSNS children requires further studies.

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P012

PROTECTION OF ISCHEMIC RENAL INJURY BY RENAL ISCHEMIC PRECONDITIONING: POSSIBLE ROLE OF NITRIC OXIDE

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Objectives of Study: Ischemic preconditioning, a phenomenon induced by brief ischemia and reperfusion periods, renders an organ tolerant to subsequent prolonged ischemia. This study evaluated different schedules of preconditioning the kidney to assess the role of nitric oxide (NO) and determine the effects of preconditioning on kidney transplantation.
Methods and Results: Ischemic preconditioning, which consists of three cycles of 2-minute ischemia followed by 5-minute reperfusion, was performed prior to 45-minute ischemia. Ischemic preconditioning significantly improved the renal dysfunction induced by 45-minute ischemia followed by 24-hour reperfusion. Histopathological examination of the kidney of ischemia/reperfusion rats revealed severe renal damage, and suppression of the damage was seen with the ischemic preconditioning treatment. NO metabolites (NOx) production in the kidney after 45-minute ischemia and reperfusion was markedly increased in ischemia/reperfusion rats with ischemic preconditioning, compared with animals not subjected to ischemic preconditioning. The levels of renal antioxidant enzymes were significantly improved by ischemic preconditioning as compared to ischemia/reperfusion rats. The improvement of renal dysfunction in ischemic preconditioning rats was abolished by the pretreatment with NG-nitro-L-arginine methyl ester (L-NAME), a nonselective NOS inhibitor.
Conclusion: Ischemic preconditioning has a protective effect on renal structure and function and these findings suggest that the protective effect of ischemic preconditioning on ischemia/reperfusion -induced acute renal failure is closely related to the renal nitric oxide production

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P013

WHAT IS THE BEST METHOD FOR QUANTIFICATION OF PROTEINURIA IN CHILDREN?

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Objectives of Study: Proteinuria is one of the most important prognostic factors in children with kidney diseases. Presently three methods for proteinuria quantification are used clinically. 24h urine collection is cumbersome, in small children is often incorrect and the exact urine collection is crucial for estimation of proteinuria (U_P). Other methods are urinary protein/creatinine (U_P/U_{Cr}) and protein/osmolality (U_P/U_{Osm}) ratios. A major advantage is that only spot urine is required for calculation of both ratios. The aim of our prospective study was to compare all three methods and to find out the best and the most reliable test for quantification of proteinuria in children.
Methods: Between April 2002 and January 2004 urinary protein excretion was examined in carefully collected 24h specimens. Altogether 1293 samples in 411 pts (aged 0.3 - 23 years, median 12.1 years) with proteinuria exceeding 4mg/m^2/h have been evaluated. In the same urine specimen we determined creatinine (mmol/l), protein concentration (mg/l) and osmolality (mmol/kg) for calculation of protein excretion (mg/m^2/h), U_P/U_{Cr} (mg/mmol) and U_P/U_{Osm} ratios (mg/mmol).
The protein concentration was measured by the quantitative turbidimetric method (450 nm) using alkylbenzylamonium. The creatinine concentration was determined by creatinine enzymatic method Advia 1650 (Bayer). The urine osmolality was measured by freezing point depression method (Fiske 2400). The proteinuria ranged from 4.1 – 2317 mg/m^2/h. We compared U_P (mg/24h), U_P (mg/m^2/h), U_P/U_{Cr} and UP/$_{Osm}$. Pearson's correlation coefficient was used for a statistical analysis. The statistical significance of correlation coefficient differences was tested by SISA software.

Results: Correlations coefficients

	U_P/U_{Cr}	U_P/U_{Osm}	
U_P (mg/24h)	0,690	0,812	$p < 0.005$
U_P (mg/m^2/h)	0,834	0,852	$p < 0.015$

- The standard evaluation of 24 h proteinuria was compared with U_P/U_{Cr} and U_P/U_{Osm} ratios.
- Much better correlations were demonstrated between the ratios **U_P/U_{Cr} and U_P/U_{Osm} and U_P (mg/m^2/h)** than in U_P (mg/24h) - an important finding for using these ratios in children.
- The best correlation was shown between **U_P (mg/m^2/h) and U_P/U_{Osm} ratio**.
- The good correlation of U_P (mg/m^2/h) and U_P/U_{Osm} ratio signifies that both methods of proteinuria quantification may be considered as nearly as identical.
- U_P/U_{Osm} ratio value 0.3 corresponds with proteinuria of 4 mg/m^2/h.

Conclusions:
- U_P/U_{Osm} ratio seems to be the best method for quantification of proteinuria in children.
- U_P/U_{Osm} ratio could replace cumbersome 24 h urine collection for estimation of proteinuria.
- 24-h urine collection may be abandoned.
- Examination of U_{Osm} is cheaper than U_{Cr} (in our conditions 2.5 fold cheaper).
- A significant U_P/U_{Osm} value in children with pathological proteinuria is > 0.3

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P014

POPULATION-BASED STUDY OF NEPHROTIC SYNDROME: INCIDENCE, DEMOGRAPHICS, CLINICAL PRESENTATION AND RISK FACTORS

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Objectives of Study: To estimate the incidence of idiopathic nephrotic syndrome (NS) and describe risk factors for its development in relation to age, sex, ethnicity and initial clinical presentation.
Methods: Using mechanisms of prospective reporting of diseases of interest by paediatricians, developed and validated by the Australian Paediatric Surveillance Unit (APSU), all new cases of idiopathic nephrotic syndrome were identified in Australia from July 1998 to June 2001. A survey was sent to all paediatricians reporting a case of idiopathic nephrotic syndrome to obtain details on demography, clinical presentation, treatment and outcomes over 1-year. NS was defined as a child aged > 3 months and < 15 years with oedema, proteinuria (> 3+ on dipstick), hypoalbuminaemia (serum albumin < 25g/L) and normal renal function (creatinine in normal range for age). To estimate incidence, population based denominators were obtained from the Australian Bureau of Statistics (ABS) 2001 census. Ethnicity classifications were devised from major ethnic groupings based on the country of birth of both parents from ABS. Data were analysed using exact 95% confidence intervals for incidence and the χ^2 test for differences in proportions.
Results: One hundred and forty five cases of NS were reported (80 boys, 65 girls), and incidence of 1.12 (95% CI 1.03 - 1.43) per 100,000 children less than 15 years. The incidence was no difference in boys (1.3, 95% CI 1.0 -1.6) than girls (1.1, 95% CI 0.9-1.4). There was a inverse linear relationship between age and incidence (χ2 trend = 63.5, 1 df p<0.0001), with an incidence of 2.38 (95% CI1.91 - 2.91) per 100,000 between 3 months to < 5 years compared with an incidence 0.84 (95% CI 0.6-1.1) cases per 100,000 in the 5-15 year age group.
Most children were from Oceania (including Australian born citizens) (70%), with 18% children from Asian countries and 12% from other countries (Europe (4%), North Africa (1%), Middle East (1%) and Africa (1%)). Preliminary analysis has suggested a higher incidence of NS in Asian children.
At initial presentation, 3% percent of children had an elevated creatinine, 21% were hypertensive (based on normal for age range) and 53% had microscopic haematuria.
Conclusion: NS has an incidence of 1.12 per 100,000 children, which varies with age but not gender. Microscopic haematuria is a common finding at clinical presentation. Incidence appears to be greater in children of Asian descent.

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P015

OUTCOMES FOR CHILDREN WITH NEPHROTIC SYNDROME - A PROSPECTIVE POPULATION BASED COHORT STUDY

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Objectives of Study: To describe the outcomes of children with nephrotic syndrome (NS) (time to remission, steroid responsiveness, chronic renal failure, infection and thrombosis) and to determine risk factors for these complications.
Methods: Paediatricians reporting a case of NS between July 1998 and June 2001 to the Australian Paediatric Surveillance Unit (APSU) were sent a follow-up questionnaire one year after the initial notification. Details were sought on steroid responsiveness, remission and relapse rates, infection, thrombosis, and patient status at 1-year. Patients were classified into five categories from best outcome to worst outcome. These categories included no relapse, infrequent relapsing (IFRNS), frequent relapsing (FRNS), steroid dependent (SDNS) and steroid resistant (SRNS). Baseline clinical presentation, age and gender were used to try to predict category of disease. Definition of NS, remission and relapse were as per ISKDC guidelines.
Results: Of the original cohort of 145, one hundred and thirty two (91%) follow-up questionnaires were received. Data was available for 126 (95%) patients with 6 (5%) lost to follow-up.
Steroid Responsiveness: One hundred and seven (81%) patients had steroid sensitive nephrotic syndrome (SSNS) while nineteen (14%) patients were classified SRNS. In the SSNS group sixty five percent of patients were under five years of age and there were 63 males (60%) and 43 females. SRNS children had a significantly higher proportion of females (63%) compared to males. Renal biopsies were performed in thirty (21%) children. Eleven (37%) children were steroid responsive while nineteen (63%) were steroid resistant. Focal segmental glomerulosclerosis was the most common histological finding in fourteen (47%) children, nine (30%) had minimal change and 6% mesangial proliferation. Creatinine, hypertension and haematuria at presentation were not significantly different between SSNS and SRNS groups.
Time to Remission: The median time to remission in all categories of SSNS children was 10 days (range 3-120). Thirty-one (31%) of children were in remission by one week, 75% by two weeks and 90% by three weeks. The remaining 10% of children went into remission between 4 to 17 weeks.
Relapse Rates: Relapses occurred in eighty seven (80%) patients with a median of two relapses (range 1-6) and a median time to relapse of 13 weeks (range 1-48). Forty (47%) patients were classified as IFRNS, 29% FRNS and 24% SDNS. The proportion of males varied significantly across all categories with the lowest proportions in the IFRNS and the SRNS categories. Time to relapse was significantly (χ2= 20.02, 2df, p< 0.0001) lower in the SDNS and FRNS categories.
Adverse Events: Eight (6 %) children from all categories developed infection (3 with pneumonia and 5 with peritonitis) with three cases occurring at the time of presentation. Pneumococcus was the organism identified in 3 (38%) patients and one patient was on antibiotic prophylaxis. Two (25%) had received pneumococcal vaccination prior to infection. Thrombotic complications did not occur in any patients. Two (1.5%) patients, both from the SRNS category had developed chronic renal failure at one year follow-up.
Conclusion: SSNS remains the most common form of NS with males more likely to have steroid sensitive disease. 90% of children responding to steroids will be in remission three weeks following presentation. Time to relapse, but not time to remission, is a reasonably reliable predictor of disease category.

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P016

UNEXPLAINED VARIATION IN THE INITIAL MANAGEMENT OF CHILDREN WITH NEPHROTIC SYNDROME

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Objectives of Study: The clinical management of idiopathic childhood nephrotic syndrome (NS) includes a combination of curative, supportive and preventative management. The objective of this study was to describe the current management regimens used by paediatricians in patients presenting with their first episode of NS.
Methods: A survey was sent to paediatricians reporting a case of NS between July 1998 and June 2001 to the Australian Paediatric Surveillance Unit (APSU). Paediatricians were requested details on planned steroid regimens, pneumococcal vaccination, antibiotic prophylaxis, the use of albumin infusions, diuretics and aspirin. NS was defined using standard criteria.
Results:
Corticosteroid Regimens: Eighty-nine (129/145) percent of paediatricians began their patients on the recommended regimen of 2mg/kg/day or 60mg/m^2/day of prednisone with dosages given daily (73%), twice daily (23%) or three times daily (4%). There was large variability in the planned duration of daily steroids with thirty-nine percent planning for 4 weeks of daily steroids, 17% for a longer duration of 5-12 weeks, 5% for shorter duration of 2-3 weeks, 4% until in remission and 35% did not specify.
Invasive pneumoccocal disease prophylaxis: Prophylaxis against invasive pneumococcal disease varied among respondents. Antibiotic prophylaxis was used in 86/145 (59%) patients, with penicillin (73) being the most widely used antibiotic compared to cephalosporins (8) and macrolides (2). Pneumococcal vaccination was used in thirty-five patients (24%) with 94% of patients over the age of two years. Sixty-six percent (23/35) of patients were clinically nephrotic and 91% receiving steroid treatment at the time of vaccination. This low vaccination rate may be indicative of differences in recommendations for pneumococcal vaccination at the time of the survey. Pneumococcal vaccination at the time of this survey was recommended for children over the age of 2 years secondary to the low immunogenic potential of the vaccine in children under 2 years.
Oedema management Albumin infusions were used in sixty (41%) patients while diuretics, primarily frusemide, were used in fifty-one (35.4%) patients.
Thrombosis prophylaxis: Aspirin as prophylaxis was used in forty-eight (33%) of patients.
Conclusion: There remains marked variability in the management of first episodes of NS despite ISKDC recommendations. Variability was not explained by regional variation in practice and may be the result of random effects, physician preference or differences in patient populations related to age and clinical presentation. The preparation of guidelines for the management of idiopathic nephrotic syndrome using available systematic reviews and where not available current clinical practice guidelines may assist in standardising current management practices

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P017

BODY GROWTH OF STEROID-DEPENDENT NEPHROTIC CHILDREN AFTER TREATMENT WITH CYCLOSPORINE –A (CSA)

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Children with steroid dependent minimal change nephrotic syndrome (MCNS) that frequently relapse usually receive high doses of corticosteroids over a long period and develop severe toxicity such as growth retardation, obesity, hypertension, etc. Steroid therapy, urinary loss of proteins, ions, and hormones bound to protein including insulin like growth factor (IGF-I) are the main factors that affect statural growth in these patients. Cyclophosphamide is recommended for children with frequently relapsing steroid-dependent MCNS. However, about 30% will still have frequent relapses. CSA is a widely accepted treatment for idiopathic steroid-dependent nephritic syndrome (SDNS) children. CSA in experimental studies increased serum IGF-I and GH.
Objectives: Compare statural growth between pre- and post-treatment of idiopathic SDNS children after one year treatment with CSA.
Methods: Criteria for inclusion in the study were normal serum creatinine and liver function. All patients were submitted to renal biopsy, after parents' consent and before starting CSA. We included patients who present less than 30% of interstitial fibrosis. CsA dosage was 4-6 mg/kg/day combined with previously prescribed prednisone which had been withdrawn. Attempted level: 50-150 through whole blood. Body height was measured using a stadiometer and weight, in the monthly consultation, but mainly before CSA (T0) and after treatment (T12). Standard deviation scores of height (SDS) were related to the growth standards of Marcondes. Body mass index (Kg/m^2) was calculated in agreement with National Center for Health Statistics (NCHS). Target height (TH) was determined using the formula: mid-parental height +10 cm for boys and -2.6 cm for girls. To asses bone maturation, bone age (BA) of all patients was determined using Greulich & Pyle standards, in T12. To asses height development in relation to the normal population, the height data were transformed to SDS, calculated according to the equation $SDS=(x_i-x_s)/SD_s$, where x_i= individual value x_s and SD_s are the sex-specific means and the SD reference population interpolated for the actual age of the subject.
Results: Fourteen idiopathic SDNS children were studied, 6 female 8 male, aged 8-16 (average 12.1 ± 3.8). Histological findings leading to the INS were**:** Minimal Change Disease (n=6), Focal Segmental Glomerulosclerosis (n=7) and Membranous Nephropathy in one. Steroids were withdrawn within 3.8 (± 2) months and all patients went into total remission. Prior to CsA, 43%received pulsetherapy with methylprednisolone in 6, cyclophosphamide in other 6 (43%). There was statistical significant improvements in the gain of height and body mass index (BMI). The SDS in T0 was -0,89 (± 1,27) and in T12 -0,51 (± 1,15), p=0,001 (Wilcoxon). The BMI in T0 was 22,4(±5,2), and in T12 20,0 (± 4,0), p=0,003 (Student T-test). Bone age and height at T12 were correspondent to target height.
Conclusions: CsA was highly effective in inducing remission and patients experienced an improvement in height and weight, possibly due to remission and suspension of steroid therapy. However CSA might play a specific role stimulating growth hormones release.

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P018

ROLE OF CALCIUM AND VITAMIN D IN PREVENTION OF METABOLIC BONE DISEASE IN CHIDREN WITH NEPHROTIC SYNDROME

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Objectives of Study: This study was conducted to evaluate the role of Calcium and Vitamin D supplements in the prevention of metabolic bone disease in children with idiopathic nephrotic syndrome (INS).
Methods: We prospectively studied 100 consecutive children with INS. These children were treated with prednisone as per the standard APN protocol. All of them were subjected to a baseline assessment for clinical, biochemical and radiological evidence of metabolic bone disease and initiated on daily Calcium (200mg /day) and Vitamin D (200 IU /day) supplements, followed by a repeat assessment at 6-12 months. The principal outcome variable was a change in delta az scores. This was calculated as the difference between the initial and follow-up BMD z scores. A univariate and multivariate analysis was done to analyze for factors predictive of improvement in delta Z score.
Results: Of the 100 children, 88 completed the study. Of these 15 were noncompliant while the rest 73 continued taking the supplements. On evaluating the delta z scores we observed, that despite prednisone therapy, the mean spinal BMD values in the study group were significantly better at the end of the study period (0.620+0.013 gm/m^2) as compared to the baseline values (0.561+0.01 gm/m^2) (p = 0.000) on Calcium and Vitamin D supplements. There was deterioration in the delta z scores in only 4/88 (4.5%) children. None of the patients in either group developed pathological fractures. We observed that on multivariate analysis, the factors which were significantly predictive of an improved BMD delta z score were younger age at BMD study (p=0.000), Calcium supplement (0.000), Vitamin D supplement (0.000), greater dietary calcium intake (0.042) and lower steroid dose per year (0.005) (R value=0.630). On comparing the group of 15 children (Group I) who had discontinued calcium and Vitamin D supplements right at the onset with the rest of the 73 (Group II), we observed that children in group II had a significantly greater total calcium intake and a positive delta z score (as compared to group I who had a negative delta z score).
Conclusion: Hence we conclude that concomitant Calcium and Vitamin D supplements are of therapeutic value in prevention of MBD in children with nephrotic syndrome.

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P019

STUDY OF THE CARRYING OF RESPIRATORY TRACT VIRUSES IN STEROID RESPONSIVE SIMPLE NEPHROTIC SYNDROME

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Objective: This study aimed to investigate the relationship between the carrying respiratory tract viruses and the quantitative change of urinary protein of the steroid responsive simple nephrotic syndrome (SRSNS), and to assess the function of respiratory tract viruses in the triggering of the SRSNS.
Methods: 37 children with nephrotic syndrome underwent the examination of respiratory tract viruses (APAAP method) once a week when they were hospitalized from August 1997 to March 1998. Among them, 17 children had simple nephrotic syndrome and 10 had nephritic nephrotic syndrome. Moreover, 18 children with respiratory infection in the same season were chosen as the control and were subjected to the examination of respiratory tract viruses by the same method.
Results: In the cases of SRSNS, the percentage for the carrying of respiratory tract viruses increases obviously in the acme; the percentage for the carrying of respiratory tract viruses of SRSNS has close relationship with the quantity of urinary protein ($P<0.05$). There is no difference between the constitution of the category of the viruses carried in the acme of SRSNS and that of the viruses in the control ($P>0.05$). The most frequently detected respiratory tract viruses in the acme of SRSNS are RNA virus, and that in the control is the same ($P>0.05$). In the acme of SRSNS, most children have no symptoms of respiratory infection although respiratory tract viruses were detected. In the SRSNS, the percentage for the carrying of respiratory tract viruses increases obviously in the acme; it has an important relationship with the quantitative change of urinary protein. The category of the respiratory tract viruses detected in the SRSNS has a relationship with the season, this may be the reason why the cases of SRSNS so easily happen and relapse in this season.
Conclusion: RNA virus perhaps plays an important role in the triggering of this disease, including the infection of respiratory tract viruses and the carrying of respiratory tract viruses without the symptom of respiratory infection.

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P020

EFFECT OF THE ASTRAGALUS INJECTION ON URINE PROTEIN IN CHILDREN EPHROTIC SYNDROME

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Objectives of Study: To explore the effect of Astragalus injection on urine protein and plasma protein in children nephrotic syndrome (NS).
Methods: 30 cases hospitalization children with nephrotic syndrome from 1996 to 2002, divided into two groups random (1) Cortisone plus Astragalus groups were given in 17 cases. (2) Cortisone group was given in 13 cases.
Results: Clinical symptom and blood biochemical marker detected in treatment group were obviously better than in control group in NS patients. The urine protein was decreased in 24h while plasm albumin was increased. Significant differences were detected between two groups ($p<0.05$).
Conclusion: The Astragalus injection is obviously effective in NS. It is worth to applicate in children NS patients.

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P021

HEMATURIA AND ITS ASSOCIATED WITH RESPONS TO CORTICOSTEROID THERAPY IN CHILDREN WITH IDIOPATHIC NEPHROTIC SYNDROME

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Objectives of Study: The purpose of the present study was to identify the number of hematuria and correlates with respond to cortiocsteroid therapy of children with idiopathic nephrotic syndrome.
Methods: The medical record of patients diagnosed with idiopathic nephrotic syndrome at Pediatric Department Hasan Sadikin General Hospital Bandung during year 1996 – 2000 were analyzed using Chi square
Results: Of 137 idiopathic nephrotic syndrome patients, 93 were males (67,0 %) and 44 were females (32,1 %). The mean age at presentation was 5 years 9 months. Hematuria was noted in 46 patients (33,6 %) and 91 patients (66,4 %) did not have hematuria. Of the hematuria group, 22 patients (16,1 %) were responsive to prednisone therapy and 24 patients (17,5 %) did not respons. Of the non-hematuria group, 61 (44,5 %) patients responded to prednisone and 30 (21,9 %) did not respond to prednisone. There was significant relationship between hematuria variable and respond to prednison therapy (p=.03).
Conclusion: The number of hematuria of the present study was similar with the previous study and the number of hematuria is less in prednisone responsive group.

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P022

FIVE YEARS MULTICENTER STUDY OF CYCLOSPORINE A EFFICACY IN CHILDREN WITH PRIMARY NEPHROTIC SYNDROME

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The aim of this research is to determine of the efficacy of cyclosporine A (Sandimmun-Neoral [S-N], Novartis Pharma, Switzerland) in children with primary nephrotic syndrome (NS) by the 5 years multicenter study.
Patients and methods: 115 pts with steroid dependent or frequently relapsing steroid sensitive (SSNS, n=63) and steroid resistant (SRNS, n=52) aged 3-16 years in different nephrology departments were treated with S-N according to the uniform protocol. Duration of NS before entry of the study varied between 1-10 years. More than half of pts had signs of steroidtoxicity. All pts with SRNS had no effect on cytotoxic therapy and pulses of steroids before S-N treatment was started. Nephrobiopsy was done in 53 pts, as a rule in SRNS. Doses of S-N were 3-5mg/kg/day (Co blood levels of cyclosporine – 80-150 ng/ml). Simultaneously pts received prednisolon (Pr) in dose of 1mg/kg/24h or 1mg/kg/48h. Duration treatment with S-N+Pr was 6-12 monthes, then part of the pts had S-N monotherapy.
Results: Complete remission was achieved in 86,8% pts with SSNS and in 28,6% with SRNS, partial remission – in 13,2% of SSNS and 32,7% of SRNS. In pts on S-N therapy Pr was abolished and signs of steroidtoxicity disappeared. Duration of remission in pts varied from 2,5 months to 4,5 years. 21 pts (18,2%) were cyclisporindependent. 43 pts (37,4%) continue S-N treatment to 1.01.2004. S-N was abolished in 19 pts (16,5%) with SRNS, who had no effect from the treatment. 16 of them had now CRI. Different results were achieved in pts with various biopsy data (table)

Morphology	MCNS (n=3)	FSGS (n=11)	MsPGN (n=22)	MbPGN (n=17)	All (n=53)
Remission n/q	3/1,0	6/0,55	13/0,59	13/0,76	35/0,66
No effect n/q	0	5/0,45	9/0,41	4/0,24	18/0,34

Conclusion: Five years multicenter study has shown high efficacy of S-N in pts with SSNS and SRNS, including children with FSGS, MsPGN, MbPGN. Some pts with SRNS who had not positive results after alkylating agents and pulses of stroids achieved complete or partial remission only with S-N. We are planning to use this treatment in the future.

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P023

CAPTOPRIL THERAPY IN CHILDREN WITH STEROID DEPENDENT NEPHROTIC SYNDROME AND THEIR LONG TERM FOLLOW UP

UK Jayantha

Objectives of Study: To find out the effectiveness of captopril in the management of steroid dependent nephrotic syndrome.
Methods: Patients with steroid dependent nephrotic syndrome were started on captopril 0.5 - 1mg /kg/day after induction of remission with steroids. A minimum dose of prednisolone was used in addition to captopril. Average relapse rate, dose of steroid dependency before and after captopril therapy was documented.
Results: There were 36 children (19 - boys, 17 - girls). Age of onset of nephrotic syndrome was range from 2.5 to 9 yrs. Duration of follow up was 1.5 - 5 yrs. Average relapse rate dropped from 3.619 per year (SD 1.4) to 1.336 (SD 1.0) per year with captopril therapy. This drop was statistically significant (paired t-test t = 8.35, df = 35, p = 0.001).
Mean prednisolone dose required to maintain in remission before introduction of captopril was 1.4889mg/kg/EOD (SD 0.62). Mean prednisolone used while on captopril was 0.4694mg/kg/EOD (SD 0.30). This drop of steroid requirement was statistically significant (paired t-test t = 9.065, df = 35, p = 0.0001). There were 4 children who developed no relapses during follow up period. There were 3 children in whom remission was maintained by captopril alone. There was no significant alteration of renal function during therapy. 3 children were responded poorly to captopril therapy.
Conclusion: This study showed statistically a significant reduction in relapse rate and the dose of steroid required in patients with steroid dependent nephrotic syndrome with captopril. Therefore captopril therapy could be use as a first line treatment in steroid dependent nephrotic syndrome, before considering more toxic drugs such as cyclophosphamide, or cyclosporins.

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P024

DOES DAILY PREDNISOLONE FOR ONE MONTH IS BENEFICIAL IN THE FIRST ATTACK OF NEPHROTIC SYNDROME?

UK Jayantha

Objectives of Study: To find out whether daily prednisolone for one month is beneficial in the management of first episode of nephrotic syndrome.
Methods: Children with first episode of steroid sensitive nephrotic syndrome presented from January 2001 to December 2002 were given a short course of daily steroid followed by every other day therapy for 6 months (gp1). This regime consisted of prednisolone 60mg/m^2/daily until 3 days of protein free urine followed by 60mg/m^2/EOD for 28 days. This dose of steroid was reduced by 10mg/m^2/monthly until reach 10mg/m^2/EOD. Duration of initial remission, percentage of relapse, relapse rate/pt/yr, cumulative dose of steroid given were documented. Patients who have completed 6 months of follow up were analyzed. Data were compared with the data of our previous study involving daily prednisolone for one month with similar reducing dose of every other day prednisolone (gp2). This regime consisted of prednisolone 60mg/m^2/daily for 28 days followed by monthly reducing course of prednisolone every other day starting from 60mg/m^2/EOD. Then the dose was reduced by 10mg/m^2/monthly until reach 10mg/m^2/EOD.
Results: Total numbers of children were 81 (gp1 – 33, gp2 – 48). Age of onset in gp1 and gp2 was 57.9 months (SD 34.0) and 50.4 months (SD 30.0) respectively (t = 0.8437, P = 0.401323). Mean duration of remission in gp1 and gp2 was 4.35 months (SD 4.36)and 12.33 months (SD 9.31) respectively (t = 3.54, P =0.000923) 44% children at the end of 6 months and 70% at the end of 12 months in gp1 experienced one or more relapses. Only 10% at 6 months and 33% at 12 months in gp2 showed relapses. At the end of 24 months of follow up 92% in gp1 and only 46% in gp2 had relapses. At 2 yrs of follow up cumulative dose of steroid received in gp1 and gp2 were 9210.8 mg and 7111.6 mg respectively.
Conclusion: Patients who received one month of daily prednisolone had statistically prolong mean duration of remission and low percentage of relapses. Hence daily prednisolone for one months is beneficial with a prolong cause of prednisolone taper.

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P026

PROLONG VERSUS STANDARD STEROID THERAPY FOR CHILDREN WITH A RELAPSING COURSE OF NEPHROTIC SYNDROME

UK Jayantha

Objective of Study: To assess the benefits and harms of two different corticosteroid regime in children with steroid sensitive nephrotic syndrome, who experience a relapsing course.
Methods: Patients with steroid sensitive nephrotic syndrome who experience relapses were included. Patients who have been treated with cyclophosphamide were excluded. Standard prednisolone regime (gp1) consisted of prednisolone 60mg/m^2/day until urine free of proteins for 3 days followed by 40mg/m^2/EOD for 28 days. Prolong steroid regime (gp2) consisted of prednisolone 60mg/m^2/day until urine free of proteins for 3 days followed by prednisolone 60mg/m^2/EOD for 28 days. Then prednisolone dose was reduced by 10mg/m^2/each month until reach 10mg/m^2/EOD for 28 days. Patients were randomly allocated to both groups. Ethical clearance was obtained from the ethics committee of the Faculty of Medicine, Galle, Sri Lanka.
Results: Study period September 1994 to September 2002. Total numbers of children studied were 95 (50 boys, 45 girls). Age of onset was range from 12 months to 133 months. Mean age of onset 45.4 months (SD 28.41). 48 patients received standard regime (gp1) and 47 received prolong regime (gp2). There were no statically differences in mean age of onset in both groups. No of frequent relapses and steroid dependent patients were not significantly different in both groups. Mean duration of remission in standard and prolong regime was 6.09 and 15.04 months respectively ($P = 0.0001093$, $t = 4.116582$). No of patients develop relapses after 3 yrs of follow up in gp1 and gp2 was 100% and 64% respectively. Mean relapses rate/pt/yr after 5 yrs of follow up in gp1 and 2 was 1.70 and 0.38 respectively ($P = 0.0000$). The rates of development of steroid induced side effects were not significantly different in both groups.
Conclusion: This long term comparison between standard and prolong steroid regime in the management of relapsing nephrotic syndrome clearly shows that prolong steroid regime is more beneficial when consider duration of remission, relapse rate and relapse rate/pt/yr.

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P027

VINCRISTINE IN STEROID-DEPENDENT NEPHROTIC SYNDROME

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OBJECTIVES OF STUDY
The treatment of steroid-responsive nephrotic syndrome continues to pose a therapeutic challenge in frequently relapsing patients. To avoid serious drug side effects, alternative treatments are needed for children with steroid-dependent nephrotic syndrome (SDNS). We retrospectively evaluated the efficacy of vincristine in patients with SDNS who continued relapsing after cyclophosphamide.
METHODS
Fourteen patients with SDNS were treated with vincristine between September 1999 and August 2003. Eight of these patients were in relapse with heavy proteinuria when vincristine was commenced. All had evidence of steroid toxicity, including growth retardation, obesity, osteoporosis, glycosuria, cataracts or behaviour problems. Prior therapy included oral cyclophosphamide in all patients, cyclosporin in 11 patients and levamisole in 4. Of the 7 patients biopsied, 6 had minimal change glomerulopathy and one had focal glomerulosclerosis. Vincristine was administered by intravenous boluses in a dose of 1.0-1.5mg/m^2 once per week for 4 weeks then once per month for 4 months.
RESULTS
Six of the 8 patients in relapse achieved complete remission within 8 weeks of starting vincristine, usually by the second or third dose. One showed no response to vincristine and one patient discontinued vincristine after six doses because of frequent relapses during the vincristine course. Of the 12 patients in remission at the end of vincristine treatment, 4 remained in remission for the median time of 12 months (range 8~37 months). Seven of the 10 patients who continued to relapse after one course of vincristine were treated with vincristine for relapses of proteinuria. Their proteinuria cleared after an additional 1 or 2 infusions of vincristine (1mg/m^2). Side effects were uncommon, but one patient suffered an extravasation burn with no long-term injury. Abdominal pain was a significant problem at high initial doses of 1.5mg/m^2, but was alleviated at the reduced dose of 1.0mg/m^2.
CONCLUSION
Vincristine is able to induce complete remission of relapse in SDNS patients, usually after 2 infusions of the drug at weekly intervals. Sustained remission may also occur after vincristine, making it a useful alternative treatment to both cyclosporin and cyclophosphamide. Several patients indicated they would prefer a few vincristine infusions to treat their relapse rather than a course of oral prednisolone or cyclosporin.

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P028

HEALTH-RELATED QUALITY OF LIFE (QOL) IN CHILDREN WITH STEROID SENSITIVE NEPHROTIC SYNDROME (SSNS)

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Data on QOL are scarce in SSNS although morbidity is significant as up to 50% of patients have a relapsing course, often requiring intensive immunosuppression.
Methods: We evaluated QOL in a group of 45 children with SSNS (mean age 9.8±1.8 years and median follow-up 5.9 (0.1–16.3) years) and also analysed the correlation to clinical variables. QOL was measured using the standardized questionnaire TNO-AZL Child Quality of life (TACQOL).
Results: Patients rated their QOL as normal in 6 of 7 subscales, except for social functioning ($p<0.05$). Parents, however reported impairment in 4 of 7 subscales, i.e. motor ($p<0.05$), cognition ($p<0.01$), social functioning and positive emotions ($p<0.0001$) as compared to healthy controls. QOL was influenced by a relapsing course with steroid dependency ($p<0.05$) and need for cytotoxic treatment ($p<0.05$).
Conclusion: QOL is impaired in SSNS and patients and parents differ in their interpretation of QOL. A significant impact of a complicated course can be documented. Intensified psychological support seems necessary especially for patients with frequently relapsing SSNS.

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P029

REPORT OF THE BRAZILIAN MULTICENTRIC NEPHROPATHIC CYSTINOSIS STUDY

MH Vaisbich, VH Koch

Objectives of study: to present an update on demographics of study participants with special interest on renal function status and response to therapy with cysteamine.
Methods: The Brazilian Multicentric Cystinosis Study Group was founded in 1998; it is currently composed of 7 Pediatric Nephrology Units throughout Brazil, which are coordinated by the Pediatric Nephrology Unit of Instituto da Criança – HCFMUSP, São Paulo. Patient recruitment to the study is based on informed consent and has been done with the help of the Brazilian Society of Nephrology, which congregates approximately 500 dialysis centers, by the creation of an electronic homepage to facilitate the contact of medical professionals and families with Instituto da Criança, by participation in medical meetings and publications in medical periodicals. Our study protocol involves the completion of an initial and follow up questionnaire, the measurement of leucocyte intracellular cystine content, initiation and follow up therapy with Cysteamine and clinical patient follow up based on a structured protocol of subsidiary exams.
Results: We have recognized 83 patients (35 F) with nephropatic cystinosis in Brazil, 53/83 children are being followed at Instituto da Criança, 15/83 pts are being followed in other participating centers and 16/83 pts are known to us only by questionnaire. 32/83 (38.5 %) have normal renal function, 16/83 (19.2 %) are in chronic renal failure conservative treatment, 13/83 (15,6%) are on dialysis (7PD, 6HD), 21/83 (25,3 %) received a kidney transplant and 1/83pt died on PD. Cysteamine therapy is currently in use by 52/83 pts, of those, 18 children started therapy before 2 yrs of age. Growth parameters were markedly improved by cysteamine, especially in the youngest pts. It is of note that only 2/18 pts started on cysteamine before 2 yrs of age are currently in need of thyroid hormone replacement.
Conclusion: Our study demonstrates the importance of a multicentric study coordinated by a specialized center, for the recruitment, diagnosis and management of rare diseases. This kind of approach reduces costs and facilitates funding by concentrating special needs in specialized centers and its results have a profound positive impact on the quality of life of affected children and their families.

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P030

EFFICACY AND SAFETY OF CYCLOSPORINE A IN STEROID DEPENDENT AND STEROID RESISTANT PEDIATRIC IDIOPATHIC NEPHROTIC SYNDROME (INS)

V Lanzarini, L Henriques, MH Vaisbich, VH Koch

Objectives of Study: to describe results of efficacy and safety of cyclosporine a (cya) in steroid resistant (sr) or steroid dependent (sd) pediatric ins patients.
Methods: A series of 18 pts (5F) is presented, mean age 4.6 yrs (1.4-11.3), mean time interval from diagnosis to CyA initiation: 47.5 mos. Initially 11/18 pts were SR (renal biopsy: FSGS 6 pts; MCD 4pts; mesangial proliferation 1 pt) and 7/18 were SD (renal biopsy FSGS 2 pts, MCD 5pts). Previously to CyA initiation, 17/18 pts were treated with cyclophosphamide(CF), which changed the response of 2 pts from SR to SD. At CyA initiation 9 /18 pts were SD and 9/18 pts were SR. CyA, at the dosage of 3-5 mg/Kg/day, was prescribed in association with prednisone (PDN). Urinary Retinol Binding Protein (RBP) (immunenzymatic assay) was evaluated on CyA initiation in 16/18 pts. A CBC, SMAC panel and urinary evaluation were repeated bimonthly. Renal Biopsy was repeated yearly. CMV, EBV and viral hepatitis serology were negative at initiation of CyA and were repeated during CyA therapy.
Results: A positive response to CyA was observed in 16/18 pts: 6/16 pts, 1 SR 5 SD, were able to discontinue PDN, of whom: 4 pts. relapsed after CyA discontinuation, 1pt is on CyA, 1 pt is in remission out of therapy; 9/16 pts despite a positive response had to be taken off CyA due to side effects, and 1/16 pt became highly steroid and CyA dependent. A negative response was seen in 2/18 pts. Elevated levels of RBP were obtained in 3/16 pts, 2 of them non responders. The main observed side effects were: CyA nephrotoxicity(4 pts), acute renal failure (2pts), renal tubular acidosis (1 pt), severe infectious episodes (2 pts), elevated LFTs due to VHC hepatitis (2pts.)
Conclusion: Our study demonstrates that CyA is a short term therapeutic option for pediatric INS. Its usage needs to be undertaken with a structured protocol for diagnosis of the multiple possible side effects. RBP might be a good prognosticator of negative response to immunosuppression in INS patients.

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P031

POLYMORPHISMS OF IL-4 GENE WITH CHILDHOOD STERIOD SENSITIVE NEPHROTIC SYNDROME

H Liu, H Xu, Y Yang, Q Shen, Huang

Objectives of Study: Recent arguments suggest that T cell dysfunction may be involved in the pathogenesis of this disease. The aim of the study was to investigate the association between the polymorphisms in variable number of tandem repeat region of IL-4 gene and childhood Steriod Sensitive Nephrotic Syndrome (SSNS).
Methods: We identified the polymorphism in the IL-4 gene using the polymerase chain reaction and direct sequencing methods in 55 Chinese children with SSNS who were followed-up for at least 1 year and 115 healthy Chinese as control. A variable number of tandem repeat (VNTR) region polymorphisms of IL-4 gene were studied, and alleles were designated IL-4 B1 and B2, corresponding to 2 and 3 repeats, respectively. IgE also identified in 48 patients before steroid treatment.
Results: 1. There was no difference in the genotype and allele frequencies between patients with SSNS and normal controls (P=0.072). 2. The frenquencies of B1B1 were significantly higher in the group who had frequent relapses (28 patients had more than 3 times relapses during the first year after onse) than in 27 patients who had low or no relapse (P = 0.004). 3. 23 patients who relapsed frenquently had higher serum IgE levels than 25 patients who had low relapse (P=0.003), and the patients with B1B1 (38 patients) also had higher serum IgE levels than patients with B1B2/B2B2 (10 patients) (P=0.026). 4. The frenquencies of B1B1 were significantly higher in Chinese (in Shanghai) than in Caucasian and Janpanese (P<0.001).
Conclusions: Our results suggest first that IL-4 VNTR B1/B1 genotype could predict the frequent relapse in childhood SSNS in Chinese.

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P032

A CASE OF INFANTILE NEPHROTIC SYNDROME THAT WAS PERFORMED SUCCESSFUL LIVING RELATED RENAL TRANSPLANTATION

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Infantile nephrotic syndrome is rare disorder and its prognosis is poor of rapid progression to end-stage renal disease (ESRD) in a short term. We report a case of infantile nephrotic syndrome diagnosed in six month of age and was performed successful living related renal transplantation at 9 kg in weight. She had a checkup at a hospital nearby in chief complaint for pyrexia. She was diagnosed as nephrotic syndrome and was initiated treatment with prednisolon (PSL). She was, however, transferred to our hospital on the fifth disease day because of massive proteinuria more than 20 g/day and severe hypoalbuminemia. Initial treatment consisted of infusion of albumin, PSL, cyclosporine, and ACE inhibitor, which were ineffective. An open renal biopsy was performed on the eighth disease day and the finding was diffuse mesangial sclerosis (DMS). The missense mutation in the WT1 gene was noticed. Thereafter, those medications were tapered. Because generalized edema and peripheral circulatory failure developed, we started continuous hemodiafiltration (CHDF) at the twelfth disease day. While she became anuric in several days, her serum albumin concentration became stable. Peritoneal dialysis was initiated one month later. Dialysate of higher concentration of glucose was needed in order to gain enough drain volume. Although we tried dialysis with icodextrin, it was not so effective. Because reabsorption of gulucose through peritoneum and abdominal distension, the quantity of her oral intake of breast milk did not increase. So she needed nasogastric tube feeding for the purpose of calorie supply. Her height SDS has been consequently almost –2SD. Living related renal transplantation from her father was performed when she was 1 year and 10 months of age, weighing 9 kg. Her native kidneys were removed at the same time. She was discharged uneventfully, and renal function is good. Her development has being improved.

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P033

DETERMINATION OF OSTEOPOROSIS RISK IN CHILDREN WITH IDIOPATHIC NEPHROTIC SYNDROME TREATED WITH CORTICOSTEROID

M Mazaheri

Objectives of Study: In this study we evaluated bone mineral density and risk of osteoporosisby determination of Z score in 39 children (30 male & 9 female) aged (5-17) years with nephrotic syndrome who received corticosteroid.
Methods: Measurement of BMD was performed by dual energy X-Ray absorptiometry (DEXA). Results were expressed as grams hydroxyl apatite divided by the projected area in squared centimeter (g/cm2) and as Z score for determination of risk of osteoporosis.
Results: Lumbar spine Z score in 64.8% of patients and femoral neck, Z score in 18.1% and distal end of radius Z score in 100% of patient was lower than –2, meaning osteoporosis risk is 4 times of normal children. There was no relation between cumulative steroid dose and densitometric determinants. There was positive relation between age at the beginning of disease and distal radius BMD (P<0.04). There was negative relation between duration of steroid treatment & distal end of radius Zscore (0.004).
Conclusion: All of patients had abnormal bone density at least in one region (distal end of radius). We can use radius densitometry as the first diagnostic test of osteoporosis in these
Children. we advise to start prophylactic regimen for osteoporosis in nephrotic children who are treated with corticosteroids.

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P034

CICLOSPORIN IN CHILDHOOD IDIOPATHIC NEPHROTIC SYNDROME – TWO YEARS FOLLOW-UP

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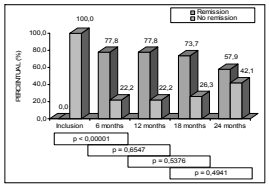
Objectives of Study: sustained proteinuria remission is the main objective in the treatment of the idiopathic nephrotic syndrome, together with the sparing of corticosteroids (CE) doses. This study is conducted to avaliate the effectivity and safety of cyclosporine A (CsA)microemulsion in the treatment of steroid-dependent (CD) and steroid-resistant (CR) children with idiopathic nephrotic syndrome.
Methods: uncontrolled cohort, with clinical and laboratorial variables measured every six months. The statistical analysis was carried out using the Mann-Whitney U-test, the chi-square test, the Fisher´s analysis, the Pearson correlation coefficient and comparison of proportions. Statistical significance was defined as p-value < 0,01. Remission of proteinuria is considered < 0,20g/24 hrs.
Results: 29 (93,6%) of the 31 children (7,3±3,5 years) included in the study completed the 6th month of follow-up, 7 (22,6%) have discontinued the study (4 were withdrawn from CsA and 3 were transfered to another center) and 19 (61,3%) completed 24 months until 15 february 2004; 14 (73,7%)males (p=0,009), 7 (36,8%) with focal glomerulosclerosis (FGS), 9 (47,4%) with minimal change disease (MCD) and 3 (15,8%) with diffuse mesangial proliferation (DMP), 8 steroid-dependent (4 with remission) e 11 steroid-resistants (7 remissions). The cyclosporinemia varied from 131,13±42,12 ng/ml in the 6th month to 109,86±109,86 in the 12th, the seric creatinin from 0,53±0,09 to 0,63±0,16 md/dl, cholesterol from 377,8±161,2 (median 343,0) to 225,7±157,3 (median 183,0) mg/dl (p<0,0001), the serum albumin from 2,5±1,1 to 3,6±0,9 g/l (p<0,0001). The median of each six months cumulative steroid dose was 175,29±86,54 mg/kg/6m pre-inclusion and 21,45±40,67 mg/kg (median 0,77) in 24th month of follow-up (p<0,0001).
Conclusion: CsA was effective and safe in the treatment of steroid-dependent and steroid-resistant childhood idiopathic nephrotic syndrome. The incidence of remission was 63% with 6 months of treatment and 75,9% for those who completed 12 months. The renal function was preserved and the morbidity was reducted. In this serie the outcome was independent from the histological diagnosis. We considered the FGS group specially benefited since it was mostly described as the poorest prognostic group.



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P036

NEPHROTIC SYNDROME AND PROTEINURIA AS CLINICAL PRESENTATION OF NEUROBLASTOMA: DESCRIPTION OF 2 CASES

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Objectives of Study: Neuroblastoma is a childhood neoplasm arising from neural crest cells. The clinical manifestations are dependent on the widespread distribution of neural crest tissue and the location of the simpatetic chain involvement. The most frequent sign of onset is a palpable abdominal mass. Proteinuria has been rarely described as presenting sign of tumor and only in one case has been associated with neuroblastoma. We describe two cases of neuroblastoma presenting with proteinuria, one of them with nephrotic syndrome.
Methods and Results: A 12-year-old boy (BW 44 Kg) have been complaining of fatigue and swelling of the peri-orbital and pretibial regions and some days. Clinical examination, abdominal ultrasound and a chest X-ray showed general oedema, ascites and right pleural effusion. Urine analysis showed proteinuria of 9.2 g/L (12.7 g/day) and microscopic hematuria. Total serum protein was 43 g/L (albumin 29 g/L). Cholesterol (368 mg/dl) and triglycerides (152mg/dl) were elevated. Complement and renal function were normal. As a standard steroid course showed no response, a kidney biopsy was performed, demonstrating minimal change nephritis, with Ig G and C4 granular deposits. Steroids and cyclophosphamide were able to reduce proteinuria to 1.5–4 g/day, but 3 months later there was a nephrotic relapse (21.24 g/L). Clinical examination showed a right supraclavicular lymph node enlargement (3x4 cm) and right pleural effusion. A CT scan of neck and chest and a spinal MNR demonstrated a right paravertebral mass and a lymph node biopsy brought to the diagnosis of disseminated neuroblastoma (stage IV). 30 days after initiation of chemotherapy, proteinuria was within normal limits associated to a good partial remission of the tumor. During treatment (chemo- and radiotherapy) the nephrotic syndrome did not recur, even after the neuroblastoma relapse, 24 months after diagnosis.
The second patient, a 5-year-old girl (BW 15.8 Kg), showed proteinuria (1 g/L) at a urine analysis performed because of swelling of the right peri-orbital region. One and half month later proteinuria (1 g/L) was still present associated to mild bilateral exophtalmus and two masses (3x4 and 1x0.5 cm) in parieto-occipital region, liver (+4 cm) and spleen (+6 cm) enlargement. Total serum protein was 57.9 g/L (albumin 33 g/L), triglycerides were 403mg/dl. A total body CT scan diagnosed a disseminated neuroblastoma and documented the compression of the left renal vein showing the presence of different masses (paravertebral, pelvis, right orbital region), bone marrow infiltration and multiple bone metastasis. 30 days after initiation of treatment proteinuria was within normal limits, and did not recur, even after neuroblastoma relapse, 15 months after diagnosis.
In the patient presenting with the nephrotic syndrome there are some atypical signs: the age, the microscopic hematuria, the relatively high serum albumin concentration, with a contemporary high proteinuria and mainly the unilateral pleural effusion.
Conclusions: Atypical signs of nephrotic syndrome have to remind the possibility of an unusual cause of this disease as a neoplastic disease. In both cases the presence of proteinuria was associated with a bulky disease and disappeared only after a significant reduction of the tumor mass; we believe that this prove the relation between the 2 diseases

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P037

SINGLE-DOSE DAILY ADMINISTRATION OF CYCLOSPORIN A FOR IDIOPATHIC NEPHROTIC SYNDROME

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Objectives of Study: It has been reported that the pharmacokinetic activity of cyclosporin A (CsA) in children is different from adults. Usually, pediatric patients appear to metabolize CsA more rapidly than adult patients, requiring higher dose of CsA (Neoral) in transplant patients. In this context, we speculate if single-dose daily administration of CsA (Neoral) would yield higher peak blood level without trough level elevation, and thereby allowed better results than that of the conventional twice daily administration procedure in the treatment of childhood idiopathic nephrotic syndrome (INS).
Methods: A total of eight children with biopsy-proven (INS) [6 with minimal-change nephrotic syndrome (MCNS) and 2 with focal segmental glomerulosclerosis (FSGS)], aged 6 to 16 years were prospectively evaluated. All the study participants initially given prednisolone (PSL) combined with CsA twice-daily administration protocol (T). However, all exhibited poor steroid-sparing effect of CsA. After obtaining informed consent, CsA administration was replaced by single-dose-daily administration protocol (S). The initial daily dose of CsA (Neoral) of the S protocol was adjusted in each individual according to the previously administered dose by the T protocol, i.e., approximately two thirds of the daily dose administered by the T protocol.
Results: Although the mean daily CsA dosage in the subjects receiving the drug by the S protocol tended to be lower than that by the T protocol (S, 2.4±1.0 mg/kg per day v.s., T, 3.4±1.2 mg/kg per day), the mean peak blood level (1-2hr after drug administration) tended to be higher by the S protocol than that by the T protocol (S, 741±117 ng/ml v.s., T, 350±276 ng/ml) without the mean trough blood level elevation. As a result, minimum PSL doses required for maintain of clinical remission in MCNS patients were significantly lower by the S protocol than that by the T protocol (S, 0.4±0.1 mg/kg per alternate-day v.s., T, 0.6±0.3 mg/kg per alternate-day, p<0.05), although two patients experienced another relapse while on the S protocol. Despite of PSL tapering, proteinuria in the FSGS patients decreased following the S protocol. Four patients who received repeat renal biopsy following the S protocol (7-21 months) revealed no evidence of CsA nephrotoxicity.
Conclusion: These clinical observations, although small and preliminary, suggest that single-dose daily administration of CsA might be an attractive and cost-beneficial protocol in at least a proportion of children with INS.

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P038

CLINICAL FEATURES OF NEPHROTIC SYNDROME IN CHILDREN AT SOETOMO HOSPITAL SURABAYA

MS Noer

Objectives of Study: Because of incidence of nephrotic syndrome is between 2 -7 cases per 100,000 children per year, this disorder is not uncommon in pediatric practice. This study is conducted to investigate the clinical features of first episodes of nephrotic syndrome in children.
Methods: A study was carried out to children with nephrotic syndrome hospitalized at Soetomo Hospital Surabaya.
Results: Of 93 children with nephrotic syndrome aged 2-15 years old (mean 6.75 years) there were 52.7% children above 6 years old and 47.3% children under 6 years old. Boys were more common than girls with ratio 1.8:1. There were 90.3% children suffered from edema. Proteinuria was detected 1.0–12 g/L where 93.6% were massive proteinuria. Serum albumin level was 0.90–3.0 g/dl where 82.8% was hypoalbuminemia < 2.5 g/dl. Serum hypercholesterolemia was detected in 91.4% children in a range of 238.0–1,007.0 mg/dl. Urinalysis showed hematuria 46.2%, leucocyturia 49.5% and oliguria 15.1%. Pathological bacteria in urine culture grew in 38.7%. Peripheral blood examination showed 14.0% anemia and 36.6% leucocytosis. Blood chemistry analysis revealed uremia in 8.6%, increased serum creatinine level in 7.5%. Serum electrolyte showed hyponatremia 6.5%, hypernatremia 1.1%, hypokalemia 20.4%, hyperkalemia 2.2%, hypocalcemia 63.4%. Hypertension was detected in 18.3% of children.
Conclusion: Clinical and laboratory features of children with nephrotic syndrome hospitalized at Soetomo Hospital Surabaya showed no significant difference with other cities in Indonesia and other countries in the world.
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P039

PREDICTORS OF RELAPSE PATTERN IN RESPONSIVE-STEROID NEPHROTIC SYNDROME

MS Noer, S. Kusumawardhani

Objectives of Study: More than half of children with nephrotic syndrome will experience relapses. Relapse can be devided into infrequent relapses (< 2 relapses in 6 months) or frequent relapses (≥ 2 relapses in 6 months). The most difficult problem in caring children with nephrotic syndrome is the occurrence of frequent relapses in patients who respond initially to steroid treatment. This study is conducted to identify predictors of relapse pattern and determine the predictive score for relapse in steroid-responsive nephrotic syndrome.
Methods: Ninety-nine children with nephrotic syndrome visiting pediatric nephrology outpatient clinic Dr. Soetomo Hospital from 1983 to 2001 were studied. There were 63 children with relapses (50 infrequent relapses, 13 frequent relapses) and 36 children with no relapse for at least 1 year after beginning steroid treatment were served as control group. The selected variables were grouped into non renal factors (age, sex, nutritional status, infection) and renal factors (histopathologic findings, hypertension, hematuria, azotemia, hypocomplementemia, rapidity of early steroid response, number of relapses within first 6 months, time-interval between early steroid response and first relapse).
Results: Using discriminant analysis function test, it was found that the statistically significant predictors of relapse pattern were the time-interval between early steroid response and the first relapse, number of relapses within first 6 months, infection within the first relapse, hematuria and sex. Predicting score can be determined using 3, 5 or 6 parameters by including the rapidity of early steroid response.
Conclusion: Relapse pattern in steroid-responsive nephrotic syndrome can be predicted using predictive scores. Using 3 parameters will give better results in predicting relapse pattern.

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P040

THE OUTCOMES OF ORAL CYCLOPHOSPHAMIDE TREATMENT IN CHILDREN WITH IDIOPATHIC NEPHROTIC SYNDROME

MS Noer, A Ninik

Cyclophosphamide has been widely used in children with idiopathic nephrotic syndrome. Recent studies showed that patient with frequent relapses nephrotic syndrome (FRNS) respond well to cyclophosphamide with 50-90% long-term remission, while steroid dependent nephrotic syndrome (SDNS) are more resistant. Cyclophosphamide had also used in steroid resistant nephrotic syndrome (SRNS) with variety of results. There is a suggestion that SRNS with minimal changes disease (MCD) are more likely to respond to alkylating agent such as cyclophosphamide than those with focal segmental nephrotic syndrome (FSGS).
Objectives of Study: The aim of this study was to evaluate the outcomes of oral cyclophosphamide treatment in frequent relapses, steroid dependent and steroid resistant nephrotic syndrome.
Method: Retrospective, observation longitudinal study has been conducted to evaluate 26 children, with 9 children FRNS, 8 children SDNS and 9 children SRNS. Each patient was treated with oral prednisone according to The International Study Kidney Diseases Of Children (ISKDC) protocol. Oral cyclophosphamide was commenced four weeks after the initial dose of prednisone for SRNS, while for FRNS and SDNS, cyclophosphamide was given three weeks after induction prednisone therapy.
Results: The results of treatment using oral cyclophosphamide therapy showed 5 (72%) of 7 patients SDNS free from dependent steroid, 1 (14%) continue to have SDNS, and 1 (14%) did not continue the treatment. From 9 FRNS, 7 (78%) had infrequent relapses while 2 (22%) continue to have frequent relapses. Six (67%) of 9 SRNS became steroid sensitive nephrotic syndrome, while 3 lost of the follow up. The most common complications of 26 children who had oral cyclophosphamide therapy were nausea 4 (25%) and vomiting 4 (25%).
Conclusion: The oral cyclophosphamide therapy seems to have a good respond either for SDNS, FRNS and SRNS

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P041

PERITONEAL FLUID HANDLING IS SIMILAR USING PH-NEUTRAL OR ACIDIC DIALYSIS SOLUTIONS IN CHILDREN

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Objectives of Study: Differences in peritoneal fluid handling can be expected if pH neutral dialysis solutions are applied because conventional acidic solution exerts toxic effects on peritoneal mesothelial cells and microcirculation. Therefore, peritoneal fluid kinetics were investigated in a group of children with both types of solutions.
Methods: Twenty-four peritoneal equilibration tests using dextran 70 as a volume marker were performed in14 children (mean age 4.0 years; SD 3.1) on stable PD for 24 months (SD 19) with pH neutral dialysis solution (Physioneal® 3.86%). As a control group 24 children (mean age 7.5 years; SD 5.0) were studied, being tested in 32 peritoneal equilibration tests with an acidic solution (Dianeal® 3.86%).
Results: Mean (± SD) transcapillary ultrafiltration was 816 ± 367 ml/1.73m^2 in the study group and 936 ± 327 ml/1.73m^2 in the control group (NS). Marker clearance (as an indicator of lymphatic absorption) was 349 ± 253 ml/1.73m^2 in the study group and 451 ± 316 ml/1.73m^2 in the control group (NS). There were no differences in D/P ratio for urea and creatinine. D_0/D_0 ratio for glucose was slightly lower for the pH-neutral solution (p=0.04).
Conclusion: It is concluded that peritoneal fluid kinetics are not altered if pH neutral dialysis solutions are applied, compared to acidic solutions. An altered transcapillary ultrafiltration, as is hypothetically possible using an acidic solution, was not established.

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P042

THERAPEUTIC EFFECTS OF MYCOPHENOLATE MOFETIL (MMF) ON MULTIDRUG-RESISTANT INTRACTABLE NEPHROTIC SYNDROME

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Objectives of Study: The introduction of cyclosporin A (CyA) in the treatment of intractable nephrotic syndrome allowed a reduction in the incidence of relapse and the total dose of steroids. However, some forms of nephrotic syndrome relapse repeatedly despite treatment with CyA, or are refractory. MMF, a newly developed immunosuppressive agent, is a metabolic antagonist of purine, and is characterized by selectively inhibiting the proliferation of lymphocytes that depend on the de novo pathway, without causing serious side effects which is sometime observed in other immunosuppressive agents. In this study, we treated such intractable or resistant patients with MMF.
Methods: The subjects consisted of 6 patients with frequently relapsing nephrotic syndrome (FRNS) and 1 patient with steroid-resistant nephrotic syndrome (SRNS). The patients (5 males and 2 females) averaged 16 (4.0 years of age. Histologically, all FRNS patients had minimal-change nephrotic syndrome, and the SRNS patient had focal segmental glomerulosclerosis (FSGS). Until the administration of MMF, all patients had received steroids and CyA for 6.0 (2.2 years, and 4 of them had received cyclophosphamide (CPM) therapy. MMF was administered in two divided doses of 750-1,000 mg/m2, and prednisolone was reduced as far as possible.
Results: In the FRNS patients, the number of relapses during the 6 months before the start of MMF therapy was 1.6 (0.8, but decreased to 0.5 (0.8 after MMF administration, allowing the discontinuation of CyA in 5 patients, and a reduction in steroid dose and withdrawal from steroid. However, 1 patient was resistant to MMF, and was treated with CPM therapy. On the other hand, in the SRNS patient with FSGS, CyA therapy and LDL adsorption therapy failed to reduce proteinuria, but MMF therapy resulted in complete remission and steroid withdrawal. In addition, the adverse effects of MMF, such as leukopenia and severe infections, were not observed. Two patients had gastrointestinal symptoms, which were transient, or were improved by MMF dose reduction.
Conclusion: MMF therapy may be useful in patients with FRNS difficult to control even with CyA, and in those with CyA-resistant SRNS. No serious side effects have been observed during the short period of observation. Long-term observation for its therapeutic effects and safety and the establishment of effective administration methods for patients exhibiting poor results are needed.

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P043

NECROTISING FASCIITIS IN A CHILD: A RARE COMPLICATION OF IDIOPATHIC NEPHROTIC SYNDROME

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Objectives of Study: In nephrotic syndrome there is an increased tendency for bacterial infections due to immunologic changes secondary to proteinuria. Necrotizing fasciitis (NF) is an uncommon, life-threatening soft tissue infection characterized by rapidly spreading inflammation and necrosis of the skin, subcutaneous tissue, and fascia. It is even rare in children with idiopathic nephrotic syndrome despite the infection prone status.
Methods: We report a case of nf as a complication of nephrotic syndrome.
Results: Fourteen month old infant applied to our center with the complaints of diffuse body swelling and diarrhea. He was using peroral prednisolone for nephrotic syndrome for one month, was admitted to hospital with the diagnosis of peritonitis and urinary tract infection (UTI). Steroid dose was reduced and intravenous amikacin and cephazolin were started. In urine culture Proteus and Klebsiella; in dialysate culture Streptococcus pneumonia were isolated. On the 3rd day of admission sepsis and disseminated intravascular coagulation ensued. On both lower extremities pink-purple coloured echymotic skin lesions with demarcating borders developped. Imipenem, vankomycin, clindamycine were added to the treatment and intravenous immunoglobulin was given for three days. The lesions on both legs first became bullous and necrosis appeared and extended rapidly up to the muscles. With surgical debridement, supportive and appropriate antibiotic therapy the wounds healed in 113 days. After the initiation of steroid treatment the nephrotic syndrome was remitted. It was thought that in this patient NF developped due to increased tendency for infection caused by immune dysfunction secondary to nephrotic syndrome and steroid treatment.
Conclusion: NF, although rare, might be seen in children with nephrotic syndrome and prompt diagnosis and appropriate medical and surgical treatment is life saving despite very high mortality.

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P044

ALBUMIN AND MANITOL TREATMENT IN DIURETIC RESISTANT EDEMA IN NEPHROTIC SYNDROME IN CHILDREN ALI ASGHAR HOSPITAL

H Otukesh, R Hoseini

Objective of Study: Nephrotic syndrome and treatment of its complications (eg. Edema) is important. Edema is more common and severe in children with minimal change disease. Because mechanisms that lead to edema are different in children in relation to adults, the treatment of edema in these two groups is different. In children decreased effective intra-vascular volume leads to increase the vasoactive hormones such as rennin, aldostrone, norepinephrine. In adults the main cause of edema is primary reabsorption of sodium and water in distal tubules. Thus treatment of edema in children contains the protocols that can increase the intravascular volume and decrease sodium reabsorption in all parts of nephron especially proximal tubule. Albumin is an appropriate therapeutic agent in children, because it's volume expanding properties reduces sodium reabsorption in proximal tubule. However, albumin has some adverse effects such as the following:1- Albumin is an expensive drug. 2- Albumin combined to furosmide in renal tubulelumen and this reduces the effects of it. 3- This drug intensifies proteinuria and tubulointerestitial nephritis. Thus nowadays, drugs that have not these disadvantages (like manitol) are more useful. Thus, in this study we assess the effects of manitol and albumin in edema treatment of nephrotic syndrome in children.
Methods: We study 42 children with nephrotic syndrome. They had resistant edema and treated with manitol or
albumin alternatively. The percent of weight loss (first and latest weight) is important for us.
Results and Conclusion: Weight loss was remarkable in both albumin and manitol protocols (Pv < 0.01). This loss of weight had not meaningful difference between these two groups of patients Pv >0.05). This weight loss had not relationship with the amount of serum albumin in these two groups. But the relationship between weight loss and urine protein was positive in albumin protocol. (Pv <0.05). 30% of patients that treated with albumin and 15.8% of manitol protocol had not respond to this drug. Complications was evident in 15.8% of patients with manitol and 17% of other patients with albumin protocol. Complications in manitol protocol were hypokalemia and hypertension whereas hypertension and dehydration were seen in patients with albumin treatment.

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P045

MYCOPHENOLATE MOFETIL IN THE TREATMENT OF CHILDREN WITH LUPUS NEPHRITIS

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Objective of Study: Treatment of lupus nephritis includes the use of corticosteroids, often in combination with cytotoxic agents such as azathioprine (AZA) or cyclophosphamide (CyP). In children the impact of prolonged steroid treatment on growth and development and the increased risks of infection, infertility and the long term risk of malignancy limits the use of CyP or other
immunosuppressive agents in the management of pediatric SLE. In addition the children need therapeutic alternatives for inadeguate response or frequent relapses with traditional therapy. MMF has shown to be safe and efficacious in clinical trials in transplant patients and recent evidence in adult patients suggests that MMF can be an effective alternative to traditional therapy. We report our clinical experience with MMF in twelve children with lupus nephritis.
Methods and Results: Diagnosis of SLE, according to ARA criteria, was established in twelve patients, 8 F and 4 M, with a mean age at disease onset of 12.4 years (range 4.8 – 19.1). Renal biopsies showed the following histologic diagnosis according to WHO classification: IV class in 7 children, II class in 4 children and VI class in 1 child. Before the treatment with MMF: all patients have received corticosteroids, 2 children had received i.v. CyP once a month for six months, 1 child AZA and another one CsA; all but the two patients treated with CyP, who exhibited in the second serial renal biopsy a decrease of histological class from IV to II , showed proteinuria > 3 g/day, decreased C3 serum levels and a significant increase in anti-dsDNA antibody serum levels (SLEDA I). Treatment outcome was monitored through assessment of SLEDAI score, renal function, proteinuria and serologic markers such as complement and anti-dsDNA antibody serum levels. MMF was administered twice daily at mean dose of 29 mg/Kg/day (range 19-40 mg/Kg/day). The mean duration of therapy was 12.4 months (range 3-20 months). All children received prednisone (P) in association with MMF. After 7 months in 4/12 children P was stopped, 8/12 children were receiving P at a dose of 0.3 mg/Kg/day. A serial second renal biopsy was performed in 2 more children, who showed a decrease in their renal histological WHO class from class IV to III and II, respectively, after 12 months of MMF therapy. All patients had a sustained remission of desease: proteinuria was absent in all, 11/12 patients showed an increase of serum C3 and C4 levels; anti-ds DNA levels decreased in 6/12 patients. We observed in all patients a considerable steroid sparing effect, clinical signs of hypercorticysm, infact, dramatically improved in all children. We observed no haematological and gastrointestinal side effects; just one patient had an opportunistic Herpes Zoster infection and MMF was withdrawn for a month.
Conclusion: in our experience MMF represents a good alternative to traditional therapy in
the treatment of SLE in children. MMF appears to be effective in controlling disease activity in children. We can't establish long-term effects of MMF, but we can assess that MMF acts as an effective drug in controlling lupus nephritis activity and as steroid sparing agent without significant short and mean term side effects.

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P046

RENIN-ANGIOTENSIN SYSTEM (RAS) GENE POLIMORPHISMS IN CHILDREN WITH STEROID-RESISTANT NEPHROTIC SYNDROME

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Objectives of Study: Polymorphisms of the genes associated with the RAS including the angiotensinogen (AGT) gene M235, the angiotensin converting enzyme (ACE) I/D and the angiotensin II type1 receptor (AT_1R) gene A1166C polymorphisms have been implicated in the progression of renal disease. We investigated the impact of genotypes polymorphisms of the RAS on outcome in 14 children with steroid-resistant nephrotic syndrome (SRNS) (8M/6F; median age 15.14 ± 2.03yrs) using polymerase chain reaction.
Methods: The patients (pts) were divided into 2 groups: 1st, 7 pts with CRF (median GFR 45.89 ± 9.67 ml/min/1.73m^2), including mesangial proliferative GN (MPGN) (n=2), focal segmental glomerulosclerosis (FSGS) (n=2), membranoproliferative GN (MbPGN) (n=3); 2nd, 7 pts with normal renal function (median GFR 104.12 ± 8.71 ml/min/1.73m^2) who has had MPGN (n=2), MbPGN (n=3) and FSGS (n=2). There was no significant difference in the duration of SRNS in pts of both groups (5.43 vs. 3.93 yrs, respectively). The ACE I/D gene polymorphisms were significantly different between two groups (table).

	SRNS with CRF (n=7)	SRNS with normal GFR (n=7)	P value
II	14.29	71.43	<0.01
ID	57.14	28.57	>0.05
DD	28.57	0	>0.05

Results: There was no difference in the genotype distribution of the AGT gene M235 and AT_1R gene A1166C in pts with CRF (AGT gene: TT=0%, MT=85.71%, MM=14.29%; AT_1R gene: AA=57.14%, AC=42.86%, CC=0%) compare with children had normal GFR (AGT gene: TT=28.57%, MT=57.14%, MM=14.29%; AT_1R gene: AA=57.14%, AC=28.57%, CC=14.29%).
Conclusion: In conclusion, the DD genotype and D allele of ACE may be a genetic susceptibility factor contributing to progression of SRNS in children to CRF. We preliminary detected no linkage of genetic polymorphisms of the AGT gene M235 and AT_1R gene A1166C to CRF in pts with SRNS.

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P047

THE RELATIONSHIP BETWEEN HYPERLIPIDEMIA AND KIDNEY DAMAGE OF IDOPATHIC NEPHROTIC SYNDROME IN CHILDREN

Q Meng, YW Zhang, Y Shen, ZF Jiang.

Objectives of Study: To investigate the characteristics of hyperlipidemia and the relationship between it and kidney damage in children with idopathic nephrotic syndrome (INS).
Methods: Plasma lipids, lipoproteins, apolipoproteins, fibrinogen, BUN and creatinine, urineβ2-microglobulin (β2-MG) and retinoid-binding protein (RBP) were examined in 100 pediatric patients with nephrotic syndrome in 2001-2002, with 30 patients as control who did not experience any diseases of nephrological and endocrino-logical and hepatic system. Also the renal function was assessed and the renal histopathological changes in 37 patients with INS were analysed by renal biopsy under the image analysis system and semiquantitive scoring system.
Results: The levels of serumTG, TC, LDL-C, VLDL-C, HDL-C, apoB100, apoCII, apoCIII, apoE and lp (a) were higher in children with INS (P<0.05) with no changes in apoAI compared to the control group, whereas the ratio of apoAI/apoB100 was decreased (P<0.01).
The levels of serum TC, TG, LDL-C, VLDL-C, apoB100 were positively correlated to serum fibrinogen (P<0.05) and negatively correlated to serum albumin (P<0.05). Serum TG, VLDL-C were positively correlated to 24 hour urine total protein (P<0.01,P<0.05). Serum apoB100 was positively correlated to the duration of disease (P<0.05).
Urinalysis showed that patients with INS had highly elevated levels of RBP andβ2-MG. Plasma TC, TG, VLDL-C, apoB100, apoCII and apoCIII were positively correlated to urineβ2-MG and RBP (P<0.05). Histopathological analysis demonstrated that 7 types of pathological changes were found with minimal change and mesangium proliferation growth as the primary changes. There was no relationship between the level of serum lipid and the types of renal pathology.
In MCNS, kidney sclerosis index was positively correlated to serum LDL-C (P<0.05) and tubule interstitial sclerosis index positively correlated to serum TG, VLDL-C (P<0.01).
Mesangium and matrix proliferation were positively correlated to serum TC and LDL-C (P<0.05). In non-MCNS, there was no linear relationship between hyperlipidemia and the renal pathological changes. ApoA1/apoB100 was positively correlated to serum albumin (P<0.05) and negatively correlated to serum fibrinogen, urineβ2-MG (P<0.05). Serum HDL-C was negatively correlated to kidney sclerosis index (P<0.05).
Conclusion: Dyslipidemia is a charicateristics of children with INS. Hyperlipidemia is closely related to the renal function and pathogenesis. In both MCNS and non-MCNS, hyperlipidemia is positively correlated to the dysfunction of tubular. The linear correlation between hyperlipidemia and mesangium and matrix proliferation exists in MCNS. These results suggest that dyslipidemia may play some important roles in the renal pathogenesis of nephrotic syndrome. HDL and apoAI can protect renal function.

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P048

NEPHROTIC SYNDROME ASSOCIATED WITH MICROCEPHALY AND CEREBELLAR ATROPHY (CASE REPORT)

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Objectives of Study: The aim of this case report was to stress the association of nephrotic syndrome, manifested after first year of life, microcephaly, and cerebellar atrophy in possible syndrome.
Methods: Our patient was a 14-months old girl, born after 38 weeks of gestation via cesarean section because of signs of EPH gestosis in the mother. The fetus was reported as retarded in growth during the pregnancy. The microcephaly and dysmorphism of face were noted soon after birth. Her chromosome analysis was normal (46 XX). According to mother statement she had a good psychomotor development until the sixth month of life. After that period she started to loose previously acquired psychomotor abilities, which started to deteriorate. She finally lost the social contact. The eye movements become disconjugated with nystagmoid movements. Her body took rather opistotonic and twisted position with stereotypic movements of arms. She was dystonic with diminished tendon reflexes. At the age of 14-months she developed neprotic syndrome, associated with gross proteinuria (24 g/day) and deep hypoproteinemia, which was steroid and therapy resistant. Despite of aggressive replacement therapy with albumins and furosemide she developed acute renal failure and was put on peritoneal and later on continuous veno-venous ultrahemodiafiltration. Ten weeks after addmition she died because of acute respiratory distress syndrome, pneumomediastinum, and subcutaneous emphysema due to artificial respiration.
Results: The kidney biopsy disclosed signs of diffuse mesangial sclerosis. MR of central nervous system showed atrophic cerebellum, especially vermis.
Conclusion: This case is example of rare, probably autosomal recessive syndrome consisting of central nervous system malformation and nephrotic syndrome. Other authors previously described the similar associations of symptoms but each of them differed in details from our reported case.

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P049

FENA, A RELIABLE MARKER FOR VOLUME STATUS IN NEPHROTICS

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Objectives of Study: To assess the intravascular circulatory volume status in nephrotic children.
Method: It is a prospective descriptive study conducted for a period of one year.All nephrotic children with normal renal function were evaluated clinically and biochemically .Urine sodium, potassium,creatinine were estimated .Fractional excretion of sodium and uk/uk + Na x100 was calculated.
Observations: 106 nephrotics,64males ,42females ratio 1.5 :1 were included .Majority were in the age group of 3-5yrs(41/10638)%,followed by 5-10yrs (32/106) 30.2% .27children were below 2yrs 25.5% ,6 were above 10yrs 5.6%.youngest was<1yr and eldest was 12yrs.All had nephrotic proteinuria.Mean s.albumin was 1.0g% and s.cholesterol 166-757mg%. Urine electrolyte ratio was calculated by using the uk/uk +Na x 100 and it varied between 18 & 84 mean 44.53 ±18.47.FENa is a marker for underlying volume status. 72/106 (67.9)had a FENa >1 indicating underlying overfilled staus..Of these 72 children,44were males and 28were females,34children(32.1%) had a FENa<1, denoting underlying hypovolemic circulatory status.Of 34children 20 were males and 14females.
uk/uk + Na x100 was calculated and 78 ± 28 had underfilling, 35 ± 17 had overfilling.
Conclusion: About two thirds (64-70%) of nephrotics with normal renal function exihibited either increased or normal intravascular volume status and the remaining one third(30-35%) had decreased intravascular volume.
Oliguria clinically and s.albumin<2.0g indicate underfilled volume status.Albumin and diuretics help edematous children. Albumin infusion can be dangerous in overfilled nephrotics as it can push these children into pulmonary edema. Diuretics alone is sufficient .A quick diagnosis of intravascular volume status can be made by bedside estimation of FENa and the urinary electrolyte ratio (uk/uk + Na x 100).These two correlated strongly with each other in predicting the underlying intravascular volume status.
It is cost effective alternative to estimation of serum aldosterone levels in planning the management of nephrotic edema.Hence it should be estimated in all edematous nephrotics prior to administrating albumin infusion.

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P050

THE INFLUENCE OF POLYMORPHISMS OF ACE GENE AND PAFAH GENE ON THE RELAPSE OF STEROID RESPONSIVE NEPHROTIC SYNDROME

Q Shen, H Xu, Y Yang

Objectives of Study: To investigate the influence of polymorphisms of angiotensin I-converting enzyme(ACE) gene and platelet-activating factor acetylhydrolase(PAFAH) gene on the relapse of steroid responsive nephrotic syndrome.
Methods: 42 steroid responsive nephrotic syndrome children and 100 normal controls were studied.ACE and PAFAH genotypes were determined by PCR.
Results: 1.There were no significant difference in the frequency of ACE genotypes between steroid responsive nephrotic syndrome children and normal controls. 2.The mean number of relapses during the first year after onset was significantly higher in the 24 patients who were ID/DD genotype of ACE than in the 18 patients who were II genotype of ACE(2.75±1.32 vs.1.61±1.04;P=0.0064). 3.There was no significant difference in the frequency of PAFAH genotypes between steroid responsive nephrotic syndrome children and normal controls. 4.The mean number of relapses during the first year after onset was significantly higher in the 15 patients who were GT genotype of PAFAH than in the 27 patients who were GG genotype of PAFAH(3.0±1.25 vs.1.85±1.20;P=0.0093). 5.Patients with both ID/DD genotype of ACE and GT genotype of PAFAH had higher mean number of relapses than the other patients(P<0.05).
Conclusions: The polymorphisms of ACE gene and PAFAH gene may contribute to the relapse of steroid responsive nephrotic syndrome.

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P051

SPIRULINA SUPPLEMENTATION IN PEDIATRIC NEPHROTIC SYNDROME

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Objective: High lipid levels are a marker of nephritic syndrome and also contribute to the process of glomerulosclerosis. Spirulina is a blue green algae and a natural antioxidant and is one drug, which has shown positive effects in reducing serum cholesterol and HDL cholesterol. The study was conducted to determine the effect of spirulina administration on the clinical course of nephrotic syndrome and its effect on the biochemical profile of the disease.
Design:Combined,concurrent and non concurrent cases for the clinical aspects were enrolled in the study.For the biochemical aspects of the study randomized prospective
Single blinded placebo were included.
Settings: Pediatric ward and pediatric nephrology clinic of a tertiary health care facility (urban medical college).
Methods: All the children satisfying the clinical and biochemical criteria of nephritic syndrome, including the first attack and the subsequent relapses were enrolled in the study. After the enrollment the children were randomized into a spirulina and a non spirulina group.The spirulina group was supplemented sprirulina orally twice a week foraperiod of four months.While the non spirulina group was supplemented with a placebo. Clinical parameters were followed once every 30 days and the biochemical profile(cholesterol,serum triglyceride, total serum proteins and serum albumin) were monitored at 0,2and 4 months both in the study and the control group.
Results: Thirty patients were included in the spirulina group and twenty-two patients were included in the control (non spirulina) group. The mean age of the patient in the spirulna group was 7.4 years. The mean duration of the follow up was 13.6 and 11.6 months respectively in the spirulina and the non-spirulina group. Mean number of relapses in the spirulina group was1.39 years per patient year, which decreased to 0.369 relapse per patient year after spirulina supplementation (statistically significant). Serum triglyceride and cholesterol levels reduced significantly in the study group. A (251.05mg/dl to 114.55 mg/dl and 369.36 mg/dl to 192.35mg/dl). A statistical difference was not observed in the non-spirulina group. The serum proteins levels including albumin levels also rose significantly in the spirulina group.
Conclusions: Spirulina demonstrated evidences of steroid sparing effect and maintaining longer remissions in nephrotic syndrome. Spirulina demonstrates the benefit on the clinical and biochemical profile of pediatric nephrotic syndrome.

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P052

IDIOPATHIC NEPHROTIC SYNDROME IN CHILDREN: LONG-TERM OUTCOMES IN A SINGLE AMERICAN PEDIATRIC MEDICAL CENTER

O Boyer, J Moulder, MJG Somers

Objectives of Study: Most children with nephrosis have Minimal Change Nephrotic Syndrome (MCNS). Recent reports suggest the prevalence of more aggressive forms of idiopathic NS is increasing. We sought to characterize the clinical features, treatment responses, and long-term outcomes in children with presumed MCNS who presented for their initial care and had regular follow-up at a single American tertiary-care pediatric medical center.
Methods: We identified all children with idiopathic NS, clinically presumed to be MCNS, with >2 yrs of documented follow-up care at Children's Hospital Boston from 1977-2002. Children with nephrosis due to infection or malignancy, congenital nephrosis, or with primary nephropathy other than MCNS or Focal Segmental Glomerulosclerosis (FSGS) were excluded. Abstracted data included demographics, clinical characteristics at presentation, relapse frequency, steroid dependency, histology on renal biopsy (bx), and height standard deviation scores (Ht SDS) at diagnosis and last follow-up. Children were grouped according to initial response to 8 wks of 60 mg/M^2/day oral prednisone: Responders (Resp) with complete NS remission; Partial Responders (Part Resp) with improved hypoalbuminemia and proteinuria but no remission; and Non-Responders (Non-Resp) with no change in NS. Resp were further classified according to relapse pattern: Primary Responders (1° Resp) maintained sustained remission after steroids with no further NS relapses; Infrequent Relapsers (Infreq) relapsed < 4 times per yr; and Steroid Dependent and/or Frequent Relapsers (SD/Freq) experienced 3 or more NS episodes including presentation during first 6 months of follow-up, or > 4 relapses in any subsequent yr period, or relapsed on or within 2 wks of stopping steroids.
Results: 185 children (64% boys; average age at diagnosis 5.2±4.4 yrs; average age at last follow-up 12.1±6.2 yrs; 71% white/15% Hispanic/8% black/6% Asian) with presumed MCNS at diagnosis were identified. 18 children (9.8%) had family history of NS. 136 children (73%) were Resp; 24 (13%) were Part Resp; and 25 (14%) were Non-Resp. When compared to Part and Non-Resp, Resp showed no difference in frequency of microhematuria or mean GFR at diagnosis. Resp were, however, more likely to be normotensive (61% vs. 15%; p<0.002), more likely to be boys than girls (80% vs. 63%; p=0.01), and more likely to be white, Hispanic, or Asian than black (75% vs. 50%, p<0.04). Of the 136 Resp, 15 (11%) were 1° Resp; 40 (29%) were Infreq; and 81 (60%) were SD/Freq. 1° Resp were older at presentation than Infreq or SD/Freq (7.1±4.1 yrs vs. 5.7±4.0 yrs vs. 3.6±3.2 yrs; p<0.05). During follow-up, SD/Freq were more likely to exhibit decreased linear growth than Infreq (-1.0±1.7 HtSDS vs. 0.6 ±2.3%; p<0.05). 80/185 (43%) children underwent renal bx. When compared to Resp, Part and Non-Resp were more apt to have FSGS on bx (74% vs. 24%, p<0.0001) and Non-Resp with FSGS were more likely to progress to ESRD (44% vs. 0%, p=0.02). FSGS was also more prevalent in blacks undergoing bx than whites (88% vs. 44%; p=0.03), in girls undergoing bx than boys (65% vs. 39%, p=0.04), and especially in Part and Non-Resp girls undergoing bx than Part and Non-Resp boys (95% vs. 50%), p=0.002). 7/185 children (2.9%) progressed to ESRD. All 7 were Non-Resp with FSGS bx.
Conclusions: We conclude that: 1) Most NS children are Resp but SD/Freq; 2) Resp are more likely to be normotensive, boys, and non-black; 3) SD/Freq tend to be younger and have more growth delay; 4) FSGS is seen more often in Part and Non-Resp, blacks, and girls; 5) In Non-Resp FSGS, ESRD is common. This data would suggest that the prevalence of NS with poorer outcome is quite influenced by the racial and gender mix of the local pediatric population.

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P053

EFFECT OF NON-STEROID THERAPY ON LONG-TERM LINEAR GROWTH IN CHILDREN WITH STEROID SENSITIVE NEPHROTIC SYNDROME

J Moulder, O Boyer, MJG Somers

Objectives of Study: Children with nephrotic syndrome (NS) often relapse frequently or develop steroid dependency and are monitored closely for steroid sequelae such as decreased linear growth. Although a fall in height velocity is often the reason why alternative therapy to steroids is begun, little data exist examining the efficacy of this approach in preserving long-term height potential. We sought to compare changes in height velocity from diagnosis to last known follow-up in children with steroid sensitive NS treated exclusively with oral steroids vs. children receiving some steroid-sparing therapy.
Methods: We identified all children with idiopathic steroid sensitive NS who were diagnosed and had at least 2 yrs of initial follow-up care at Children's Hospital Boston between 1983 and 2002. Children with steroid resistant disease, partial steroid responsiveness, secondary nephrosis, or congenital nephrosis were excluded. Abstracted patient data included demographics, therapeutic regimens, relapse frequency, steroid dependency, and height standard deviation scores (Ht SDS) at diagnosis and at last follow-up. Children were grouped according to four patterns of steroid response: infrequently relapsing NS (IRNS), frequently relapsing NS (FRNS) characterized by 3 or more NS episodes including presentation and first 6 months of follow-up, steroid dependent NS (SDNS) characterized by NS relapses while on steroids or within 2 weeks of stopping steroids, and steroid dependent frequently relapsing NS (SDFRNS) characterized by both relapses and steroid dependency.
Results: 119 children (66% boys; average age at diagnosis 5.0±4.0 yrs; average follow-up 5.1±4.3 yrs) with idiopathic steroid sensitive NS were identified. In terms of NS relapse patterns, 55/119 children (46%) had IRNS, 9/119 (7.5%) had FRNS, 10/119 (8.5%) had SDNS, and 45/119 (38%) had SDFRNS. For therapeutic regimens, 88/119 children (74%) received oral steroids alone whereas 31/119 (26%) received at least one additional medication because of steroid sequelae. All 55 children with IRNS and 8/9 children with FRNS received steroid therapy exclusively. Of 31 children who received non-steroid medications, 8 received a single steroid-sparing agent other than an alkylating agent (Alk) or calcineurin inhibitor (CNI), 8 received a single course of Alk, and 15 received a single course of Alk and subsequently needed therapy such as CNI. At diagnosis, IRNS children were significantly older than children with FRNS, SDNS, or SDFRNS (6.1±4.0 yrs vs. 2.7±0.7 yrs vs. 3.3±1.8 yrs vs 3.8±3.6 yrs; p<0.05). No difference in gender distribution existed among groups. Mean Ht SDS showed a small increase in height velocity in IRNS children from diagnosis through last follow-up (0.4±2.3 SDS) whereas Ht SDS fell in children with SDNS (-0.4±1.4 SDS), with FRNS (-1.3 ±1.9 SDS), and with SDFRNS (-1.3±2.1 SDS; p<0.05). In SDFRNS children, no significant difference in loss of height velocity existed among children who were treated with steroids alone (-1.2±1.8 SDS) vs. children treated with a single steroid-sparing agent other than Alk or CNI (-1.5±2.7 SDS) vs. children treated with a single course of Alk (-0.6±1.7 SDS) vs. children treated with Alk and then CNI (-1.8±2.6 SDS).
Conclusions: We conclude that: 1) Half of children with idiopathic steroid sensitive NS exhibit frequent relapses and/or steroid dependency; 2) Children with FRNS, SDNS, and SDFRNS are significantly younger at presentation than children with IRNS; 3) Normal growth is maintained in children with IRNS treated with steroids alone; 4) Height is lost in children with FRNS, SDNS, and SDFRNS regardless of the additional therapy used to spare steroid burden. This data would suggest that in FRNS, SDNS, and SDFRNS children, the choice to switch from steroid therapy alone to alternative therapeutic regimens should not be based on superior long-term growth potential.

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P054

POLYMORPHISMS IN RAS GENES IN CHILDREN WITH IDIOPATHIC NEPHROTIC SYNDROME

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Objective of Study: Over-activity of the local RAS may be of crucial importance in kidney damage. Physiologic actions of angiotensin II including cell growth, inflammatory response and fibrosis could be influenced by polymorphism in angiotensin- I converting enzyme (ACE) gene. Most of angiotensin II actions in kidney, including fibrosis are mediated through angiotensin II type 1 receptors (ATR1). The aim of this study was to analyze ACE I/D and ATR1 A1166C gene polymorphisms in children with idiopathic nephritic syndrome.
Methods: Twenty-three patients with idiopathic nephrotic syndrome divided in two groups: A-focal glomerular sclerosis (14 pts.) and B-minimal change nephritic syndrome (9 pts.) were included in the study. Genomic DNA was isolated from the whole blood. Genotyping for ACE I/D polymorphism was performed by polymerase chain reaction (PCR) using a modified three-primer method. For detection of ATR1 gene polymorphism we used allele specific PCR performed in two parallel reactions. PCR products were visualized on agarose gels stained with ethidium bromide.
Results: Frequencies distribution for ACE II, ID and DD genotypes were 16.7%, 50.0 % and 33.3% in group A, and 22.2%, 55.6% and 22.2% in group B, respectively. I/D allele frequencies were 0.417/0.583 and 0.500/0.500 in groups A and B, respectively. Genotype frequencies for ATR1 AA, AC and CC were 50%, 50%, 0% in group A, and 55.6%, 44.4% and 0% in group B. A/C allele frequencies were 0.750/0.250 and 0.778/0.222 in groups A and B, respectively. Comparing the frequencies of ACE genotypes, we found significant difference between analyzed groups. Frequency of DD genotype was higher within group of patients with focal segmental glomerulosclerosis. There were no differences in I/D allele, ATR1 genotypes and A/C allele frequencies between analyzed groups.
Conclusion: Higher frequency of DD ACE genotype might influence local angiotensin II over-activity and contribute to progressive nature of focal glomerular sclerosis.

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P055

ADOLESCENT ONSET NEPHROTIC SYNDROME IN INDIA: CLINICAL FEATURES AND HISTOPATHOLOGICAL SPECTRUM.

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Objectives of Study: The adolescent population signifies the transitory period where the frequency of occurrence of different histopathological lesions in patients with nephrotic syndrome is different from that seen in the paediatric population less than 12 years of age as well as that seen in adults. The types of glomerular pathology encountered in adolescent population with nephrotic syndrome have not been well characterised.
Methods: We evaluated clinical features, laboratory data and histopathology of 163 patients with nephrotic syndrome having its onset between 12 to 18 years of age seen at this Institute over 2 years.
Results: The commonest cause of idiopathic nephrotic syndrome was minimal change disease (MCD) in 49 (33.1%) patients followed by focal segmental glomerulosclerosis (FSGS) in 43 (29%), membranoproliferative glomerulonephritis (MPGN) in 26 (17.6%) cases, mesangial proliferative glomerulonephritis in 15 (10.1%), membranous glomerulopathy (MGN) in 6 (4.0%), sclerosing glomerulonephritis, crescentic glomerulonephritis and IgA nephropathy in 3 (2.0%) patients each. Fifteen (9.2%) of these 163 patients had a secondary cause for their nephrotic syndrome. Secondary amyloidosis (53.3%) was the commonest followed by lupus nephritis (33.3%) and diffuse proliferative glomerulonephritis (14.4%). Of the 56 (37.8%) patients with microscopic hematuria, 18 (32.1%) had MPGN, 15 (26.8%) had FSGS and 7 (12.5%) had MCD. Of the 48 (32.4%) patients with hypertension, the commonest lesion was MPGN in 18 (37.5%) cases followed by FSGS in 12 (25%) cases and only 6 (12.5%) patients had MCD. The commonest cause of steroid resistant state was FSGS seen in 13 (35.1%) patients followed by MPGN seen in 8 (21.6%) cases. Among the steroid dependent patients MCD was seen in 57.1% and FSGS in 28.6% of cases. When biopsies done after 1996 were compared with those done before 1996, the incidence of FSGS was seen to have increased from 19.2% to 40.0% ($p<0.01$).
Conclusions: We conclude that the commonest cause of idiopathic nephrotic syndrome among adolescents is MCD (33.1%) closely followed by FSGS (29%). Fifteen (9.2%) of cases had a secondary cause. Adolescent nephrotics with microhematuria, hypertension at presentation as well as a steroid resistant state have lesions other than MCD. The incidence of FSGS has increased in the second half of last decade. In view of the high incidence of lesions other than MCD it preferable to biopsy all adolescents with nephrotic syndrome initially rather than treat them empirically with steroids.

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P056

ASSESSMENT OF SERUM INTERLEUKIN –7 CONCENTRATION IN DEPENDENCE ON COAGULATION STATE IN CHILDREN WITH NEPHROTIC SYNDROME

Barbara Tomaszewska, Anna Wasilewska, Walentyna Zoch-Zwierz

Objective of study: Clinical significance of interleukin-7 (IL-7) and its source in the serum have not been fully known. The aim of the study was to evaluate serum IL-7 levels in children with the symptoms of nephrotic syndrome (NS) and to determine a correlation of its concentration with platelet count, other homeostatic exponents and NS intensity.
Methods: The study was performed in two groups: I - 26 children with NS (12 boys , 14 girls) aged 6.8 ± 2.1 years, subjected to examination twice: A - before treatment, B - during treatment with prednisone (60 mg/kg/24h after albuminuria regression, C - control group of 20 healthy children. Serum IL-7 level was assayed by ELISA method using a R&D Quantikine set**.**
Results: In group I, IL-7 level in examination A (33.33 ± 33.24 pg/ml) was higher than in control subjects ($p<0.01$). In examination B, IL-7 concentration was reduced to the level of 10.86 ± 5.22 pg/ml and did not differ from control ($p<0.05$). A positive correlation was observed between IL-7 and platelet count and serum fibrinogen level. A negative correlation was noted with antithrombin III concentration. No correlation was found with serum levels of albumins and cholesterol or urine protein.
Conclusions: In NS children, serum IL-7 level increases proportionally to the elevated platelet count and other homeostatic exponents, but shows no correlation with serum albumins or urine protein.

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P057

A RETROSPECTIVE REVIEW ON LONG TERM STEROID RESPONSIVENESS OF PAEDIATRIC PRIMARTY NEPHROTIC SYNDROME

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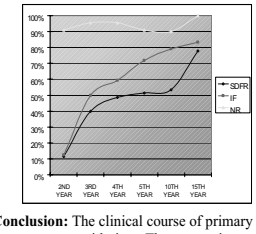
Objectives of Study: To study the clinical course of steroid-sensitive nephrotic syndrome in the first 5 years and to describe their clinical outcome at 10 or more years of follow-up.
Methods: Patients who were admitted to Princess Margaret Hospital and Queen Elizabeth Hospital with diagnosis of primary nephrotic syndrome from Jan 1978 to Dec 1993 were included for retrospective review. They were stratified into non-relapser group(NR), infrequent relapser group(IF) and steroid dependent/frequent relapser group(SD/FR) according to their clinical behaviour in the first year by ISKDC classification. Their subsequent clinical behaviours were profiled yearly to fifth year or more. Steroid resistant patients in the first year were excluded from the study.
Results: A total of 89 patients were included in this review. The number of patients stratified into SD/FR, IF and NR group in the first year was 35 (39%), 32 (36%), 22 (25%) respectively. The M:F ratio is 3:1. The age at presentation was 4.6yr +/- 2.6. The duration of follow-up was 12.4yr +/- 3.7. The time to first relapse was 3.3 mth +/- 1.5 for SD/FR group and 5.5 mth +/- 2.7 for IF group. The renal biopsy rate was 83%, 28 % and 9% in SD/FR, IF and NR group respectively. 70% were due to Minimal Change Glomerulonephritis. In the SD/FR group, levamisole was used in 49%, cyclophosphamide in 46% and cyclosporin A in 29% of the patients. Two patients (6%) in SD/FR group changed to steroid resistant and were converted back to become steroid sensitive after a course of cyclophosphamide. The rate of no relapse at the fifth and tenth year in SD/FR group was about 50%. In IF group the rate was 70% and 80% respectively. The rate of clinical recovery (no relapse for 3 consecutive years) at 5 year is 33%, 40% and 86% respectively for SD/FR, IF and NR group.



Conclusion: The clinical course of primary nephrotic syndrome in paediatric patients exhibited transition among groups with time. The conversion to steroid resistance occurred in SD/FR group only. The majority of NR group remained relapse-free and will not convert to SD/FR. The rate of clinical recovery at end of fifth year for SD/FR, IF and NR group was 33%, 40% and 86% respectively.

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P058

CONGENITAL AND INFANTILE NEPHROTIC SYNDROME IN THAI INFANTS

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Objective: To review clinical manifestations, course, and outcome of nephrotic syndrome in some Thai infants.

Patients and Methods: We retrospectively reviewed children diagnosed congenital and infantile nephrotic syndrome who attended Pediatric Nephrology Clinic in Songklanagarind Hospital, Thailand 1983-2002.

Results: There were 5 girls and 5 boys; seven were diagnosed with congenital nephrotic syndrome and three with infantile nephrotic syndrome. Two had congenital nephrotic syndrome secondary to congenital syphilis. All had edema, ascites and failure to thrive. Four patients had an opisthotonos position, and of the three patients tested for thyroid function, all showed hypothyroidism. Two patients developed renal failure. Renal tissue was examined from 4 patients from 3 biopsies and 2 autopsies; only one patient showed tubular microcysts. Symptomatic therapy was performed concurrently with penicillin therapy in two patients having congenital syphilis. Prednisolone, cyclophosphamide and enalapril had been tried in some patients, with little effect. Five patients died from respiratory failure complicated by later infection, one patient died from renal failure, and four patients were lost to follow up.

Conclusion: Nephrotic syndrome in the first year of life in the eastern world is rare. Prognosis of nephrotic syndrome in Thai infants at this time is still poor.

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P059

GLUCOCORTICOID RECEPTOR EXPRESSION IN CHILDREN WITH RECURRENT NEPHROTIC SYNDROME

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Objectives of study: The immunosuppressive action of glucocoricosteroids follows their binding to the intracellular receptor GCR. The aim of the study was to evaluate the expression of GCR in lymphocytes (CD3/GCR) and monocytes (CD14/GCR) of the peripheral blood in children with recurrent nephrotic syndrome.
Methods: The study involved 38 children with recurrent nephrotic syndrome (NS) treated with prednisone (60 mg/m^2 body surface). The patients were divided into group I - 19 children aged 5.6 ± 3 years with pathological albuminuria regressing after 8-10 days of treatment; it was their first or second NS attack (mean 1.42 ± 0.6) and total prednisone dose they had received since the disease onset was 177.0 ± 80.7 mg/kg b.m, and group II – 19 children aged 6.7 ± 4.2 years with albuminuria regression after 20-25 days of therapy; a subsequent NS event (mean 4.8 ± 0.6) and total prednisone dose 536.3 ± 223.1 mg/kg b.m. Flow cytometry was employed twice to determine the number of CD3+ lymphocytes and CD14+ monocytes and the percentages of these cells with CD3/GCR and CD14/GCR: A – before treatment and B – directly after albuminuria regression during prednisone administration.
Results: In group I, normal levels of CD3/GCR (61.7 ± 19.5%) and CD14/GCR (56.6 ± 19.7%) before treatment (A) decreased significantly during prednisone therapy (B) ($p<0.05$). In group II, before treatment (A), the mean expression of CD3/GCR (27.6 ± 28.8%) and CD14/GCR (31.9 ± 15.3%) was markedly lower compared to control group ($p<0.05$). In examination B, percentages of the receptor-presenting cells were still low ($p<0.05$). A negative correlation was observed between CD3/GCR expression and total prednisone dose: in group I $r=-0.563$, $p<0.01$, in group II – $r=-0.538$, $p<0.01$. A positive correlation was noted with serum cortisol level.
Conclusion: Long-term prednisone therapy has a depressive effect on the expression of receptors in mononuclear cells of the peripheral blood. It was shown that children with frequent recurrence of NS had markedly higher percentages of CD3/GCR+ and CD14/GCR+ lymphocytes and monocytes compared to children with fewer NS recurrences.

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P060

FETUIN-A DEFICIENCY IN CHILDREN WITH PROTEINURIA

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Background: There are hints that the extracellular calcium-regulatory protein fetuin-A (alpha-2-Heremans Schmid glycoprotein) is a potent soluble inhibitor of calcification in vitro and in vivo, and could therefore interfere atherogenesis.
Ketteler et al. (Lancet 361 (2003): 827-33) reported a possible connection between fetuin-A deficiency and accelerated atherosclerosis in uremia.
Because fetuin-A is a protein with a molecular weight of 60 kDa a loss of fetuin-A during proteinuria is possible. We aimed to investigate the hypothesis that proteinuria may lead to fetuin-A deficiency.
Methods: Fetuin-A was determined by immunoassay (Limbach Laboratory, Heidelberg, Germany) in 11 children and adolescents with different degrees of proteinuria (91-1028 mg/d).
Three groups were differentiated according to fetuin-A serum levels and risk for vascular calcification: group 1: fetuin-A < 0.4 g/l (high-risk group); group 2: fetuin-A 0.4 – 0.7 g/l (mid-risk group) and group 3: fetuin-A > 0.7 g/l (low-risk group). C-reactive protein (CRP) was also determined in all 11 subjects.
Results: In all 11 children, fetuin-A levels were lower than 0.7 g/l with a mean value of 0.39 g/l. In 7 of the 11 children, fetuin-A levels were lower than 0.4 g/l (group 1) with a mean value of 0.26 ± 0.07 g/l. Proteinuria in these 7 children amounted to a mean of 481 ± 321 mg/d. In 4 of the 11 children, fetuin-A levels were between 0.4 – 0.7 g/l (group 2). Proteinuria in these 4 children amounted to a mean of 155 ± 78 mg/d. Both the mean value of fetuin-A and the mean value of proteinuria differed significantly between these two groups, proven by t-test ($p = 0.001$ and $p = 0.037$ resp.).
CRP values in all children were under 5 mg/l, with the exception of 1 child; this child belongs to the high-risk group.
Discussion: In all 11 children with proteinuria, fetuin-A levels were lower (0.13–0.57 g/l) than the levels cited in the literature for normal adult–aged subjects (to date, there are no fetuin-A reference values for children at hand). Therefore, patients with proteinuria may bear an additional risk for vascular calcification that may even be intensified by a disturbed lipid metabolism. Furthermore, there seems to be a correlation between the extent of proteinuria and fetuin-A deficiency.
Conclusion: This pilot study comprising of 11 children and adolescents with proteinuria seems to confirm the hypothesis that proteinuria causes fetuin-A deficiency.

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P061

STUDY ON RENAL TISSUE GENE EXPRESSION PROFILE IN CHILDHOOD MINIMAL CHANGE NEPHROSIS

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Objectives of Study: To explore the pathogenesis of minimal change nephrosis in children through studying renal tissue gene expression profile in childhood minimal change nephrosis with internationally recognized standardized gene chips provided by Affymetrix Co.
Method: (1) patients group and control group were set up with 7 cases diagnosed through renal biopsy as primary minimal change nephrotic syndrome assigned in the patients group. Since there were limited available renal tissue, the 7 patients were further divided into 3 groups (3, 2, and 2 each); in which there are 5 males and 2 females. Regular corticosteroid therapy and follow-up were carried out at least for more than 6 months. The control group consisted of 2 sub-groups, congenital hydronephrosis and tumor patients with normal renal tissue far off the pathological tissue. Both the renal biopsy specimens from the patients group and renal tissue of the control group were immediately put into RNAlater Staplization Reagent manufactured by QIAGEN Co. and stored under –20℃ ; (2)extraction of RNA, quality control, and reverse transcription into cDNA were carried out and in vitro transcription and synthesis of single stranded cRNA probe performed; (3) synthesized cRNA probe after treated with fragmentation were hybridized with 5 HG-U133A chips provided by Affymetrix Co., including equivalent to 18,400 known human gene, they were hybridized for a total of 5 times with renal tissue from 2 sub-groups in the normal control group (with a total of 2 cases) and 3 groups of minimal change nephrosis (with a total of 7 cases); (4) comparison was carried out between the expression profile of each patient group and the 2 normal control groups respectively, cross-comparative analysis and comprehensive analysis in three kinds of signals, i.e. increase, decrease and without changes, and their intensity were carried out.
Results: Differentially expressed genes were identified by calculating generalized t tests ($P < 0.001$). In the 18,400 known human gene, 47 over-expressed genes and 173 low expressed genes were singled out from the 6 comparisons in the results of expression profile between each patient group and that of the 2 control groups. Several gene expression signatures were readily identifiable in each patient group, including signatures associated with cell communication, cell growth, cytokine/chemokine expression, and immune response. Of particular interest was the association of a gene expression signature enriched for genes such as angiopoietin-like 3, apolipoprotein H and c-maf with patients experiencing frequent relapse of nephrotic syndrome, which have not been reported yet. To confirm GeneChip results, real-time PCR and Immuno-histochemical method of selected genes were performed.
Conclusion: Our data provide a first report of gene expression profiles of renal tissues in childhood minimal change nephrosis.

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P062

ANGIOTENSIN CONVERTING ENZYME GENE POLYMORPHISM IN NEPHROTIC CHILDREN

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Background: Genetic variability in renin-angiotensin system may affect the course of renal disease. In this study we evaluated the association between clinical characteristics and ACE genotypes in steroid sensitive nephrotic syndrome (SSNS) and focal segmental glomerulosclerosis (FSGS) patients and we compared the results with 30 healthy control subjects.
Methods: ACE genotype polymorphism was assessed by PCR amplification on genomic DNA isolated from blood leukocytes in 25 children with SSNS diagnosed according to ISKDC criteria (15 males, 10 females, mean age: 7.3 ± 3.5 years) and in 25 children with biopsy proven FSGS (18 males, 7 females, mean age: 9.6 ± 5.3 years). Response to steroids (n: 9) and response to different cytotoxic drugs, cyclophosphamide and chlorambusil (n: 10) as a second line of treatment and cyclosporine A (n: 6) used as a third line of treatment was also analyzed in FSGS patients.
Results: The allele frequencies were as follows:

Group	DD genotype (%)	ID genotype (%)	II genotype (%)
SSNS	40	44	8
FSGS	40	28	11
Controls	23	60	17

A similar distribution of ACE polymorphism was found between SSNS patients, FSGS patients and controls. There was also no association between ACE genotype and response to therapy in FSGS patients. The clinical course of FSGS was assessed according to the presence of hypertension (n:6), hematuria (n: 13) and decline in renal function (n: 5) in FSGS patients. The correlation between ACE polymorphism and the course of FSGS according to hypertension, hematuria and renal function was not significant.
Conclusion: Our preliminary results suggest that ACE polymorphism does not predict the clinical outcome in childhood SSNS and FSGS patients.

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P063

HEMOLYTIC UREMIC SYNDROME (HUS) IN CHILE.
EIGHT YEARS´EXPERIENCE IN A SINGLE PEDIATRIC CENTER

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Objectives of study: To evaluate our clinical experience with HUS affected children in a pediatric center during an 8 years period, with emphasis in predictive elements of severity, medical treatment decisions, intra-hospital evolution and follow up.
Methods: Medical records were analyzed retrospectively in patients younger than 15 years old admitted to hospital with HUS between January 1995 and December 2002. From these charts, data regarding demographic and past medical information, laboratory and imaging studies, urine output, medical therapy including acute renal replacement and use of drugs, complications, days of hospitalization and follow up. Statistical comparison was made between patients that underwent dialytic therapy and those with conservative therapy
Results: 61 patients were admitted in this period (7.6 patients/year), but the study analysis was made with 43 patients whose medical records were found. The mean age was 26 months (1- 69 mo.), 60% younger than 2 year old. Male:female was 1:1. D+(post diarrhea) HUS was diagnosed in 95% of patients. Of them, 80.5% had prodromic bloody diarrhea, 41% with positive stool culture, mainly enterohemorrhagic E.coli, and 70% received antibiotics preceding HUS diagnosis. Hypertension was found in 37.2% during the hospitalization; red blood cells transfusion was given to 81.4%,Since admission, 30.2% developed anuria and 16.3% oliguria; 72% received some kind of diuretics. Acute renal replacement therapy was used in 40% of patients: 64.7% only peritoneal dialysis, 11.7% only continuous hemofiltration, and 23.5% both. No deaths or recurrences were detected in our study, 11.6% developed chronic renal failure in a mean follow up of 54 months.
Comparison between dialyzed and dialyzed patients is shown in the next table:

	Dyalisis (mean)	No dialysis (mean)	p value
Admission BUN	70	47	0.035*
Max. BUN	91	52	0.006*
Admission creatinine	3.94	1.15	0.001*
Max. creatinine	4.6	1.15	0.001*
Min. hematocrit	19.7	19.6	0.911
Admission WBC	16700	13550	0.157
Max. K+	4.2	4.15	0.823
Days of hospitalization	18	6.5	0.001*

	% of patients on dialysis	p value
Prodromic diarrhea	41.5	0.511
Bloody diarrhea	45.5	0.269
Previous use of antibiotics	40	1
Hypertension	62.5	0.026*
Anuria	100	0.001*
Oliguria	42.86	1
Normal diuresis	0	0.001*

Conclusion: In our study, HUS is primarily D+, with 40% requiring acute replacement therapy. When comparing dialyzed vs. non-dialyzed patients, the first group had higher admission and maximal BUN and creatinine values, longer hospitalization and more frequently had hypertension and anuria. Our mortality rate and development of chronic renal failure in these patients is similar to developed countries.

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P064

PNEUMOCOCCAL – ASSOCIATED HAEMOLYTIC-URAEMIC SYNDROME IN SINGAPORE

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Objective: Haemolytic-uraemic syndrome (HUS) is an important cause of acute renal failure in children. This is a retrospective descriptive study of our cases of HUS from 1994 to 2001 in two tertiary paediatric nephrology units in Singapore.
Methods: Case notes were reviewed and data collected with respect to demography, clinical feature and outcome.
Results: There were 13 cases; 9 (69%) were associated with invasive *Streptococcus pneumoniae* infection, while 3 were atypical HUS. Only 1 (8%) was diarrhoea-assoicated. Of the 9 cases associated with pneumococcal infection, 7 had lobar pneumonia complicated by empyema. One had meningitis, while the remaining patient had spontaneous bacterial peritonitis complicating relapse of nephrotic syndrome. The clincial features of these 6 children included anaemia, thrombocytopaenia and hepatic involvement (100%), hypertension (78%), oliguria and / or anuria (67%), of whom 5 (56%) required acute dialysis, and encephalopathy with seizures (33%).
2 (22%) died from overwhelming sepsis and multiorgan failure; there was no recovery of renal function in another. Of the remaining 6, all have normal renal function at 2 months to 6 years of follow-up.
Conclusion: Invasive *Streptococcus pneumoniae* infection is the commonest cause of HUS in our population. Greater awareness, early diagnosis and hence, appropriate management of this complication in children with pneumococcal pneumonia will lead to a better outcome.

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P065

DIARRHEA-ASSOCIATED HEMOLYTIC UREMIC SYNDROME (D+ HUS) IN 2003 IN KOREA

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Objectives of Study: D+ HUS is characterized by hemolytic anemia, acute renal failure and thrombocytopenia following a preceding food borne infection by enterohemorrhagic *Escherichia coli* (EHEC). Because of the gradually increasing annual incidence of D+ HUS during the last several years in our country, the Korean Society of Pediatric Nephrology (KSPN) and Korea Center for Disease Control and prevention (KCDC) had constructed a national surveillance system for D+ HUS in early 2003. And a small outbreak was detected in 2003 by the surveillance system. In this paper, we analyzed the epidemiological and clinical features of the outbreak.
Methods: Thirty nine hospitals covering whole country except Jeju island participated the surveillance system, and all the children with clinical diagnosis of D+ HUS from March to December 2003 were registered. The epidemiological study was done by KCDC, and the clinical data were analyzed by the KSPN.
Results: Total 30 children with D+ HUS were registered during the period. Twenty six (87%) of them were less than 5 years old, and the male and female ratio was 14:16. Nineteen patients (63%) resided in Seoul and Kyunggi-Do area, the northwestern region of Korea. Twenty five cases (83%) were clustered during the end of May to the beginning of July. Multiple cases were detected in one center for crippled children (3 cases), one nursery school (2 cases) and one junior high school (3 cases), but not in any families. Various kinds of foods were suspected to be the source of infection in 15 cases, but EHEC was not detected from any of those foods. The stool culture grew EHEC in 7 patients (24%), and the serotype was O104 in 3, O121 in 2, O119 in 1, and O26 in 1. The stool PCR for verotoxin was positive in 10 cases (33%). The clinical features of the patients were as follows; pallor 67%, abdominal pain 60%, vomiting 40%, petechiae 33%, fever 23%, hepatomegaly 17%, seizure 13%, altered consciousness 7%, and splenomegaly 7%. Bloody diarrhea was accompanied in 63% of patients. Laboratory examination revealed various degrees of hemolytic anemia, acute renal failure and thrombocytopenia in all patients. Anuria was accompanied in 10 cases (33%) for 1 to 12 days, and dialysis was performed in 8 (27%, hemodialysis in 6 and peritoneal dialysis in 2). Packed red blood cells were given 26 cases (87%) and fresh frozen plasma in 7 (23%). Thirteen cases (43%) received parenteral or oral antibiotics for 2 to 30 days. Three patients (10%, Group 1) died during the acute stage, and 15 (50%, Group 2) had persistent mild proteinuria, hematuria and/or hypertension after recovery. Eleven cases (37%, Group 3) recovered fully without any sequelae. All the clinical findings and laboratory data were compared among 3 groups of patients, and the degree of thrombocytopenia, hyperuricemia, and azotemia showed significant differences between Group 2 and 3. (P<0.05).
Conclusion: We experienced an outbreak of D+ HUS in Korea in 2003. Although the scale of the outbreak was not big, it was the first one detected in our country. Considering the gradually increasing incidence of D+ HUS during these several years, D+ HUS may be one of the major health problems in our country in near future. So, the nationwide surveillance system for D+ HUS should be essential for the control and prevention of this disease.

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P066

EFFECT OF PLASMA EXCHANGES INTENSIFICATION ON THE PROGNOSIS OF ATYPICAL HUS IN CHILDREN.

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Objectives of Study: D-HUS often leads to end-stage renal failure (ESRF) and relapse on the transplant followed by graft loss. Confronted to the case of a girl with D-HUS having already 2 sisters with ESRF due to the same illness, we have intensified the usual plasma exchanges (PEs) protocol and applied PEs preventively in 4 patients presenting with D-HUS.
Patients: none of them had complement activation or factor H or von Willebrand factor-cleaving proteasis deficiency. Three of them were the 3 sisters of the above-mentioned family (patient 1,2 and 3). Two patients were treated for D-HUS on native kidneys (patient 3 and 4) whereas PEs were performed in relation with transplantation for the other two.
Method: PEs were performed with fresh frozen plasma; 40 ml/kg/session; frequency: a) D-HUS on *native kidneys*: daily PEs until decrease of plasma creatinine, thereafter progressive tapering until one session/2w pursued indefinitely; b) *Tx*: first PEs before Tx, followed by 7 daily sessions and tapering as in a); in case of relapse: as in a).
Results: a) *native kidneys*: patient 3 (identical twin of patient 2): normal GFR 3 years after initial episode and after one relapse; patient 4: plasma creatinine = 60 mcmol/L after 17 months of treatment (from which 6 w of daily PEs) b) *Tx:* patient 2: plasma creatinine 100 mcmol/L 16 months after Tx despite a relapse; *patient 3:* after 2 months of satisfactory graft function, graft loss secondary to D-HUS relapse related to late initiation of daily PEs because of delayed diagnosis (no haematological HUS signs).
Conclusions: PEs intensification and preventive use in D-HUS allow restoration of a normal function in case of initial episode as well as successful Tx on condition to initiate daily PEs early after the diagnosis of relapse.

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P067

ATYPICAL PRESENTATION OF A RELAPSE OF FAMILIAL ATYPICAL HUS AFTER TRANSPLANTATION.

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Introduction:D-HUS often leads to end-stage renal failure and can relapse after transplantation. The risk of graft lost is then very high. As the shift of immunosuppression and/or the performance of plasma exchanges (PEs) might have a favourable effect on D-HUS, it is essential to make an early diagnosis in order to take adequate measures before graft lesions become irreversible.
Case report: a 12 year-old girl presenting with a familial D-HUS was successfully transplanted 6 years after a first graft failure because of immediate HUS relapse. For the second Tx (but not for the first), PEs were used preventively before and after Tx. Two months after Tx and concomitantly with a reduction of PEs frequency, plasma creatinine increased from 70 µmol/L to 194 µmol/L in 2 weeks without thrombocytopenia or signs of haemolytic anaemia. The patient had minimal clinical symptoms. Biological and imaging studies were compatible with graft rejection. A biopsy was performed after the sixth methylprednisolone pulse because of an ongoing increase of plasma creatinine suggesting a steroid-resistant rejection. Surprisingly, a typical picture of microthromboangiopathy was found without any sign of rejection. Transplantectomy had to be performed after a few days despite daily PEs and conversion of CsA to tacrolimus.
Conclusions: in our patient, a D-HUS relapse occurred after Tx without characteristic hematological features, leading to a wrong diagnosis, and therefore to a delay of daily PEs initiation and of immunosuppression shift with graft lost as final consequence. This observation emphasizes the necessity of a transplant biopsy in case of a non-explained decrease of renal function after Tx for D-HUS in order to avoid a fatal delay for taking adequate measures.

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P068

HEMOLYTIC UREMIC SYNDROME IN CHILE: FOLLOW-UP OF RENAL FUNCTION AND PROGNOSTIC FEATURES

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Hemolytic Uremic Síndrome (HUS) is characterized by acute renal failure, microangiopathic hemolytic anemia and thrombocytopenia, is the main cause of acute renal failure in childhood. The aim of this study was to describe the demographic and epidemiologic aspects and to know the renal outcome associated HUS for at least 1 year of follow-up. Analysis of 472 patients clinical records in a twelve years period (1990 to 2002), 288 were analyzed (184 had not follow- up) 53% females , mean age was 2 y (2 m-8 y), 90% had D(+) HUS, 33% occured in summer; peritoneal dyalisis was required in 75, hemodyafiltration in 12,1%, hemodyalisis in 8,7% and plasmapheresis in 1,4% Neurological involvement was seen in 24% being convulsive syndrome the most common acute problem. Etiological agent was identified in 22% of cases, enterohemorragic E.coli was the most frequent isolated agent. 50% had hypertension at the beginning and 5,8% remain hypertensive at 1 year followed up. Mortality was 4,3%, multiorganic failure and refractary epilepsy were the principal death cause. One and five years renal function followed up showed : chronic renal failure 12% and 21% respectively , proteinuria with normal renal function 6% to year and 3% to five years. A positive correlation was found between anuria , seizures, hypertension and chronic renal failure ($p<0.005$); do not was differences between HUS D+ vs HUS D-, patients transplanted with IRC by SHU versus transplanted patients with IRC by other diagnoses; no patient presented recidiva of the disease after transplant.
HUS is still an important public health problem in our country with a significative morbidity and mortality.

P069

PROGNOSTIC FACTORS AND OUTCOME 2 YEARS AFTER HEMOLYTIC UREMIC SYNDROME IN GERMANY AND AUSTRIA

A Gerber, H Karch, F Allerberger, LB Zimmerhackl for the HUS study group[#]

Objectives of study: Hemolytic uremic syndrome (HUS) is the most common cause for acute renal failure in children. Clinical characteristics during the acute disease that influence long term outcome and prognostic determinants are still under discussion world wide.
Methods: From 1997 to 2002, 628 pediatric patients with HUS were evaluated in a prospective multicenter surveillance study in Germany and Austria. Clinical data and laboratory parameters including STEC (shiga toxin-producing *Escherichia coli*) analysis in stool and serum were assessed. After the acute disease yearly follow-up examinations were analysed.
Results: 59/231 (26%) patients showed any kind of sequelae 2 years after acute HUS: Arterial hypertension in 13%, proteinuria >0.2 g/l in 21%, impared renal function (GFR <80 ml/min/1.73 m²) in 11% and neurological residues in 4%. A significantly increased risk to develop long term sequelae was found in patients without preceding diarrhea (47% vs. 24%, $p<0.05$, χ^2 test; relative risk 1.97, 95% CI 1.16-3.36), patients with renal insufficiency requiring dialysis (30% vs. 12%, $p<0.005$, χ^2 test; relative risk 2.52; 95% CI 1.26-5.04), patients without STEC infection (49% vs. 20%, $p<0.005$, χ^2 test; relative risk 2.39, 95% CI 1.51-3.76) and in patients with very high leukocyte counts >20,000/µl during acute disease (39% vs. 23%, $p<0.05$, χ^2 test; relative risk 1.68, 95% CI 1.08-2.64). There was no difference regarding long term outcome in patients who fell ill during the summermonths or in patients who received antibiotic treatment 2 weeks prior to HUS onset. Patients with CNS involvement in the acute phase were at higher risk to develop arterial hypertension and proteinuria. Dialysis duration longer than 10 days was associated with an increased rate for arterial hypertension. Patients younger than 1 year of age at disease onset showed an increased risk for hypertension, proteinuria and especially neurological residues.
Conclusion: Long term sequelae after HUS are frequent. Diarrhea-negative HUS, renal insufficiency requiring dialysis, STEC-negative HUS and high leukocyte counts are associated with an increased risk for long term sequelae 2 years after HUS onset. If patients without any sequelae 2 years after the acute disease could be discharged from further follow-up, is not predictable at the moment. Follow-up examination for several years after the acute disease are recommendable to identify more prognostic factors and to optimize therapy and long term outcome.

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P070

FACTOR H ABNORMALITIES IN TYPICAL HEMOLYTIC UREMIC SYNDROME (HUS) IN GERMANY AND AUSTRIA

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Objectives of study: Factor H deficiency has been identified as a cause of familial and recurrent forms of HUS. Factor H is an inhibitory protein of the alternative complement pathway. In this study the role of factor H in typical HUS was investigated.
Methods: From 1997 to 2002, 628 children with HUS were evaluated prospectively in a multicenter surveillance study in Germany and Austria. For the last 2 years the patients in this study have been facultatively screened for abnormalities in factor H concentration and protein structure.
Results: 11/117 analysed patients demonstrated abnormal mobility in westernblot indicating defective factor H protein. None of 94 tested patients showed a reduced factor H concentration. In addition, none of 39 analysed patients had low von Willebrand factor cleavage protease activity. 10/11 patients showed preceding diarrhea during the acute disease and in 8 patients STEC (shigatoxin-producing Escherichia coli) infection was found. 8/11 patients developed acute renal insufficieny requiring dialysis. Acute clinical course was uncomplicated in 3 patients. 4 patients required dialysis therapy for more than 6 weeks. 3 patients showed severe CNS involvement and 2 patients had accessory pancreatitis. 8/11 patients showed a unusually severe course of disease: 1 patient died due to shock and sepsis, 2 patients developed end stage renal disease and received renal transplantation, 2 patients went into chronic renal failure combined with severe hypertension, 3 patients had persistent arterial hypertension, one of them additionally suffered from persistent hemiparesis after cerebral infarction. Only 3/11 patients recovered completely after the acute phase.
Conclusion: Abnormal factor H protein structure in westernblot is found in patients with typical HUS and is associated with a high risk for severe disease and long term sequelae. HUS is a complex of diverse pathogenic mechanisms, one important is factor H.

[#] Supported by Biomed 2, BMBF, APN and the Universities Freiburg and Innsbruck.

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P071

EXPRESSION OF ADAMTS13 IN NORMAL RENAL CORTEX AND IN THROMBOTIC THROMBOCYTOPENIC PURPURA

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Objectives of Study: Thrombotic thrombocytopenic purpura (TTP) is a syndrome characterized by thrombocytopenia, hemolytic anemia, fever, neurological and renal manifestations. Congenital TTP has been associated with mutations in ADAMTS13, a von Willebrand factor cleaving metalloprotease mainly synthesized by the liver. The purpose of this study was to investigate the expression of ADAMTS13 in normal and TTP renal cortex.
Methods: Renal cortex was available from a patient with congenital recurrent TTP. This patient has a known compound heterozygote mutation affecting the ADAMTS13 gene in exons 9 and 12, respectively. The biopsy was taken at the age of two years during an acute episode of TTP. Renal cortex from one adult and two pediatric controls were used for comparison.
In situ hybridization with a probe directed against exons 25-29 was used to investigate *ADAMTS13* mRNA expression. In order to study the ADAMTS13 protein expression we performed immunohistochemistry using a pool of two polyclonal rabbit antibodies directed against two peptides in the protein.
Results: *ADAMTS13* mRNA is expressed in normal renal cortical tissue mainly in glomerular endothelium and mesangium, and less so in the tubuli. ADAMTS13 protein expression was detected in the glomerular capillary endothelium. A weak signal was also found in the mesangium and the tubuli. There was no visible difference in mRNA and protein expression pattern between normal and TTP renal cortex.
Conclusion: The *ADAMTS13* gene mutation found in this TTP patient affects protease bioactivity but does not appear to influence mRNA and protein expression in the renal cortex.

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P072

FACTOR H-ASSOCIATED HEMOLYTIC UREMIC SYNDROME. A CASE REPORT.

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Objectives of Study: Hemolytic uremic syndrome (HUS) in children is mostly caused by Shiga toxin-producing E. coli. The non-diarrhea-associated, atypical HUS is much less frequent, but tends to relapse and has a poor renal prognosis. Recent studies have identified a factor H-associated form of atypical HUS, caused by gene mutations that cluster in the C-terminal region of factor H, an essential regulator of the alternative pathway of complement. These mutations are mostly heterozygous. Reduced expression or activity of factor H favors complement activation on vascular surfaces after an endothelial injury leading to thrombotic microangiopathy. Here we describe laboratory data and clinical course of a child with a sporadic form of factor H-associated HUS.
Case Report: The 13 years old boy developed HUS for the first time. He never suffered from a serious illness before. 5 days before admission he developed inappetence and vomiting, but no diarrhea, and one day before fever and back pain. At admission the general condition was reduced, the blood pressure in the normal range. He showed the typical trias of HUS with hemolytic anemia, thrombocytopenia and acute renal failure. Shiga toxin-associated HUS, lack of von Willebrand factor cleaving protease and lupus erythematodes were excluded by specific investigations. Complement factor C_3 (0.9 g/l) was within the normal range (0.9 - 1.8 g/l). Renal biopsy on day 8 revealed focal segmental glomerulosclerosis and focal tubulointerstitial injury which could be signs of glomerular thrombotic microangiopathy in repair. The clinical course was uncomplicated. The patient received neither hemodialysis nor red cell transfusions or other specific therapies. Hemoglobin, platelets and creatinine were normalized after 2 weeks. Follow up examinations up to 12 months showed no relapse or residual symptoms. Investigations after admission showed an increased serum concentration of factor H (644 µg/ml, normal range 284 – 528 µg/ml). Factor H Western blot, however, displayed an unusual mobility pointing to a mutated functionally defective protein. The genetic analysis confirmed a heterozygous single point mutation (R1210C) in the hot spot (SCR20) of factor H gene. In this case the mutated protein is normal secreted, the functional defect, however, results in reduced function. Under normal circumstances reduced protein activity is sufficient to maintain tissue integrity. Upon insults, like stress and infections, reduced functional activity of factor H causes insufficient protection of endothelial cells leading to HUS.
Conclusion: This case report shows that reduced serum levels of complement factor C_3 and factor H are not regularly associated with factor H gene mutations. Only detailed functional and genetic analysis can show an involvement of this regulator in the pathogenesis of HUS.

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P073

THE EXPERIENCE OF DIAGNOSIS AND TREATMENT ABOUT CHILDREN WITH HEMOLYTIC UREMIC SYNDROME

LI Qiu , YANG Xiqiang

Abstract: Objective: To investigate the diagnosis and treatment experience of children with hemolytic uremic syndrome. Methods: The clinical manifestation, diagnosis and treatment of 9 cases children with hemolytic uremic syndrome were summarized and analysized. Results: All cases had the typical clinical manifestations, such as hemolytic anemia, thrombocytopenia, acute renal failure. Several cases with of hemolytic uremic syndrome followed upper respiratory tract infections or diarrhea. The diagnosis of this syndrome was based on synthetical analysis of history, clinical manifestations and laboratory tests. It was very importance in therapy to recover normal renal function, redress anemia and anticoagulants. Corticosteroid was not selected at first. Conclusion: The prognosis of this syndrome is dependent on correct diagnosis and treatment.

Key words: hemolytic uremic syndrome, diagnosis, treatment.

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P074

COMPLEMENT FACTOR H AND HUS: COMPOUND HETEROZYGOUS INHERITANCE AND DECREASED FH-ENDOTHELIAL BINDING

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Objectives of Study:
1) Mutation analysis of complement factor H (FH) gene in a previously described family with low FH and HUS (see reference).
2) Investigation of the binding properties of mutated FH.

Methods: DNA was extracted from whole blood by probe method. PCR products obtained using specific primers followed by bi-directional dideoxyfingerprinting. PCR products were cloned and sequenced by automated fluorescent method. Western blot of FH in family members using monoclonal anti-FH antibody. FH-binding ELISA using human umbilical vein endothelial cells incubated with plasma from family members, followed by monoclonal anti-FH antibody.
Results: Linkage analysis had previously shown that the index cases and an affected sibling shared the same haplotype. Mutation analysis in the index case revealed a base pair change T2768G in exon 15 predicted to substitute aspartate for tyrosine (Y899D). This was inherited from his unaffected mother. Additionally, a mutation in exon 20 (G3654A), replacing a polar with a non-polar residue in CCP 20 (G1194D), was inherited from his unaffected father. FH plasma concentration in the index cases was consistently low, while parental concentrations were variable. Western blotting identified an orthotopic single band of FH in the index case. Adjusted for plasma concentration, FH in the index case showed significantly reduced binding to HUVEC compared with normal human FH.
Conclusion: The low concentration of FH in the index cases is not fully explained. However, reduced binding to endothelium is probably accounted for by the mutation in CCP20. Reduced endothelial binding of mutated FH may have a role in pathogenesis.

Reference. Warwicker P *et al* Nephrol Dial Transplant 1999 14; 1229-33

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P480

LIPOPOLYSACCHARIDE FROM ENTEROHEMORRHAGIC ESCHERICHIA COLI BINDS TO AND ACTIVATES PLATELETS

A-L Ståhl, C Svanborg, M Svensson, P Tarr, R Johnson, A-C Sjögren, D Karpman

(This abstract concerns Haemolytic Uraemic Syndrome and is printed on page C212)

P075

ARTERIAL BLOOD PRESSURE AND RENAL FUNCTION IN RECURRENT HEMOLYTIC UREMIC SYNDROME (recHUS)

F. Prüfer, S. Sautter, R. Würzner, A.Bauer, P. Zipfel, M. Kirschfink, A. Gerber, W. Rascher, R. Beetz, M. Bulla, G. Reusz, G. Offener, H. Fehrenbach, T. Müller, J. Janda, B. Klare, M. Kemper, T. Neuhaus, B. Tönshoff, J. Misselwitz, W. Radauer, L.B. Zimmerhackl in collaboration with the European recHUS registry and APN

Background: HUS is he main cause of acute renal failure in children, with an incidence of 0,7/100000 children < 15 years in Germany and Austria. Most patients recover completely. 3-5% develop a recurrent disease with a high risk to develop terminal renal insufficiency. RecHUS, defined as having at least 14 days of complete remission before recurrence, is a heterogeneous group of disorders. The pathogenesis of the disease is not completely understood. Complement activation and mutations of complement inhibitors (factor H, MCP) are of major importance.
Methods: Patients with recHUS were evaluated in a multicenter, retro- and prospective study in Austria, Germany, Switzerland, Hungary and Czechia from 2001 to 2004. None had evidence of diarrhoea caused by shigatoxin-producing E.coli. Clinical data of the first episode of HUS, relapse, outcome after 12 month from diagnosis and transplantation were acquired by standardized questionnaire. Analysis of alternative complement pathway (ACP) was done by measurements of C3, CH50 and Factor H, of classical pathway (CCP) by C4. In addition vWF protease was determined.
Results: Data from 39 patients (20m, 19f) could be analyzed. The age at onset of the disease was 4,2 years (median) with an earlier begin in boys (3,7 versus 4,7y). In 36,8% diarrhoea was reported, bloody diarrhoea in 5,4% (significanly different to our EHEC HUS group. CCP was unaffected in all patients. ACP was activated in 44,8%. lternative complemC4 concentration was within normal limits, measurements of C3 showed lower level in a subgroup of patients (44,8%). Factor H analysis were performed in 26 patients, In 7,7% the concentration was lower than normal 19% demonstrated pathologic protein mobility. The first relapse occurred after 5,4 month (mediatn, the age at the first relapse was 5,5 y. The time between the relapses diminished. One year from diagnosis 46,4% showed elevated blood pressure. Mild 25%, moderate 10,7% and severe 10,7%). 50% had moderate to heavy proteinuria. Dialysis was necessary in 30%. GFR was 90ml/min*1,72qm (12,2-107,5 Innerquartile range). Below 80 were 13/25 (52%). In 9 children (27,2%) 18 transplants with recurrence in 13 TX (72% HUS relapse).
Conclusion: The pathogenesis of recHUS is heterogenous. In a subgroup of the patients activation of the ACP can be detected. Long term outcome is poor. Combined efforts are needed to improve the clinical situation

P076

EFFECTIVE ULTRASONOGRAPHIC PREDICTOR FOR THE DIAGNOSIS OF ACUTE LOBAR NEPHRONIA

C.-H. Cheng, Y.-K. Tsau

Objectives of Study: Correct identification of acute lobar nephronia (ALN) is necessary to prevent progression to renal abscess. The goal of this retrospective study was to determine whether the sonographic finding of severe nephromegaly (i.e. renal length greater than mean +3SD) is a preselection criterion for computed tomographic (CT) scanning in diagnosing pediatric ALN among children with an acute upper urinary tract infection (UTI).
Methods: We evaluated a new imaging work-up scheme to detect pediatric ALN. All patients with UTI were evaluated using ultrasonography (US). If a markedly enlarged kidney or focal mass was present sonographically, CT scanning was done immediately. CT scanning was also performed when the patient had borderline nephromegaly and remained febrile for 72 hours after start of antibiotic treatment. ALN diagnosis was made according to positive CT findings.
Results: Thirty patients with ALN (13 left, 7 right, 10 bilateral) and one with acute pyelonephritis were identified. ALN in all patients resolved after 3 weeks of antibiotic treatment. Thirty-nine of the 62 kidneys evaluated showed severe nephromegaly and 10 had focal renal masses. Taking CT diagnosis of ALN as the reference standard, the sensitivity of severe nephromegaly was 90.0% and the specificity was 86.4% (see Table). When the focal renal mass was added as a combining predictor, the sensitivity further increased to 95%.
Conclusion: Pediatric ALN was effectively predicted using sonographic findings of severe nephromegaly and/or focal mass prior to CT scanning

Table Prediction of computed tomography (CT)-diagnosed acute lobar nephronia (ALN) using specific ultrasonographic findings of nephromegaly[a] in 62 kidneys

Results of Ultrasonography	Results of CT		Total	
	ALN (+)	ALN (–)		
Nephromegaly (+)	36	3	39	PPV 92.3%
Nephromegaly (–)	4	19	23	NPV 82.6%
Total	40	22	62	
	Sensitivity 90%	Specificity 86.4%		

a: Nephromegaly was defined as renal length of greater than the mean + 3 SD for age
+: presence –: absence
PPV: positive predictive value, NPV: negative predictive value

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P077

ULTRASONOGRAPHIC ASSESSMENT OF KIDNEY SIZE IN THE FIRST 6 MONTHS OF LIFE

K Drnasin, M Saraga, V Capkun

Objectives of Study: The aim of this study was to assess the normal values of kidney lengths in healthy infants in the first 6 months of life with respect of sex, and side.
Methods: The ultrasonographic screening has been performed in 935 healthy infants (1870 kidneys). Out of that number 476 males, and 459 females aged between 0,5-6 months entered the study. The observed group was consisted of healthy unselected termed infants. All 1870 kidneys have been analyzed by Sonoline Prima Siemens ultrasound machine with semi convex probes of 5,0 MHz and 7,5 MHz. Kidneys were divided in groups according to sex, age, and side. Kidney lengths were measured three times in both, supine and prone position. The longest measure was recorded. All recorded data were classified in six age groups. Average, minimal and maximal values (5. and 95. centilla) and standard deviation were determined by statistic program SPSS 9.0. Variables were compared by t-test with threshold of significance of $p<0,05$.
Results: We found statistically significant connection between kidney lengths and age in boys and girls and in left and right kidneys ($p<0,05$). In the first 3 months of age we found the statistically significant difference of kidney lengths between boys and girls. Boys had larger kidneys than girls ($p<0,05$). In next three months the difference between kidney lengths were not statistically significant ($p>0,05$). A significant difference was found between left and right kidneys in male and female infants. Left kidneys were longer than right kidneys ($p<0,01$). In 63,3% examined children left kidney was longer than right kidney, in 21,5% kidneys were equal, while in 15,2% kidneys right kidney was longer than left kidney.
Conclusion: This study, based on respectable number of measurements resulted with four different nomograms on kidney size, which does not confirm the results of some previous studies.

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P078

PROPYLTHIOURACIL-INDUCED ANCA POSITIVE CRESCENTIC GLOMERULONEPHRITIS: A CASE REPORT AND LITERATURE REVIEW

S Chim[1], TL Lee[1], KW Chan[2]

Objectives of Study: We report a case of an 11-year-old Chinese girl with antineutrophil cytoplasmic antibody (ANCA) positive crescentic glomerulonephritis after treatment with propylthiouracil (PTU) for Graves' disease. As it is a rare complication of propylthiouracil treatment, a literature review was conducted to look for the clinical outcome in children to guide our treatment plan.
Methods: An 11-year-old Chinese girl with Graves' disease on propylthiouracil and thyroxine presented in December 2003 with gross haematuria and pallor, preceded by right knee arthritis two months before. On admission, she was pale with peri-orbital and ankle oedema, right knee swelling but no cutaneous vasculitis. Haemoglobin was 6.8g/dl and serum creatinine rose to a maximum of 124umol/l from a baseline of 44umol/l. Proteinuria was 1.3g/day and serum albumin was 32g/l. ESR and C-reactive protein were raised. ANA and anti-ds DNA were negative and p-ANCA was positive with MPO-ANCA titre >1600RU/ml. Renal biopsy showed pauci-immune crescentic glomerulonephritis with cellular and fibrocellular crescents in 26 out of 33 glomeruli, compatible with PTU-induced ANCA positive crescentic glomerulonephritis. A few case reports and a retrospective review were found from literature review.
Results: Eleven children from four publications were reviewed. All had positive p-ANCA, high MPO titre and crescentic glomerulonephritis on renal biopsy. Seven of them had vasculitis involving other organs, and one died of complications of severe systemic vasculitis. All children were treated with oral steroid and some of them with additional pulse steroid, plasma exchange or oral cyclophosphamide. Nine out of the ten children recovered and some of them had decreased MPO-ANCA titre. One patient resulted in end stage renal disease requiring haemodialysis. No relapse of the glomerulonephritis was reported. Outcome of children with idiopathic ANCA-associated glomerulonephritis was shown to be worse from another review. 84% of patients achieved remission and 39% of responders relapsed. 29% of the overall progressed to end stage renal disease.
PTU was stopped in our patient. She was treated with IV pulse methylprednisolone, followed by oral prednisolone and IV monthly pulse cyclophosphamide. Her serum creatinine dropped to 65umol/l and haemoglobin rose to 10.6g/dl. MPO-ANCA titre decreased gradually during treatment. Proteinuria still persists at recent follow up, two months after initiation of treatment.
Conclusions:
1. Propylthiouracil-induced ANCA positive crescentic glomerulonephritis in children is rare. Patient can progress to renal failure although not common. Early detection and administration of immunosuppressive therapy would be beneficial.
2. MPO-ANCA titre may be used as an indicator for treatment response. Prolonged immunosuppressive maintenance may not be necessary as the risk of relapse is low.

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P079

EFFECT OF GLYCINE SUBSTITUTIONS ON ALPHA5(Ⅳ) CHAIN AND STRUCTURE-PHENOTYPE CORRELATIONS IN ALPORT SYNDROME

J Ding, YF Wang, F Wang, DF Bu

Objectives of Study: Alport syndrome (AS) is a progressive hereditary nephritis presented with hematuria, sensorineural deafness, ocular lesions and progressive renal failure. The most majority cases of AS are caused by mutations in the COL4A5 gene on the X chromosome which encodes α5(Ⅳ) chain. A greater incidence of missense mutations in the COL4A5 gene which caused glycine substitutions have been detected in the X-linked AS (XLAS). The phenotype variety caused by glycine substitutions prompted the complexity of structure changes of α5(Ⅳ) chain. However, the structure and function changes of the consequent α5(Ⅳ) chain with missense mutations are little to know. This study aimed to detect the protein structure encoded by COL4A5 gene with missense mutations which caused the glycine substitution and to analyze the effect of gene mutations on the secondary structure of α5(Ⅳ) chain and structure-phenotype correlations.
Methods: Two XLAS patients with different missense mutations (g.3246 G > T resulting in p.G1015V and g.3290 G > A resulting in p.G1030S, respectively) and with different phenotype severity, juvenile-onset and adult-onset XLAS, respectively, as well as a control were included in this study. The fragments of cDNA of COL4A5 gene containing the two mutations, respectively, and that of corresponding cDNA from the control were expressed in *Escherichia coli*. The secondary structures of the recombinant proteins were analyzed with circular dichroism (CD) spectroscopy.
Results: The spectrum of the recombinant protein with the normal amino acid sequence showed a negative peak near 200nm and without peaks of α-helix characteristic, which suggested very little α-helix component in the structure of this protein. However, the spectrum of the recombinant protein containing G1015V mutation, which was identified in patient 1 with juvenile-onset XLAS, exhibited a clear shift in negative peak from 200nm to 220nm, suggesting a relatively high amount of α-helix component in the protein structure. The spectrum also showed a decrease in magnitude of the negative peak from -9000deg×cm^2×dmol^{-1} to -4000deg×cm^2×dmol^{-1} as compared to the control. Estimation of the secondary structures based on the spectra exhibited that the recombinant protein containing G1015V mutation presented 12.9% α-helix whereas the control did not. The spectrum of the recombinant protein containing G1030S mutation, which was identified in patient 2 presented with adult-onset XLAS, was slightly different from that in the control in that the curve was broader around 200nm, and the magnitude of negative peak increased from -9000deg×cm^2×dmol^{-1} to -11000deg×cm^2×dmol^{-1}. Estimation of the secondary structures exhibited the increase of the random coil fraction and the decrease of β-sheet in the recombinant protein containing G1030S mutation when compared with the control.
Conclusion: Two kinds of glycine substitutions caused by different missense mutations of COL4A5 gene, even though in the same domain of α5(Ⅳ) chain, displayed the distinct different secondary structures of α5(Ⅳ) chain. The changes of the secondary structure of α5(Ⅳ) chain could explain the phenotypic diversities of AS, which would be hardly understood solely by analyzing genomic DNA or mRNA of α5(Ⅳ) chain.

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P080

CLINICOPATHOLOGIC FEATURES AND OUTCOME OF FILIPINO CHILDREN WITH RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS

RH Francisco, JS Elises, ZL Antonio, SB Gonzalez, MR Cruz

Objectives of Study: To describe the clinicopathologic profile of children who presented with rapidly progressive glomerulonephritis at the Philippine Children's Medical Center from January 1990 to December 2001 and determine their outcome, survival patterns and possible risk factors.
Methods: Medical records of pediatric patients diagnosed to have rapidly progressive glomerulonephritis from 1991-2001 were reviewed. Data were collected on initial consult and at the time of last follow-up or time of death. All patients underwent renal biopsy and specimens were studied by both light microscopy and immunofluorescence.
Results: Twenty-eight patients, 13 males and 15 females (M:F=1:1.15), age range= 2.8-15.2 years (mean, 8.57±3.09 years) were included in the study. Initial clinical features were edema, hypertension, gross hematuria, oliguria, pallor, and hypertensive encephalopathy, microscopic hematuria and proteinuria but were not correlated with the outcome. Anemia, low GFR (mean, 10.22±4.90 ml/min/1.72 m^2), elevated BUN and serum creatinine (mean, 51.05±38.72 mmol/L and 8.68±5.77 mg/dl, respectively) were correlated with a poor outcome. On light microscopy, glomerulosclerosis was most pronounced among those in the D group (78.71%) and this was the only histopathologic finding that was significantly correlated with the outcome (P=0.0002). Initially, 60.7% underwent acute peritoneal dialysis (PD), 42.8% required chronic PD, 89.3% received intravenous pulse methylprednisolone, all were started on oral prednisone and 2 were given oral cyclophosaphamide.
On follow-up, 18 (64.3%) recovered and of these, 12 (42.9%) had normal renal function and 6 (21.4%) had impaired renal function. Ten (35.7%) died with a mean GFR of 11.58±7.54 ml/min/1.72 m^2. and a mean duration of survival
Conclusions: Sixteen (64%) children who presented with RPGN had CrGN on biopsy. On follow-up, 18 (64.3%) survived, 12 (42.8%) recovered while 6 (21.4%) had persistent impairment of renal function. Factors associated with a poor outcome were: a. severe anemia (hemoglobin level < 9 g/L), b. azotemia, c. markedly low GFR (<10 ml/min/1.73 m^2), d. the need to undergo dialysis at onset, and d. extensive glomerulosclerosis. We have found no evidence that the other clinical features, extent of crescent formation, therapeutic regimen and type of underlying disease conditions are associated with the outcome.

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P081

EPITOPE ANALYSIS OF MPO-ANCA IN GRAVES' DISEASE TREATED WITH PROPYLTHIOURACIL

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Objective of Study: This study aimed to elucidate the relationship between epitope profiles of MPO-ANCA and clinical manifestations in childhood onset Graves' disease treated with propylthiouracil (PTU).
Methods: Epitope analysis of sera from 16 patients with MPO-ANCA-positive childhood onset Graves' disease treated with PTU was examined by enzyme-linked immunosorbent assay (ELISA) using a panel set recombinant deletion mutants of MPO. Patients were grouped into 10 without clinical vasculitis and nephritis (non-vasculitis group) anf 6 with biopsy-proven pauci-immune necrotizing crescentic glomerulonephritis (vasculitis group).
Results: The high frequency sites were regions on the upstream of Met341 (Ha region) near the N-terminus of the heavy chain, and regions on the downstream of Gly598 (Hf and Hg regions) near the C-terminus. Most patients in non-vasculitis group had polyclonal MPO-ANCA recognizing both above linear site and other epitope sites of the heavy chain of MPO. There were only one of 10 patients in non-vasculiits group, and four of 6 patients in vasculitis group who had MPO-ANCA recognizing only linear site of the heavy chain of the MPO molecule (Ha, Hf and/or Hg). Of the 4 patients in vasculitis group, two had nephritis like rapidly progressive glomerulonephritis and one had alveolar hemorrhage. **Conclusion:** These findings suggest that most patients with childhood onset Graves' disease treated PTU who manifest no vasculitis and nephritis have polyclonal MPO-ANCA recognizing both the linear and other epitope sites of the heavy chain of MPO. However, some patients who develop nephritis have MPO-ANCA recognizing only linear site of the heavy chain of MPO. This clonality of MPO-ANCA may be risk factor that induces clinical vasculitis and nephritis in the patients treated with PTU. Therefore, patients exposed to PTU should be monitered for MPO-ANCA level and eitope.

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P082

GLOMERULONEPHRITIS CAUSED BY CENTRAL VENOUS CATHETER CONTINUOUS INFECTION WITH STAPHYLOCOCCUS EPIDERMIDIS

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Objectives of Study: We present a case of glomerulonephritis probably caused by central venous (CV) catheter continuous infection with Staphylococcus (S.) epidermidis.
Case Report: A 12 years old boy was referred to our department because of macroscopic hematuria and proteinuria. He had been diagnosed as MMIHS (megacystis-megaureter-intestinal hypoperistalsis syndrome) by the surgeon in our institute. He had been treated with intravenous hyperalimentation via CV catheter from infancy. Physical examination was not remarkable. Laboratory test showed anemia (Hb 8.7g/dl), hypoalbuminemia (3.1g/dl), mild renal dysfunction (BUN 27.4mg/dl, creatinine 0.68mg/dl), elevated CRP (5.6mg/dl), high PR3 ANCA (33EU/l), macroscopic hematuria, and proteinuria (0.68g/day). Renal biopsy showed mesangial proliferation with crescent formation. Immunofluorescence showed IgM, C1q, C3, and fibrinogen deposits in the glomerular mesangium. We could not find granuloma in his respiratory system. Therefore, he was not diagnosed having Wegener's granulomatosis, although he had high PR3 ANCA. Clinical findings were not consistent with SLE or other collagen diseases. We started immunosuppressive therapy with prednisolone and mizoribin. Although the treatment decreased proteinuria and CRP level immediately, severe hypocomplementemia appeared. S.epidermidis was detected in his blood culture. As glomerulonephritis sometimes occurs in patients with hydrocephalus placed with ventriculo-atrial shunt (shunt nephritis), we suspected that his CV catheter was contaminated and caused glomerulonephritis by the same mechanism as the shunt nephritis (unknown but immunecomplex may be related). We stopped the immunosuppressive therapy and replaced his CV catheter.
Results: soon after the replacement of cv catheter, his macroscopic hematuria was disappeared, and his blood culture became negative.. His hypoalbuminemia, renal dysfunction and hypocomplementemia also improved, and levels of crp and pr3 anca were normalized**.**
Conclusion: This is, as far as we know, the first report of glomerulonephritis caused by CV catheter continuous infection.

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P083

CLINICOPATHOLOGICAL EVALUATION OF 16 CHILDREN WITH C1Q NEPHROPATHY

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Objectives of Study: The clinicopathological correlation of C1q nephropathy (C1qN), which was proposed by Jenette, has not yet been clarified.
Aim: The aim of our study is to clarify the clinicopathological correlation in 16 children with C1qN.
Methods: Sixteen children, aged 3-18 years (mean: 9.2 years), who met the definition of C1qN proposed by Jenette, were enrolled in this study. The clinical manifestations and renal histological findings including edema, blood pressure, urinalysis, concentration of serum protein and creatinine were evaluated. Four of these 16 children received twice renal biopsy.
Results: 1) The mode of onset was nephrotic syndrome (NS) (frequent relapser) in 9 children, asymptomatic hematuria and/or proteinuira in 5 and asymptomatic hematuria in 2. 2) Renal pathological findings were minor glomerular abnormalities (MGA) in 10 children, mesangial proliferative glomerulonephritis (MesPGN) in 5 and focal glomerulosclerosis (FGS) in 1. 3) In 2 of 4 children with MGA at the first biopsy, C1q disappeared at the second biopsy. 4) One of these 4 children showed FGS at the second biopsy, and the remaining 3 had still MGA at the second biopsy. 5) During the mean floow-up period of 9.8 years, chronic renal failure was evident in 2 children (one with FGS and one with MesPGN), frequent relapsing NS in 6 with MGA, persistent urinary abnormalities in 6 (2 with MGA and 4 with MesPGN) and normal urinarysis in 2 with MGA.
Conclusion: A large number of cases with C1qN show frequent relapsing NS as reported by Jenette, and some cases develop renal failure. Our study provides a novel information that there are some cases showing the disappearance of C1q deposits through the follow-up.

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P084

HYPOCOMPLEMTAEMIA AND SEPTICAEMIA

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Objective of Study: Association of chronic hypocomplemtaemia with bacterial septicaemia.
Methods: Review of 9 patients with chronic hypocomplemtaemia and membranoproliferative glomerulonephritis over 48 patient years. A small survey of paediatric nephrologists.
Results: Two of nine children with chronic hypocomplemtaemia developed meningococcal septicaemia. This is a greatly increased risk over healthy population with presumed normal complement levels. Four out of five experienced paediatric nephrologists had seen and experienced cases of septicaemia in children with chronic C3 depletion.
Conclusion: Paediatric nephrologists need to be conscious of the association of septicaemia with hypocomplemtaemia, to consider sepsis in the presence of purpuric rashes, to warn parents of this association, to recommend meningococcal C and pneumococcal vaccines for chronically hypocomplemtaemic children.

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P085

MEMBRANOUS NEPHROPATHY IN IRANIAN CHILDREN

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Abstract:
The correlation between the clinicopathological features and the outcome of membranous nephropathy was reviewed in children. Between 1972 – 1995, 73 out of 2118 kidney biopsies were diagnosed as membranous nephropathy. Male to female ratio was 2:1, aged 1.5 -14 years. 45 out of 73 cases were idiopathic membranous nephropathy. 28 out of 73 were secondary included systemic lupus erythematosus (13 cases) and HBsAg positive (12 patients). The most common features in both groups were nephrotic syndrome and hematuria . In idiopathic group remission occurred in 26% and renal failure occurred in 9.6% during a mean of 2.13 years of follow up. We found no correlation between the sex, age, hypertension, hematuria and pathological staging with the outcome (P>0.05), but there were statistically significant correlation between the degree of proteinuria at the onset with the outcome (P=0.024). In secondary membranous nephropathy, the outcomes were variable depending on the etiology

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P086

CLINICOPATHOLOGIC ANLYSES ON THE MECHANISMS OF DEVELOPMENT & PROGRESSION IN CYANOTIC NEPHROPATHY

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Objectives of Study: Cyanotic nephropathy (CN) is often accompanied by congenital cyanotic heart diseases (CCHD), although its mechanisms of development and progression are still unclear. The purpose of this study was to clarify the mechanisms of development and progression in CN.
Methods: Thirty patients with CCHD (M/F=16/14, mean age: 19.8 years old) were examined. We defined CN as showing significant proetinuria (urinary protein/urinary creatinin > 0.25) or renal dysfunction (CCr < 80 ml/h/1.73m^2), and then, analyzed the risk factors for the development of CN among clinical and laboratory findings. We also examined 10 renal biopsy specimens obtained from patients with CN to elucidate the mechanisms of progression in the disease. Glomerular size was evaluated by a point-counting method. The number of glomerular capillaries per glomeruli was also counted. Kidney tissues obtained from 6 autopsies served as histologic controls.
Results: Patients with CN showed significantly higher hematocrit levels (62.2 vs.54.3 %, P=0.035), and significantly lower mean corpuscular hemoglobin concentration (MCHC) (32.5 vs. 33.7 g/dl, P=0.042). The renal plasma flow (RPF) in patients both with and without CN was higher than control (357.8 vs. 371.8 ml/min, normal range: 385-639). However, the filitration fraction (FF) in patients with CN was significantly lower than those without CN (0.223 vs. 0.355, P=0.003, normal range: 0.20-0.22), indicating the failure of compensation for reduced RPF by hyperfiltration in CN. Biopsy specimens with significant proteinuria (N=7) showed significantly larger glomerular size and significantly more glomerular capillaries per glomeruli compared to those without significant proteinuria (N=3) and control specimens (N=6) (Glomerular size: 76.3 vs. 53.4 vs. 48.5 points, P<0.01 vs. the other 2 groups, glomerular capillary numbers: 104.4 vs. 67.8 vs. 58.8, P< 0.01 vs. the other 2 groups).
Conclusion: Hyperviscosity by polycythemia and tissue hypoxia by hypochromic anemia could be responsible for the development of CN. These pathologic conditions might induce an angiogenic increase of glomerular capillary beds, leading to glomerular hypertrophy. In addition, the failure of compensation mechanism for reduced RPF by hyperfiltration could be accompanied by the development and progression in CN.

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P087

CLINICAL PROFILE AND OUTCOME OF ACUTE NEPHRITIC SYNDROME

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Objectives of Study: To analyze retrospectively clinical profile of acute nephritic syndrome in hospitalized children and to correlate various clinical and laboratory parameters with the outcome.
Methods: Children with acute nephritic features like oliguria, edema, hematuria and hypertension who were hospitalized during last four years were included in the study. History of precedent infection was noted. Duration of symptoms, presence of systemic features like skin or joint involvement and complications like hypertensive encephalopathy (HE), congestive cardiac failure (CCF), acute renal failure (ARF) (serum creatinine >1.5 mg%) were recorded. Urine analysis, renal functions, serum electrolytes, nephrotic parameters, ASLO and C3 complement levels were tested. Renal biopsy was done if there was either non-resolution or progression or if presentation was atypical. Incomplete recovery was defined as presence of either one of features like significant proteinuria, hypertension or abnormal renal functions at the end of three months. The significance of variables like history of precedent infection, presence of systemic features, raised ASLO, low complement levels, presence of, nephrotic range proteinuria, biopsy findings and complications like HE, CCF, ARF; was tested against the above mentioned end points using univariate analysis applying Chi-square test and Fischer's exact test. The p value of <0.05 was considered significant.
Results: 111 children were studied. 71 were males and 40 were females. The mean age of patients was 114.79 months (SD 42.92). 32 patients had a preceding history of infection as pyodema or pharyngitis. 13(11.7%) had associated systemic features. Complications like HE, CCF & ARF were seen in 9.9 , 4.5 & 23.4 % respectively, ASLO was positive in 67 patients,C3 complement levels were low in 66 patients . 34 patients had nephrotic range proteinuria.. At the end of three months, 84 patients had completer recovery whereas in 23 patients it was incompolete. Renal biopsy findings were diffuse proliferative glomerulonephritis (DPGN-12), MPGN-2, crescentric nephritis-4, IgA nephropathy-2, lupus nephritis-2, vasculitis-1, mesangial hypercellularity-2.
Conclusion: Acute nephritis may present with complications in the acute stage. With optimal management however, majority recover completely by three months. In our study, none of the above mentioned clinical variables could predict complete or incomplete recovery except biopsy findings of changes other than DPGN (p value < 0.05). Thus renal biopsy plays a major role in prognostication of acute nephritis. Patients with incomplete recovery need long-term follow-up.

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P088

PROTEINASE 3 AND NEUTROPHIL EXTRACT FROM PATIENTS WITH ANCA-ASSOCIATED VASCULITIS INDUCES KININOGEN PROTEOLYSIS

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Objectives of Study: The contact system is activated in vivo on endothelial cells and neutrophils when high-molecular-weight kininogen (HK) is cleaved by kallikrein thus liberating bradykinin. Bradykinin is a potent mediator of inflammation that binds to receptors on endothelial cells. We have found that the contact system is activated in children with vasculitis, however the events that trigger this process in vivo have not been elucidated. Vasculitis is an inflammation in and around the vessels. Neutrophils are believed to play a crucial part in this disease. Many patients have anti-neutrophil-cytoplasmic-antibodies (ANCA) directed towards proteinase 3 (PR3) or myeloperoxidase (MPO). In this study we investigated contact system activation in pediatric and adult patients with ANCA-associated vasculitis. Furthermore the possibility that neutrophil-derived peptides induce contact system activation in vasculitis patients was studied.
Methods: Blood and neutrophils were obtained from adults and children with vasculitis. (n=10 of which n=6 ANCA positive and n=4 ANCA negative) Neutrophils were stimulated to degranulate by triton-x 100 or fMLP in order to obtain neutrophil extracts. Neutrophil extracts from the ANCA positive and the ANCA negative and adult controls (n=3) were incubated with normal plasma or the patients' own plasma and analyzed for HK breakdown by immunoblotting. Purified PR3 (pPR3) was incubated with purified HK (pHK) and HK breakdown and bradykinin release were analyzed by immunoblotting and ELISA respectively. pPR3 was also preincubated with anti-PR3 before adding to pHK. Levels of PR3 were measured in neutrophils extract by ELISA.
Results: We found that plasma from all patients, three adults and seven children, with vasculitis showed proteolysis of HK by immunoblotting indicating systemic contact system activation. Neutrophil extracts from the ANCA positive patients, but not from the ANCA negative patients or the adult controls, induced breakdown of HK. A neutrophil extract from an ANCA-PR3 positive patient incubated with the patient's plasma led to total breakdown of HK. Incubation of PR3 with purified HK resulted in total breakdown of HK and bradykinin release. Breakdown of HK could be inhibited by preincubation of PR3 with anti-PR3. No differences were measured between patients and controls regarding PR3 levels in neutrophil extracts.
Conclusion: These findings suggest that PR3 and peptides derived from neutrophils induce contact system activation in ANCA positive vasculitis and that the ANCA auto-antibodies that patients have do not seem to block this activity.

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P089

MUTATIONS IN TYPE IV COLLAGEN GENE IN KOREAN ALPORT SYNDROME PATIENTS

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Objectives of Study: Alport syndrome is an important hereditary disorder of progressive nephritis. And primarily linked to the X chromosome, but autosomal forms have been reported. The X-linked form is associated with mutations in the COL4A5 gene that encodes the α5 (IV) collagen chain. The gene is approximately 250kb length and divided into 51 exons by 50 introns, producing 6.5kb mRNA. The entirely translated product has 1,658 amino acid residues, which consist of a signal peptide from the N-terminal residue to the 26th residue, and a 1,430 residual collagenous domain having 22 short non-collagenous domains (interruption region) and a 229 residual C-terminal non-collagenous (NC1) domain. To date there have been many reports of various kinds of mutations, including one point mutation and deletion or insertion of large or small nucleotide sequences. The detection of a mutation of this large COL4A5 gene, of which the full sequence has not yet been established, is laborious, and 90% of the mutations are expected to be subtle ones. This study was performed to investigate the characteristic features of the COL4A5 gene mutations in Korean Alport syndrome patients.
Methods: Alport syndrome patients, 12 patients of 9 unrelated families were screened in this study. Screening for mutations in all exons (1 to 51) of the COL4A5 gene was carried out by SSCP & heteroduplex analysis.
Results: A mobility shift was observed in 5 of 9 families, and their genomic DNA were directly requenced using dideoxy chain method. We found three novel mutations and a reported one with three silent mutations. Two of these had missense mutations, one was a change from isoleucine to serine in a interruption region of exon 20 (Ile444Ser), the other was a change from glycine to glutamic acid in exon 32 (Gly911Glu). One was a frame shift mutation caused by one base (adenine) deletion in exon 29 (2524/delA). This mutation was found in 2 unrelated families. This frame shift mutation changes thereafter the 17th amino acid codon leucine to stop codon, consequently making truncated protein. Three silent mutations in exon 19, 27, 39 are accompanied with the Ile444Ser patient, which is identical with the already reported mutation in Japan
Conclusion: Molecular genetic analysis of Alport syndrome is useful in the correct diagnosis of the disease. Furthermore, genetic counselling of the families and detection of asymtomatic carriers can prevent the disease. This is the first study of the DNA sequencing of COL4A5 gene in Korea, and it may contribute the understanding of molecular pathogenesis of Alport syndrome.

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P090

A CLINICAL AND RADIOLOGIC STUDY OF ACUTE FOCAL BACTERIAL NEPHRITIS IN CHILDREN

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Objectives of study: Acute focal bacterial nephritis (AFBN) is a nonliquefactive renal parenchymal infection causing focal renal swelling. Most cases results from ascending infection, usually by gram-negative bacteria. Since the clinical presentations are similar to those of other upper urinary tract infections, radiologic studies are essential in diagnosis AFBN. To aware the clinical importance and the need of proper management of AFBN, we analyzed 30 AFBN patients and 30 other upper urinary tract infection (UTI) patients by comparative studies.
Methods: The medical records of 592 children under fifteen years of age, diagnosed as UTI were reviewed. Thirty cases of AFBN patients aged from 1 month to 12 months have been selected. As a control group, 30 cases of UTI patients with no radiologic abnormalities have been selected and matched by age and sex.
Results: 1) The incidence of AFBN was 5.6% and more common in boys than girls. 2) Since both groups had similar symptoms, it was difficult to diagnose AFBN by clinical presentations alone. 3) ESR and CRP were significantly higher in AFBN patients. 4) The positive results of urine cultures were seen in 22 cases of AFBN patients, with no significant difference compared to control group. The most common causative organism was *E.Coli* in both groups. 5) On the sonographic findings, the most lesions were seen on the upper lobe of kidney, more frequently, on left kidney. The lesions of 29 cases showed globular or wedge shaped increased echogenecity compared with the adjacent normal renal cortex. 6) 99mTc-DMSA scan could detect earlier lesion of AFBN which was not seen on ultrasonography at initial diagnosis. It showed the complete coincidence of the location, size and shape in all cases compared to the findings of renal sonography. 6) 14 of 18 cases who had radiologic follow up showed improvement by antibiotics therapy alone.
Conclusion: The roles of renal sonography and DMSA scan were very important, and ultrasonography was excellent as an initial diagnostic tool for AFBN. Since the degree of infection in AFBN is more severe than other urinary tract infections and evolution into a renal abscess is possible, the early diagnosis and appropriate antibiotics therapy is essential.

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P091

THE CONTRIBUTIONS OF IL-12/IL-23 AND IL-18 TO THE PATHOGENESIS OF EXPERIMENTAL CRESCENTIC GLOMERULONEPHRITIS

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Objectives of Study: Experimental crescentic glomerulonephritis (GN) results from a delayed type hypersensitivity (DTH)-like, Th1 response to self or foreign antigens (Ag). We have shown that interleukin (IL)-12p40 (the common cytokine chain for both IL-12 and IL-23) mediates experimental crescentic GN. These studies sought to determine the relative roles of endogenous IL-12/IL-23 and IL-18 in this lesion.
Methods: Experimental crescentic GN was induced in normal C57BL/6 mice (WT), IL-12p40-/-, IL-18-/- and mice deficient in both IL-12p40-/- and IL-18-/- (IL-12p40/IL-18-/- mice). This lesion was induced by planting heterologous globulin planted on the glomerular basement membrane (GBM) as a foreign nephritogenic antigen in sensitized mice (known as "accelerated autologous anti- GBM GN").
Results: IL-12p40-/- mice developed reduced crescentic glomerular injury (WT 39 ± 3, IL-12p40-/- 13 ± 4% glomeruli affected), while crescent formation in IL-18-/- mice (29 ± 4%) was not significantly reduced compared with WT mice. No additional protection from crescent formation was afforded by deficiency of both IL-12p40 and IL-18. DTH reactants (CD4+ cells and macrophages) in glomeruli were reduced in both IL-12p40-/- and IL-18-/- mice, with the reduction being greater in IL-12p40-/- mice.
Systemic immune responses to the nephritogenic Ag showed reduced dermal DTH, most apparent in IL-12p40-/- mice. Splenocyte IFN-γ and GM-CSF production was reduced in IL-12p40-/- and IL-12p40/IL-18-/- mice and serum IgG2a levels were progressively reduced from WT, IL-12-/-, IL-18-/- to IL-12p40/IL-18-/- mice.
Compared with WT mice, IL-18-/- and IL-12p40/IL-18-/- mice showed trends to decreased glomerular immunoglobulin deposition, while C3 in glomeruli was reduced in IL-12p40/IL-18-/- mice.
Analysis of mRNA from nephritic kidneys showed reduced TNF and IL-1β in all genetically deficient groups, compared with diseased WT mice. The anti-inflammatory cytokine IL-10 was undetectable in WT and IL-18-/- mice, but upregulated in both IL-12p40-/- and IL-12p40/IL-18-/- mice. IL-18 had more effect on intrarenal chemokines than IL-12/IL-23, with IL-18-/- and IL-12p40/IL-18-/- mice showing reduced MIP-1α, MIP-1β and MIP-2. Surrogate markers of early renal fibrosis were assessed. All groups of deficient mice had decreased mRNA for TGF-β1 (a powerful profibrotic agent) and α1(1)procollagen, suggesting that both IL-12/IL-23 and IL-18 may contribute to progressive injury in this lesion.
Conclusions: IL-12p40 is the key cytokine chain determining Th1 nephritogenic cell-mediated responses and crescentic glomerulonephritis. Additional deletion of IL-18 has no further immediate benefit. While IL-18 is not a key cytokine in crescent formation, it may have pathogenetic effects in forms of GN where complement fixing IgG subclasses induce injury. In addition, IL-18 may induce renal chemokine expression and contribute to progressive renal injury.

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P092

ANALYSIS OF CLINICAL CHARACTERISTICS IN THE INFANTILE NEPHRITIC SYNDROME

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Objective of study: To explore the clinical characteristic of infantile nephrotic syndrome and relationship among pathology type and responsive for hormone.
Methods: 32 cases with infantile nephrotic syndrome clinical were noted. 14 cases renal needle biopsy. All patients were treated by middle-long term protocol. 19 cases combined with cyclophosphamide.
Results: Clinical feature of infantile nephrotic syndrome is mainly nephritis nephrotic syndrome and non-minimal change disease in most cases. About 60% cases is not responsive for steroid hormone and needed to combine with immuno-suppressive drugs, for instance cyclophosphamide.
Conclusion: Infantile nephritic syndrome has different with other children's nephritic syndrome. It should be paid attention in clinical practice.

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P093

FUNCTIONAL INACTIVATION OF COMPLEMENT FACTOR H RESULTS IN MPGN II

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Objective: Deficiency of complement regulator factor H is associated with membrano-proliferative glomerulonephritis type II (MPGN II), and the disease usually occurs early in life. The lack of plasma factor H is due to a block in protein secretion. Responsible for the intracellular accumulation of factor H are framework mutations in complement control protein (CCP)-domains 9 and 16. A similar disease can be induced by factor H-autoantibodies. – Here we report a new cause for MPGN II.
Case Report: The daughter of consanguineous healthy Turkish parents developed microhematuria and mild proteinuria starting at 5 years of age. Renal function was unaffected. Renal biopsy revealed typical findings of membrano-proliferative glomerulonephritis type II with intra-basement membrane deposits ("dense deposit disease"). A younger sister developed similar symptoms as newborn (no renal biopsy). Laboratory investigations in the siblings showed increased C3d (130 and 117 mU/l) and APH50 levels (34 and 23%), respectively, which are both indicative for unrestricted activation of the alternative pathway of the complement system. Factor H plasma levels were low but still within normal range (420 and 399 □g/ml). SDS page and Western blotting analysis of factor H showed normal mobility and no additional bands. However, genetic analysis revealed the same homozygous mutation of factor H gene with deletion of nucleotides 743-745 resulting in the elimination of amino acid Lys 224 located within CCP-domain 4. This domain is involved in the regulation of protein function, and it's modification affects the complement regulatory activity of factor H. Both parents are heterozygous for this mutation. Based on the postulated half life of factor H, substitution of factor H by means of regular transfusions of fresh frozen plasma every 2nd week was initiated. After an observation period of about 3 months the siblings are in good condition, however, microhematuria and proteinuria are still present. Side effects of the treatment were not observed.
Functional Analysis of Factor H: Heparin binding of factor H, which is involved in the development of different factor H associated diseases, was analyzed. Using heparin affinity chromatography, factor H derived from the siblings and the parents showed identical elution profiles indicating normal heparin binding activity of the mutated protein.
For analysis of cofactor activity of factor H, C3b binding characteristics of factor H were analyzed. Factor H derived from the siblings showed weak (10% of wild type protein), factor H derived from the parents showed stronger binding to C3b. Since CCP-domain 4 harbors one of the three known C3b binding sites of factor H and the deleted amino acid Lys 224 is also located within this domain, this result indicates a correlation between the identified mutation and the impaired cofactor activity of factor H.
Conclusion: Although mobility and concentration of factor H appears to be normal, functional inactivation of factor H obviously can cause MPGN II. Thus, we here describe a new pathomechanism for the development of MPGN II. Based on this finding patients with MPGN II should be screened for factor H gene mutations. Substitution of the deficient or inactive factor H by means of fresh frozen plasma transfusion might be a therapeutic option for these patients potentially preventing or at least delaying onset of end stage renal disease and start of dialysis treatment.

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P094

CLINICAL SIGNIFICANCE OF THINNING OF GLOMERULAR BASEMENT MEMBRANE IN GLOMERULAR DISEASES

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Objective of Study: To investigate the results of diagnostics in glomerular diseases obtained from renal biopsy specimens showing diffused thinning of glomerular basement membrane (GBM)(thickness of GBM<250nm) under electron microscope.
Methods: 32 renal biopsy specimens showing only minimal pathological changes under light microscope were investigated using electron microscope. All specimens observed showed diffused thinning of GBM (thickness of GBM<250nm and range of thinning>50%). In skin biopsies of 7 cases which had a positive family history (maternal) of microscopic hematuria, indirect immunofluoresence technique was employed to investigate the expression of ⟨1(IV) andα 5(IV) chains in epidermal basement membrane(EBM).
Results: (1) Two of the 32 cases investigated showed negative skin biopsy result confirming the diagnosis of Alport Syndrome (AS). (2)8 specimen were subjected to immunohistochemical studies and examined by electron microscope, of which 5 were discovered to be an IgA nephropathy with diffuse thinning of GBM. And the other three cases with positive family histories were diagnosed as thin basement membrane nephropathy (TBMN) complicated by IgA nephropathy. (3) Electron-dense bodies deposits were observed in the mesangium in 9 cases investigated. Given the clinical manifestations of these patients, thin basement membrane nephropathy complicated by glomerulonephritis was considered as a diagnosis. (4) In 3 renal biopsies, diffuse segmental thinning of GBM (range of thinning>50%) and extensive fusion of foot processes were observed by electron microscope. And the clinical manifestations and the findings by light microscopy also revealed focal segmental glomerulosclerosis (FSGS). (5) Segmental and splitting bodies in GBM was observed in the 2 cases of the glomerular basement membrane thinning whereas this was not observed in the 30 cases.
Conclusion: (1) When using electron microscope to diagnose glomerular diseases, first diagnose of thin basement membrane nephropathy should be considered in the cases of family historical thinning GBM. (2) Caution should be exercised to rule out Alport Syndrome from TBMN and also to consider the possibility of IgA nephropathy, glomerulonephritis or FSGS complicating thinning of GBM. (3) Segmental and splitting bodies along the glomerular basement membrane should also lead to a suspicion of AS after test of IV collagen.

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MEMBRANOUS GLOMERULONEPHRITIS IN JAPANESE CHILDREN

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Objectives of Study: The aim of our study was to clarify frequency, clinicopathological findings, effect of drug therapy and prognosis of patients with idiopathic membranous glomerulonephritis (MGN) in Japanese children.
Methods: All consecutive percutaneous renal biopsy specimens in our hospitals from 1979 to 2003 were evaluated. We diagnosed MGN by light microscopy (LM), immunofluorescence study (IF), and electron microscopy (EM) findings. We retrospectively investigated patients with idiopathic MGN.
Results: A total of 1,604 patients had their first renal biopsy in our hospitals during the study. We had 28 (1.7%) children (18 boys and 10 girls) with idiopathic MGN. The mean age at onset was 7.6 years old (range from 2.8 to 16.0 years old). IF findings showed diffuse granular deposits along the capillary walls of IgG (96%), IgA (32%), IgM (42%), C1q (42%), C4 (28%), C3 (67%) and Fibrinogen (42%). By EM findings, 24 patients were classified as stages I trough IV. We diagnosed 7 patients (29%) as stage I, 12 patients (50%) as stage II, 5 patients (21%) as stage III and no patient (0%) as stage IV. The other 4 patients obtained no glomeruli in the specimens for EM. All patients except one had proteinuria. Ten patients (36%) showed nephrotic syndrome and 4 of them also had hematuria. Fourteen patients (46%) had asymptomatic proteinuria and hematuria and 4 patients (14%) had asymptomatic proteinuria only. The only one patient (3.6%) showed recurrent macroscopic hematuria. We treated patients with nephrotic syndrome using corticosteroid. After the therapy for 4 or 8 weeks, 3 patients (11%) had corticosteroid-resistant nephrotic syndrome. We can now follow clinical data in 26 patients. Mean follow-up period is 13.6 years (range from 3.8 to 24.4 years). Sixteen patients have normal urinalysis and 10 patients have mild proteinuria (<1g/day). No patients have renal insufficiency or massive proteinuria in the present.
Conclusion: The outcome of patients with idiopathic MGN was good. Our results indicate that idiopathic MGN often presents nephrotic syndrome, but seems to have good prognosis in Japanese children.

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RENAL ERYTHROPOIETIN RECEPTOR EXPRESSION CORRELATES WITH THE SEVERITY OF HUMAN GLOMERULONEPHRITIS

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Objectives of Study: Erythropoietin (EPO), the principal hematopoietic cytokine that regulates erythropoiesis by binding to its transmembrane receptor (EPOR), promotes the proliferation and differentiation of erythroid precursor cells while also inhibiting their apoptosis. Recent evidence has suggested that the biologic effects of EPO are not limited to the regulation of erythropoiesis. Expression of functional EPOR has been reported in several nonhematopoietic cell types, including vascular endothelial cells, neuronal cells, myoblasts and kidney cells. The renal cell types that have been shown to express EPOR include tubular epithelial cells, glomerular endothelial and mesangial cells. These findings suggest that EPO produced primarily in mature kidney may act on the behavior of renal intrinsic cells under some physiological or pathological conditions. The purpose of the present study was to evaluate clinical specimens from a series of patients with glomerulonephritis (GN) for EPOR expression and characterize the relationship between renal EPOR expression and several clinicopathological parameters.
Methods: We studied a total of 66 renal biopsies from the patients with childhood-onset GN (5 minimal change disease, 9 focal segmental glomerulosclerosis, 9 non-IgA mesangial proliferative GN, 20 IgA nephropathy, 10 lupus nephritis, 6 membranous nephropathy, and 7 membranoproliferative GN). Renal EPOR expression was assessed by immunohistochemistry and in situ hybridization. Immunohistochemical expression of α-smooth muscle cell actin (α-SMA), a marker for monocytes and macrophages (CD68), and podocyte markers (synaptopodin and WT-1) were also examined.
Results: In normal kidney and minimal change disease, immunohistochemical expression of EPOR was weakly observed in vascular endothelial cells, tubular epithelial cells and some glomerular endothelial and epithelial cells. The localization of EPOR mRNA expression was consistent with the area showing positive immunostaining. In proliferative forms of GN including IgA nephropathy and lupus nephritis, an increased expression of EPOR protein and mRNA was observed in the lesions of mesangial proliferation, crescent formation, some podocytes and tubulointerstitium. Serial section and confocal microscopic analysis exhibited expression of podocyte markers in EPOR-positive epithelial cells at the periphery of glomerular tufts and co-expression of EPOR with α-SMA in mesangium. Glomerular EPOR expression was occasionally found dominantly in podocytes especially in focal segmental glomerulosclerosis and severe proliferative GN. By quantitative analysis, the glomerular expression score of EPOR was significantly correlated with the degrees of mesangial proliferation, segmental sclerosis, capsular adhesion and macrophage infiltration.
Conclusion: Glomerular EPOR expression is markedly upregulated in proliferative forms of GN and this correlates with glomerular histologic damage. This result suggests novel mechanism of mesangial activation and podocyte injuries in proliferative GN.

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TUBULOINTERSTITIAL CHANGES IN KIDNEY BIOPSY SPECIMENS OF CHILDREN WITH PROTEINURIC NEPHROPATHIES

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Objectives of Study: Abnormal glomerular permeability to plasma proteins and tubular traffic of filtrated proteins can elicit the profibrotic response that causes tubulointerstitial injury. Epithelial-mesenchimal transdifferentiation of tubular epithelial cells might contribute to tubulointerstitial fibrosis. We present preliminary data of the prospective study which aim is to elucidate tubulointerstitial changes in proteinuric nephropathies in children.
Methods: Renal biopsy specimens of 20 patients were analyzed. Biopsies were performed in the early course of the diseases in all except one patient. In 14 patients glomerular disease was confirmed by kidney biopsy (minimal change disease in 3 pts, focal segmental glomerulosclerosis in 4 pts, M.Henoch Schonlein in 2 pts., mesangioproliferative GN in 2 pts., IgA disease in 1 pt., systemic lupus erithematodes in 1pt., focal segmental necrotizing GN in 1 pt., rapidly progressive GN and APSGN in 1 pt). According to the grade of proteinuria at the time of biopsy, patients were divided in 2 groups: group A (9 pts.) with nephritic proteinuria and group B (6 pts.) with low grade proteinuria. Renal biopsy specimens of renal grafts of 5 pts in acute rejection were used as an unproteinuric disease controls (Group C). Renal biopsy specimens of renal grafts of 5 pts in acute rejection were used as unproteinuric disease controls (Group C). Morphology in renal specimens was evaluated by hematoxylin and eosin, periodic acid-Schiff and silver methenamine staining. Masson staining was used to evaluate the presence of interstitial fibrosis. For immunohistochemistry in paraffin-embedded tissue, primary antibody for Alfa smooth muscle actin (Alfa-SMA) was employed. Interstitial cell infiltration and fibrosis and tubular atrophy and degeneration were classified in three groups according to their extent. Score of tubulointerstitial changes represent sum of each for interstitial infiltration, interstitial fibrosis and tubular dilatation and atrophy. Number of glomerular, tubular and interstitial Afa-SMA positive cells were counted separately and classified in three groups. The intensity of score and distribution of staining were expressed as the mean ±SE. Urine collected for 24h of each patient was checked for proteinuria on the day of biopsy. Proteinuria was expressed as proteinuria/creatinuria ratio (mg/mg).
Results: At the time of kidney biopsy mean proteinuri/cratinuria ratios were: 8.26 (group A), 0.15 (group B) and 0.27 (group C). Mean score of tubulointestitial changes in group A was 2.00±1.64, in group B 2.00±0.98 and in group C 3.40±2.29. Scores of Alfa-SMA positive cells were: in glomerulus 1.11±0.68, 0.83±0.78 and 0.60±0.38, in tubules 0.67±0.43, 0.50±0.35 and 0.0.60±0.38 and in interstitium 1.11, ±0.57 , 0.67±0.30 and 1.00 ±0.65 in group A, B and C respectively. There were no significant differences in any of analyzed glomerular, tubular or interstitial markers of injury between groups. The scores of Alfa SMA positive glomerular, tubular and interstital cells were the highest in group A, but did not reach statistic significances. Tubular Alfa-SMA positive cells were found in 11 out of 19 patients. There was not significant difference in the mean score of tubulointerstitial changes between patients with, and without tubular Alfa-SMA positive cells in analyzed kidney biopsy specimens.
Conclusion: There is a tendency of higher scores of Alfa SMA positive cells in kidney biopsy specimens of children with nephritic proteinuria at the time of biopsy. Further analysis of grater number of biopsy specimens of children with proteinuric nephropathies and especially of repeated biopsies in the later course of the disease, could clarified the importance of this observation.
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MEMBRANOUS NEPHROPATHY ASSOCIATED WITH PENICILLAMINE THERAPY AND AUTOIMMUNE THYREOIDITIS – CASE REPORT

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We present 16-year-old girl with Wilson disease (H1069Q/H1069Q), who was treated with penicillamine for 2.5 years period. She concomitantly suffered autoimunne thyreoiditis (AIT). Clinical symptoms of nephrotic syndrome (eyelids and pre-tibial swellings, weight increasing) occurred then. Hypoalbuminaemia, hypercholesterolaemia, nephrotic range of proteinuria, normal GFR and blood pressure were present. The patient also presented antibodies against thyroid gland (a-TPO) with antinuclear antibody (ANA) positivity, but ds-DNA-Ab were negative. It wasn't possible to unambiguously decide whether pathogenic process of nephrotic syndrome was triggered due to penicillamine therapy or AIT. We replaced penicillamine with zinc therapy and simultaneously started symptomatic therapy with angiotensin-converting enzyme inhibitors (ACEI). Proteinuria decreased from 8.456 g/d to 1.932 g/d (albuminuria from 2630 to 561 mg/l), GFR remained normal, in the range 143.4 –106.8 ml/s/1.73 m^2, cholesterol decreased from 8.2 to 6.8 mmol/l, albuminaemia sustained in stable level (27 - 28 g/l). One month later proteinuria increased again to 8-10 g/d, albuminaemia and cholesterol gradually normalised. Renal biopsy was indicated and revealed membranous nephropathy (WHO stage 3) with positive immunofluorescence (IgG, IgA, C_3). Incubation of patient's serum with normal kidney resulted in intensive fluorescence of glomerular and tubular cell's nuclei and revealed evidence of ANA antibodies. Corticosteroid therapy (prednison 1 mg/kg/day for 6 weeks) with switching to alternative regime (1mg/kg/48 hours) was started. Proteinuria didn't disappeared during the 6-month period after discontinuation of penicillamine and during steroid therapy (persisted in nephrotic range of 3-12 g/d) whilst a-TPO became negative. We decided to introduce angiotensin II type 1 receptor antagonist therapy (ARB) in therapeutic regime. Proteinuria very shortly decreased below 1g/d during next 3 weeks and completely disappeared after 3 months therapy.
Conclusion: We do present a very rare case of membranous nephropathy (MN) in a patient with Morbus Wilson and autoimmune thyreoditis (AIT). One only can speculate about causative relations of penicillamine therapy for triggering of MN and the role of AIT. Penicillamine could serve as a hapten antigen for stimulation of autoimunne antibodies. Penicillamine induced MN is very rarely in children and adolescents. Also, MN associated with AIT is rare and typical for adults. Corticosteroid therapy didn't effect on nephrotic range proteinuria but combination of ACEI and ARB very shortly resulted in complete remission of proteinuria which persisted until now (2 years) and GFR remained stable**.**

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HEREDITALY NEPHRITIS IN A FAMILY WITH HEAVY PROTEINURIA AND CHARACTERISTIC GLOMERULAR BASEMENT MEMBRANE

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Introduction: We report a family with hereditary nephritis characterized by unique clinical course and histological findings different from previous reports.
Case report: ***Case1 (Mother)*** School urinalysis revealed mild proteinuria at age of eight years old. Prednisolone(PSL) was commenced but failed to reduce urinary protein. Proteinuria and hematuria gradually deteriorated (5g/day) after twenty years old of age, subsequently she developed end stage renal failure and started CAPD when she was thity-four years old. ***Case2 (Elder Daughter)*** She had chance hematuria and heavy proteinuria, and developed nephrotic proteinuria (3g/day) when she was three years old PSL and cyclophosphamide were not effective, but her urinary protein was reduced to 0.3g/day with administration of cyclosporinA(CsA). Hearing difficulties was found recently. ***Case3 (younger son)*** Spontaneous hematuria was noticed at two months of age, and he developed moderate hematuria and nephrotic proteinuria(456mg/dl) at nine months. ACEI was initially commenced but failed to reduce urinary protein. CsA was administered at age of two years old, and lead to significant reduction of urinary protein(0.2g/day).
Methods of study: Serial renal biopsy was done to see histology, immunofluorescence(IF) including type IV collagen (α2 and α5), and EM.
Results: ***Case1 (Mother)*** Renal biopsy was performed three times at age of ten, twenty three, and thirty years old. The first biopsy showed no remarkable result. Second and third biopsies indicated mild mesangial proliferation and irregular glomerular basement membrane(GBM). We investigate collagen type IV in last biopsy, and no aberrancy was there. ***Case2 (Daughter)*** The first biopsy was performed when she was three, and it was focal proliferative glomerulonephritis with thickness of GBM. We performed biopsies four times. The latest one at age of ten years old showed segmental mild mesangial proliferation and thickness of GBM. IF was negative, and type IV collagen was normal. ***Case3 (son)*** We performed renal biopsies when he was five months and five years old. The first result showed segmental moderate mesangial proliferation, and GBM was rough, thinned and partly meshed. The biopsy of the age of five years old revealed the result was improved. IF was also negative in this patient, and type IV collagen was normal.
Discussion: Majority of children with hereditary nephritis develops proteinuria in their adolescence. The family we reported had unique features as follows; they had heavy proteinuria since their childhood. GBM was irregular and partly thinned, however IF did not indicate typical absence of type IV collagen. Administration of CsA in Case 2 and 3 leads to remarkable reduction of urinary protein. Those findings were different from other cases with hereditary nephritis previously reported.

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ATYPICAL MANIFESTATION OF ACUTE POSTINFECTIOUS GLOMERULONEPHRITIS IN CHILDREN

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Objectives of study We reviewed the data of children with acute postinfectious glomerulonephritis admitted in our hospital during January 2000 to August 2002 to identify the cases with severe or atypical manifestation.
Materials and methods A total of 9 boys and 6 girls, age 5-14 years old (mean age 10.9 years) were admitted with the diagnosis of acute postinfectious glomerulonephritis.The diagnosis was made by clinical manifestation of edema,hypertension and hematuria developing after history of respiratory tract infection,sore throat or skin infection.ASO titers were done in all cases and were positive in 5 of 15 cases.Anti-Dnase B titers were done in 6 cases and were positive in 2 cases.The other laboratory results,clinical courses and pathology reports were reviewed.All patients were followed for more than one year.
Results Six patients (40%) had severe or atypical manifestation including heavy proteinuria,nephrotic syndrome,rapidly progressive glomerulonephritis (RPGN) and recurrent disease.All of them had nephrotic range proteinuria in which 3 cases developed nephrotic syndrome.Three patients had RPGN,2 of them had persistent proteinuria and developed chronic renal insufficiency.One of these two had recurrent disease one year after the first attack.Kidney biopsy was performed in 5 cases and indicated of acute postinfectious glomerulonephritis in all 5 cases with crescentic glomerulonephritis in 2 cases.The patient with recurrent disease had the second kidney biopsy done which confirmed the diagnosis.
Conclusion There was a tendency of more severe clinical manifestation of acute postinfectious glomerulonephritis.In this review we found 3 cases of RPGN from a total of 15 cases (20%) in the period of two and a half years.After follow up,2 cases turned to have chronic renal insufficiency and one of these had recurrent disease.The definite etiology should be identified, aggressive treatment may be needed in patients with atypical manifestation and long term follow up is necessary.

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THE CLINICAL SIGNIFICANCE AND INFLUENCE OF TRIPTERYGIUM WILFORDII HOOK F. ON GLUCOCORTICOID RECEPTORS IN CHILDREN NEPHRITIC SYNDROME

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Objective: To study the mechanism of Tripterygium Wilfordii Hook F. (TWHF) combined with prednisone for the treatment of children's nephrotic syndrome (NS) and the possibility of TWHF replacing part of glucocorticoid (GC) for NS.
Methods: All the cases of NS were divided into three groups: the group A (11cases was added TWHF 1.5mg/kg·d after prednisone 2mg/kg·d was used for two weeks), the group B (10 cases was added TWHF 1.5mg/kg·d after prednisone 1mg/kg·d two weeks), the positive contrast group (11 cases only was treated with predisone 2mg/kg·d). 57 of health children were selected as the normal control. The patients' venous blood was taken at 8:30 am, used heparin as the anticoagulant. The glucocorticoid receptors (GCRs) of peripheral blood mononuclear cells (PBMCs) was measured with the radio ligand binding assay method, ^{3}H-Dex as the ligand.
Results: Before using GC, the GCRs of PBMCs in the group A,B and the positive contrast was respectively 4942±1839dpm, 4983±1846dpm,4854±1755dpm, and the GCRs of the normal control was 6573±2813dpm. The GCRs in all patients was significantly less than in the normal control ($p<0.05$), but there was no significant difference among the group A,B and the positive contrast ($p>0.05$). When treating with GC for one week, the GCRs was further decreasing. The GCRs was respectively 2348±1012dpm, 2366±1026dpm, 2297±869dpm in the group A, B and the contrast. The GCRs of the group A, B increased after three weeks of using of GC and one week of TWHF. There was a significant difference of GCRs in group A and B between after one week and three weeks of using of GC with one week of TWHF ($p<0.05$). The lasting time of proteinuria in the group A and B was shorter than in the contrast ($p<0.05$). There was no significant difference of the lasting time of proteinuria between the group A and B ($p>0.05$). The relapse times in the group A and B were much less than in the contrast ($p<0.05$), but there was no significant difference between the group A and B ($p>0.05$).
Conclusion: TWHF for NS was obviously effective, when used with GC, the dosage of prednisone could be reduced and the lasting time of proteinuria could also be shortened and the relapse times could be reduced. The mechanism may be that TWHF resists the down-regulation of GCRs by GC.

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THE GENE EXPRESSION OF ELASTASE IN PERIPHERAL BLOOD LEUKOCYTE AND RENAL TISSUE FROM RATS WITH NEPHRITIC SYNDROME INDUCED BY RESPIRATORY SYNCYTIAL VIRUS AND RELEVANCE FOR PROTEINURIA

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Objectives: To investigate the gene expression of elastase (EA) in peripheral blood leukocyte and renal tissue from rats with nephritic syndrome induced by respiratory syncytial virus (RSV) and the effect of EA on glomerular structure and function.
Methods: A rat model was induced by nasal drip and intraperitoneal injection with RSV suspension. Thirty-five SD male rats weighed 150-210g were randomly divided into five groups: RSV4 group that was killed at day 4 after administration of RSV (n=8), RSV8 at day 8 (n=6), RSV12 at day 12 (n=5), the negative control at day 8 after administration of normal saline (NG, n=8), normal control (NORMAL, n=8). RSV groups were infected with RSV suspension on 1st, 2nd, 3rd day consecutively. NG was given normal saline by the same way. NORMAL was given nothing. The gene expression of EA of peripheral blood leukocytes (PBLs) and renal tissue was assayed respectively by the way of RT-PCR and the products were purified and given the sequence detection. The levels of proteinuria per 24 hours of all rats were measured daily. The renal histology and Alcian-Blue stain were observed.
Results: (1) The gene expression of EA in PBLs of the RSV4 (0.9251±0.1724) were the highest among all groups and had significant differences in contrast to RSV8 (0.5482±0.1149), RSV12 (0.4271±0.0836), NG (0.3392±0.0627) and NORMAL (0.3664±0.0987) respectively ($p<0.05$). Then the gene expression of EA decreased gradually. The differences among RSV8, RSV12, NG, and NORMAL had no statistical significance respectively ($p>0.05$). (2) The gene expression of EA in renal tissue of the RSV4 (0.6628±0.0842) were the highest among all groups and had significant differences in contrast to RSV8 (0.2169±0.0537), RSV12 (0.1862±0.0628), NG (0.1311±0.0374) and NORMAL (0.1272±0.0468) respectively ($p<0.05$). Then gene expression of EA in renal tissue decreased gradually. The differences among RSV8, RSV12, NG and NORMAL had no statistical significance respectively ($p>0.05$). (3) Gene expression of EA in PBLs was apparently higher than that of renal tissue in each group ($p<0.05$), respectively. (4) The gene expression of EA in PBLs and renal tissue had positive correlation with the quantification of urinary protein (r_{blood}=0.47, r_{kidney}=0.39, $p<0.05$). (5) Fusion of epithelial foot processes of glomeruli were noted in RSV12 and a reduction in Alcian-Blue staining of glomerular HSPGs were observed simultaneously.
Conclusions: (1) The gene expression of EA in PBLs and renal tissue from rats with nephritic syndrome induced by RSV increased especially on day 4. (2) The result that EA expression in PBLs was higher than that of renal tissue indicated that EA existed mainly in PBLs. (3) Higher EA expression, more the urinary protein. There was a positive correlation between the expression of EA mRNA and the urinary protein. The results suggested that the model of nephritic syndrome induced by RSV has an excessively release of EA that may cleave the HSPGs and neutralize the GBM negative charges with its positive charges. The damage of GBM negative charge barrier may lead to the change of renal histology and the occurrence of proteinuria.

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CLINICAL SIGNIFICANCE OF THE DETECT T LYMPHOCYTES SUBSETS WITH CHILDREN NEPHRITIC SYNDROME

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Objective: For the purpose of studying the clinical significance on detect of the T lymphocytes subsets with children nephrotic syndrome（NS.

Methods: T lymphocytes subsets were detected with flow cytometor. The change of the T lymphocytes subsets in serum was measured with ELISA from 25 children nephrotic syndrome patients at active stage and 20 of them at remission stage.

Results: The percentage of CD3+cell, CD4+cell, CD(16+56)+ cell and the ratio of CD4+/CD8+ were significantly lower at NS active stage than those at NS remission stage and in the control group ($p < 0.01$)

Conclusion: Clarification the cellular immunity of NS patients was reduced. The detect of the T lymphocytes subsets can be used as an index of nephrotic syndrome activation.

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LEFT RENAL VEIN ENTRAPMENT SYNDROME IN CHILDREN WITH GLOMERULAR DISEASES

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Objective To analysis left renal vein entrapment syndrome (LRVES) in children with glomerular disease and the relationship with glomerular disease.
Methods 16 patients diagnosed as left renal vein entrapment syndrome by using Doppler ultrasound sonography were retrospectively analyzed .
Results 16 patients were divided into two groups Group A: 8 patients without underlying glomerular disease. Among them 7 cases with nonglomerular hematuria, 1 case with orthostatic proteinuria.Group B: Another 8 patients with underlying glomerular diseases . A mong them 2 cases with nephrotic syndrome, 3 cases had acute glomerulonephritis history, 2 had delayed glomerulonephritis and in 1 case renal biopsy was preformed (mesanginal proliferative glomerulonephritis), Another with long lasted hematuria case renal biopsy was preformed minimal change disease. The 5 years younger sister of. this patient had also hematuria,but no left renal vein entrapment syndrome was detected by using Doppler ultrasound sonography.the mean left renal vein (LRV)ratio (the diameter of the left renal vein at the hilar portion of LRV to the aortomesenteric angle divided by the diameter of the LRV ratio) of group A and group B WERE 4.64±1.15 and 4.03±1.36. Differences were not statistically signific between the two groups ($p>0.05$).
Conclusion left renal vein entrapment syndrome may be detected in patients with underlying glomerular disease. It may be associated with some factor of heredity .Forther study should be taken. We suggest that Doppler ultrasound sonography should be taken by patients with long lasted hematuria and chronic proteinuria even if the patients had been diagnosted glomerulonephitis or nephrotic syndrome.

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A CASE REPORT: GLYCOGEN STORAGE DISEASE ASSOCIATED WITH ONSET OF NEPHRITIC SYNDROME

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A masculine patient, aged 4.5, suffered from tympanites, hunger and polyphagia in his earlier ages. However, he began to remains small in stature after one year old, and typical symptoms related to nephrotic syndrome appear after 33 months. He ever got “acidosis”. His clinical manifestations are listed as follows: microsoma in stature (-2SD), body weight +2SD, high dropsy, hepatomegaly and splenomegaly (8cm, 3cm under ribs), double kidneys tumefaction (113cm), urinary albumin 4.16g ration in 24 hours, non-selective proteinuria and tubular proteinuria, serious hyerproteinemia (Alb14g/L), hyperlipoidemia (CHO10.95mmol/L, TG7.03mmol/L), glycopenia (3.64mmol/L), serumal uric acid 287μmol /L, normal CO_2CP, normal BUN and SCr,etc.
The biopsy operation on nephridial tissues shows that the nephridial tissues seem wan, the fluorescence of pathologic immunity remains negative. Electromagnetic lens observation: symptoms as absorptive vacuoles, lipid vacuoles, endoplasmic reticulum expansion and most sedimentary glycogen granules can be seen on glomerular epithelium, endothelial cells and renal tubular epithelial cells. The glomerular basement membrane and the renal mesenchyme have no obvious pathologic changes. The foot process of the epithelial cells takes on periodical confluence (few). Diagnoses: renal damage resulted from Glycogen Storage Disease.
At the beginning, the patient was treated with prednisone irregularly and a great deal of albuminuria appeared and lasted about two years. And the prednisone remained 3+ after 8 weeks’ treatment with enough hormone. Receiving one treatment course of methylprednisolone(0.5g/day ×3) concussions treatment, the patient’s urinary albumin became negative slowly. With the confirmed diagnosis, the patient was required to orally take 2g cornstarches per day and reduce prednisone little by little, and his urinary albumin kept negative. One year later, the patient grew taller from 96cm to 104cm and liver recovered 2cm. Two years later, the patient’ urinary albumin reappeared positive because of stopping taking cornstarches. The patient was required to take cornstarches and 1mg/kg prednisone again, and his urinary albumin became negative two weeks later. Three years later, the patient re-suffered from dropsy because of irregular taking of cornstarches (only in supper), and his urinary albumin reached 3$^+$, at the time, the patient took 15mg prednisone every other day and kept normal in height (114cm), weight (32kg) and intelligence, 6cm under liver and ribs. The patient’s urinary albumin reappeared negative through repeated treatment mentioned above.
Most patients, who get hepatic glycogen storage disease resulting in renal damage, belong to Type Ia (GSD I a), and the damaged kidney show in earlier days: renal tubules keep in dysfunction, and the renal tubules will suffer a lot from the dysfunction and plentiful albuminuria often occurs at the age of 12 or over. However, the boy showed nephritic syndrome associated with tubular proteinuria in infant stage with his renal function was normal. The boy could remain in remission with the usage of cornstarch along with certain dose of prednisone. When dose of cornstarch decreased and infection was accompanied, he relapsed. If the therapy was recovered, he would again remained in remission. The medial mechanism should be further analyzed in the future.

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CLINICAL ANALYSIS OF CHILDREN WITH TYPE I MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS

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Objective of study: To observe the clinical presentation in type I membranoproliferative glomerulonephritis (type I MPGN) and go further into the therapeutic methods.
Methods: Five patients (3 boys, 2 girls) with type I MPGN treated in 2001-2003 were analyzed retrospectively. The mean age at the time of diagnosis was 12 years old (range from 8 to 13 years old). The clinical characteristics, laboratory data, pathological findings and therapeutic methods were investigated.
Results: The results showed that the clinical onset often was the very image of the acute nephritis. The clinical findings showed that the five patients had the nephrotic-range proteinuria (>50mg/kg· day), edema and haematuria (macroscopic haematuria 3, microscopic haematuria 2), 4 patients had hypertension and low serum C_3, 2 patients with azotemia. Five patients had the nephritic syndrome, which failed to respond to 6 weeks of oral prednisone therapy. Four cases received methyprednisolone (10-20mg/kg per dose up to a maximum of 1g) given by intravenous infusion 3 days a week for 2 weeks, followed by a combination of intravenous pulse cyclophosplamide (8-12mg/kg·day) 2 days a month for 6 months and alternate-day prednisone therapy (maximum dose of 60 mg). Three of the five patients obtained stable remission after 3 months, and one did not respond. Another one only received intravenous pulse cyclophosphamide (for 3 months) and oral prednisone had remission of edema but continued to have proteinuria (partial remission).
Conclusion: These results suggest that the commonest presenting symptoms of type I MPGN are edema, nephritic-range proteinuria, haematuria, hypertension and low-serum C_3. The patients with type I MPGN may obtain clinical benefits from the described protocol.

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THE EXPRESSION OF PODOCIN, NEPHRIN AND SYNAPTOPODIN IN MEMEBRANOUS NEPHROPATHY

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Objectives of Study: Nephrin and podocin are major components of slit diaphragm. Nephrin dissociates from actin, and its expression is reduced in passive Heymann nephritis, a rat model of human membranous nephropathy(MN). We examined the expression of nephrin and podocin in human MN.
Methods: We generated rabbit polyclonal antibodies against conjugated peptides derived from the human podocin and nephrin. We used these antibodies to immunohistochemistry for MN, compared with synaptopodin, podocyte membrane protein.
Results: By immunohistochemistry, nephrin, podocin and synaptopodin were detected in a linear pattern along the glomerular capillary loop in normal glomeruli. Among 11 patients, podocin was strongly expressed in glomeruli in 9 patients, while decreased in 2. The staining pattern was shifted from linear to granular. The expression of synaptopodin was similar to that of podocin with sometimes decrease staining. On the other hand the expression of nephrin was either decreased(5/8) or absent(3/8) in MN. There was no statistical significance between the expression of these proteins and clinical paramaters. clinical stages in MN.
Conclusions: Nephrin may be a target of injury in MN.

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FREQUENT VACCINATION AND IMMUNE COMPLEX DEPOSITION IN UNILATERAL NEPHRECTOMIZED MICE

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OBJECTIVES: The aim of this study is to investigate the influence of increased number and frequency of vaccination on the immune complex deposition in choroid plexuses and glomeruli in non-nephrectomized and unilateral nephrectomized mice.
METHODS: Fifty-five non-nephrectomized, 40 unilaterally nephrectomized and 7 control Swiss albino mice were used. Half of each group was vaccinated only with diphteria-tetanus, and the other half with multiple vaccines, which are in use in pediatric practice. Each group was divided into subgroups, which were vaccinated with increasing frequency.
RESULTS: No immune deposits were detected in the choroid plexus of any vaccinated mice. There was immune deposits in glomeruli in 2/55 (3.6%) of the nonnephrectomized and in 3/40 (7.5%) of the nephrectomized mice (p=0.199). The difference between the diphteria-tetanus and multiple vaccine groups in non-nephrectomized (p=0.236) and nephrectomized (p=1.000) mice was not significant. A significant positive correlation between increased frequency of vaccination and glomerular immune complex deposition 8 weeks after the last immunization was detected in multiple vaccine group of the nephrectomized mice (p=0.048, r=0.447).
CONCLUSION: Our results suggest that, the increased number and frequency of vaccines do not increase the deposition of immune complexes in choroid plexuses and glomeruli in nephrectomized mice and non-nephrectomized mice. However, the deposition of immune complexes in glomeruli 8 weeks later may correlate with the increased frequency of vaccinations in multiple vaccine group of the nephrectomized mice.

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THE INVESTIGATION OF CD68 POSITIVE MONOCYTES/ MACROPHAGES CONTAINED IN URINE AND INFILTRATED RENAL TISSUE OF VARIOUS KIDNEY DISEASES IN CHILDREN.

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Objectives of Study: This study was performed in order to investigate the participation of monocytes / macrophages (Mo/MΦ) in the progression of various kidney diseases.
Methods: The Mo/MΦ contained in urine and that infiltrating renal tissue were both measured as the number of CD68 positive Mo/MΦ ($CD68^+$ Mo/MΦ), using anti-CD68 antibody. We measured $CD68^+$ Mo/MΦ on light microscopy.
Results: The number of $CD68^+$ Mo/MΦ infiltrated in one glomerulus was more significantly in HSPN ($p<0.01$) in comparison with that in MCNS. The $CD68^+$ Mo/MΦ infiltrated in one millimeter square of tubulo-interstitium area was significantly more in number in HSPN ($p<0.05$), FSGS ($p<0.01$), Alport's syndrome ($p<0.01$), respectively, than that in MCNS. The $CD68^+$ Mo/MΦ contained in one milliliter of urine correlated significantly more in number with both that infiltrating in the glomerulus and in the tubulo-interstitium (both $p<0.01$). Moreover, the number of urine $CD68^+$ Mo/MΦ in a clinically active stage was significantly more than that in a inactive stage in the AGN ($p<0.05$), IgAN ($p<0.05$), HSPN ($p<0.05$), non-IgAN ($p<0.01$) and MPGN groups ($p<0.05$), respectively.
Conclusion: (1) It was suggested that the infiltrating Mo/MΦ in renal tissue participated in the development of various kidney diseases. (2) It was supposed that $CD68^+$ Mo/MΦ in urine reflected both the number of Mo/MΦ infiltrating in the glomerulus and that in the tubulo-interstitium. (3) It was suggested that the number of $CD68^+$ Mo/MΦ in urine indicated clinical activity in proliferative glomerulonephritis groups of children.

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LIPOPROTEIN(A) SIMULATED THE PROLIFERATION OF HUMAN MESANGIAL CELLS BY ACTIVIATING MAPK SIGNAL TRANSDUCTION

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Objectives of study: Mesangial hypercellularity is a critical early histopathological finding seen in human and experimental glomerular diseases. Hyperlipidemia and the glomerular deposition of lipoproteins are commonly associated with mesangial hypercellularity and play an important pathobiological role in the development of glomerular diseases. The activated cytoplasmic mitogen-activated protein kinase (MAP kinase), including mainly extracellular-signal regulated protein kinase (ERK), c-Jun amino-terminal kinase (JNK), and p38, has been thought to translocated into the nucleus and activate various transcription factors and protooncogenes associated with cell growth and proliferation. Lipoprotein(a) [Lp(a)] has been shown to stimulated proliferation of mesangial cells, but the events of Lp(a) signaling have not yet been characterized. The purpose of this study is to investigate the signal transduction pathways involved in Lp(a)-induced cell proliferation and provide an evidence for the participation of Lp(a) in intracellular signaling pathways for mesangial cell proliferation. **Methods:** Lp(a) was isolated from a patient who was being treated with LDL-apheresis by density gradient ultracentrifugation and then chromatography as described before. Human mesangial cells were isolated by the sequential sieving technique from patients undergoing tumor nephrectomy and stimulated with Lp(a) in different concentration and time course. The DNA synthesis of the cells was measured by [^{3}H] thymidine incorporation for detecting the proliferation, and the expression of MAPK, including ERK, JNK, and p38, and their phosphorylation were detected by Western blotting.
Results: Lp(a) could induce a significant dose-dependent proliferation of human mesangial cells. The ^{3}H-TdR incorporation was 1.64 ± 0.31, 1.69 ± 0.48, 3.59 ± 0.68 ($p<0.01$), 4.14 ± 0.78 ($p<0.01$), 4.05 ± 0.55 ($p<0.01$), 4.40 ± 0.47 ($p<0.01$) (10^3cpm) at the Lp(a) concentration of 0μg/ml, 5μg/ml, 10 μg/ml, 25μg/ml, 50μg/ml, respectively. Lp(a) caused an increase in ERK phosphorylation between 5 and 60 min, and in JNK phosphorylation between 10 and 30 min after incubating with human mesangial cells. But the level of p38 and its phosphorylation was not changed.
Conclusion: The study revealed that Lp(a) could simulate the proliferation of human mesangial cells by activiating the phosphorylation of MAPK signal transduction cascade, such as ERK and JNK, but not p38.

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EFFECT OF ALTITUDE ON RENAL AND BONE HISTOPATHOLOGY IN RATS WITH ABLATION NEPHROPATHY

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Objectives of Study: We aimed to evaluate the effect of physiologic changes due to high altitude on the residual renal tissue and renal osteodystrophy in ablation nephropathy model.

Methods: Thirty male Wistar rats were divided into two control (C1 and C2, n=7 for each) and two study (S1 and S2, n=8 for each) groups. After blood and 24-hour urine samples were obtained, 5/6 nephrectomy and sham operations were performed in the study and control groups, respectively. While C1 and S1 groups were kept at sea level, C2 and S2 groups were followed at an altitude of 1200 m above sea level. Three months later, after second blood and 24-hour urine samples were obtained, all the animals were sacrificed and renal and bone tissues were obtained for pathologic examination. In addition, daily protein excretion and GFR of the animals were determined at the onset and end of the study.

Results: GFR was lower and proteinuria was higher significantly in the study groups at the end of the study. However, these parameters did not differ among the S1 and S2. Likewise, although renal histopathological parameters (glomerulosclerosis, glomerular hypertrophy and interstitial changes) were significantly prominent in the study groups, S1 and S2 did not differ with respect to these parameters. The ratio of osteoid tissue to trabecular bone was increased in the study groups. However, this difference was only significant in S1 compared to C1. S1 and S2 groups were not different with respect to bone histopathology.

Conclusion: We concluded that high altitude did not adversely effect renal functions and renal and bone histopathology in this model of ablation nephropathy in rats.

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P112

CATIONIC CHARGE-PREFERENTIAL REABSORPTION OF IGG IN THE RENAL PROXIMAL TUBULES.

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Objective: The brush border of the renal proximal tubules has a poly-anionic charge. Since IgG molecules have a wide range of charge diversity, rebabsorption of urinary IgG molecules are supposed to be influenced by the electrostatic interaction.

Methods: Charge diversity of serum and urinary IgG molecules in patients with various renal diseases and premature neonates were analyzed, by isoelectric focusing and Immunoblotting.

Results: In patients with glomerular diseases, urinary IgG were solely composed of neutral and anionic IgG, whereas that of the cationic part (isoelectric point > 8) was not observed. By contrast, in patients with proximal tubular diseases (Dent's disease and idiopathic Fanconi Syndrome), the proportion of the cationic IgG was similar to that of serum IgG. In addition, the cationic part of IgG in the urine was found in the neonates with a gestational age of 28 and 31 weeks, but not found in that of 35 weeks.

Conclusion: The results suggest that renal proximal tubules reabsorb the urinary IgG in a cationic preferential way and this mechanism requires renal maturation.

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ELEVATED RENAL HGF/TGF-β1 IN RATS WITH NEPHROMEGALY AFTER COMMON BILE DUCT LIGATION

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Objectives of Study: Our recent data on infants with subacute or chronic liver injury revealed the extent of nephromegaly correlated positively with plasma hepatocyte growth factor (HGF) but negatively with plasma transforming growth factor-β1 (TGF-β1) (Am J Kidney Dis 2001; 38: 279). The reciprocal relationship of plasma HGF and TGF-β1 in this situation may reduce antiproliferative effect of TGF-β1 and thus potentiate proliferative action of HGF resulting in nephromegaly. In the present study we examined an animal model of liver injury using ligation of common bile duct (CBD) in young rats to observe the nephromegaly and to search for a possible mechanism.
Methods: 25-day-old male Wistar rats were performed CBD ligation and sham operation (6-12 rats in each group) via ventral laparotomy. They were sacrificed at one week, two weeks and three weeks after surgery. Blood was collected and both kidneys were removed after saline perfusion during sacrifice. Body weight and total kidney weight were measured. Kidneys were homogenized with TRI reagent and extracted for tissue lysate. Plasma HGF and TGF-β1, renal tissue protein and DNA, and renal tissue HGF and TGF-β1 were then measured using various specific EIA kits. Renal enlargement was assessed by kidney weight/body weight ratios and renal hyperplasia or hypertrophy by protein/DNA ratio.
Results: There was a tendency of, but not significant decrease in body weight and total kidney weight in CBD ligation rats compared with sham operation controls. Plasma HGF was significantly decreased at one week (p=0.004) and plasma TGF-β1 was persistently low through one week (p=0.01) and two weeks (p=0.013) after surgery in CBD ligation rats. Renal HGF/TGF-β1 started rising since one week (p=0.135) and reached a significantly higher level (p=0.015) than sham controls at 2 weeks postoperatively. This was followed by a higher kidney weight/body weight ratio (p=0.021) and an elevated renal protein/DNA ratio (p=0.015) at 3 weeks after surgery in surviving CBD ligation rats.
Conclusion: Our data suggest renal hypertrophy as a cause of nephromegaly in this rat model of liver injury, and may relate renal hypertrophy to these causative growth factors. Although complete obstruction of CBD ligation is somewhat different from partial obstruction of biliary atresia with Kasai operation, our data provide evidence that increased renal HGF/TGF-β1, high HGF and/or low TGF-β1 after liver injury may be the most important factor to result in nephromegaly in this situation.

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P114

HEAT SHOCK PROTEIN 25 (HSP 25) AND HSP 70 EXPRESSION IN LOW PROTEIN FED-RATS

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Heat shock protein (HSP) synthesis is increased in response to cell injury from a variety of renal insults. Previous studies have shown that Angiotensin II can induce expression of these HSPs in renal cells. Malnutrition, a stressful condition to the kidney, includes enhanced expression of the genes that encode for various components of the Renin Angiotensin System
Objective of the study: We investigated the impact of LP consumption on HSP25 and HSP70 expression in the kidney.
Methods: The study was performed in three groups of rats: 1) LP fed-rats (protein 8% for fifteen days), 2) control rats (CP) protein 24%, and 3) protein recovery group (RP) with re-administration of 24% protein (nephrectomy was carried out at days 3, 7 and 15 of the recovery period). Angiotensin II AT_1 expression by RT-PCR was performed. HSP25 and HSP70 protein expression were evaluated by Western Blot and immunohistochemistry.
Results: Densitometric analysis of the AT_1 Angiotensin II receptor mRNA corrected for B-actin expression (relative densitometric units) showed a significant two fold increase on LP cortex compared to CP: 2.27 ± 0.2 vs 1.13 ± 0.1 ($p<0.01$). Lower expression was shown in LP medulla compared to CP: 1.47 ± 0.1 vs 1.09± 0.07 ($p<0.05$). Readministration of 24% protein (15 days) in the diet showed increased AT_1 receptor expression in kidney cortex related to control: 2.51 ± 0.3 vs 1.13 ± 0.1 ($p<0.01$), slight AT_1 receptor increase expression was found in the LP kidney medulla: 1.87±0.10 vs 1.09±0.07 ,$p<0.05$. In LP, immunohistochemistry revealed that HSP25 expression increased in intensity in the endothelium and medial smooth muscle of the renal arteries in the cortex. In medulla the immunoreaction for HSP25 appeared with an increasing intensity gradient when the MCDs were closer to the papilla. HSP25 immunostaining was observed in IMCDs with a tendency to be higher in the membranes. HSP70 was clearly observed in the cytoplasm and nuclei of CCDs and PCTs (with high expression at the brush borders of PCTs) in LP related to control. In LP rat medulla, HSP70 appeared in the cytoplasm of OMCD cells. The amount of HSP70 protein from LP relative to that of controls were near two-fold higher in cortex (1.75 ± 0.2 n:4 $p<0.05$, in medulla 2.37± 0.2, n 4 $p<0.01$. Re-administration of 24% protein showed intense inmunoreaction for HSP25 and HSP70 in the tubular lumen in the form of cylinders in the OMCDs and IMCDs. The observation of cylinders strongly reactive for HSP70 appeared at 3 days but higher numbers were showed at 7 and 15 days. At 3 days of recuperation, there were few cylinders but the ducts showed the strongest HSP70 immunoreaction. In addition, HSP70 protein levels in renal medulla were 2.5 fold higher than control, $p<0.01$ at the 3rd day of recovery, 1.6 fold and 1.5 fold higher than control, each $p<0.05$ each, seven and fifteen days after recovery respectively.
Conclusions: These results support the concept that HSP25 and mainly HSP70 are involved in the adaptative response of the kidney to injury caused by low protein feeding. Moreover, the increased expression of AT_1receptor suggests Angiotensin II modulation.

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THE CORRELATION OF RENAL CORTICAL BLOOD FLOW AND PROTEINURIA DEVELOPMENT IN NEPHRITIC RAT

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Objectives of Study: To identify which one plays a more important role, blood pressure or renal cortical blood flow (RCBF), in the development of proteinuria, and study the material mechanism of thus effect.
Methods: □twenty-eight SD rat (150~180g) were included in nephrotic group and proteinuria was induced by single vein injection of Adriamycin (7mg/kg),meanwhile the rat in control group were injected 0.9%NaCl;□ Urinary protein was quantitated every 24 hours;□ rat blood pressure was measured through a PE50 tube, which was inserted into left carotid artery, constrictive and relaxant pressure was measured 3 times by multi –function monitor and then a mean data was made based on them; in the same time renal cortical blood flow was measured directly on kidney by laser –doppler specnoscopy; □Rat plasma Endothelin(ET)and nitric oxide (NO) were measured by radioimmunoassay, while renal ET and NO were assessed on renal cortical homogenate by Griess`s method. All of measure above (□□□) were performed respectively on 4 time points_ day 4,8,32and 56 in simple_ that is 4,8,32and 56 days after adriamycin injection, and every time group included 7 nephrotic rats; □ The correlation between RCBF and urinary protein,ET or NO was compared respectively.
Results: □Urinary protein is normal on day 4, increase on day 8,show a peak-value on day 32(it indicates a nephrotic proteinurial) and decrease again on day 56 ;□The normal rat arterial blood pressure of the 4 time points is 118,119,118 and 117mmHg ,and that is 116,124,129 and 121 mmHg in nephrotic rat. There is no difference between nephrotic and normal rat($P<0.05$);□RCBF(Pu) of the 4 time points is 66,68,67 and 70 in normal control and is 58,52,19 and 23 in nephrotic rat, there is no significant differences between the two group at day4, but that of nephrotic rat are significant lower than that of normal rat on all day8,day32 and day56($P<0.05$);□plasma ET of the 4 time points in nephrotic rat is 134,150,538 and 445 ng/ml, with significant increase on day 32 and 56 compared to control(126~129 ng/ml, $P<0.05$);The ET of renal cortical homogenate of the 4 time points is 365,653,1527 and 1394,significantly higher than that of control(235.9~246.1 ng/ml, $P<0.05$); □Plasma NO in nephrotic rat was 40,36,8 and 11 nmol/ml, significantly lower than that of control(42~46 nmol/ml) from day 32 to day 56.The NO of renal cortex is 80, 69, 8, 25(nmol/ml), that is significantly lower than that of normal rat (114~124 nmol/ml)even from day 4 before proteinurial had been induced($P<0.05$). □RCBF has significant negative correlation with urinary protein on day32, day56 and with plasma ET on day32; it has a close positive correlation with Plasma NO on day32, day56, it has also close relationship with both ET and NO of renal cortical homogenate on all 4 time points ($P<0.05$); there is no significant correlation of rat arterial blood pressure with the change of RCBF, urinary protein, plasma and renal cortical homogenate ET and NO.
Conclusion: RCBF plays a more important role in development of proteinuria. The change of RCBF relates closely to the change of ET and NO, particularly to that in renal cortical.

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P116

EXPRESSION OF CASPASE-3 IN RAT KIDNEY WHICH RENAL TUBULAR DEMAGE INDUCED BY LIPOPOLYSACCHARIDE AND HYPOXIA

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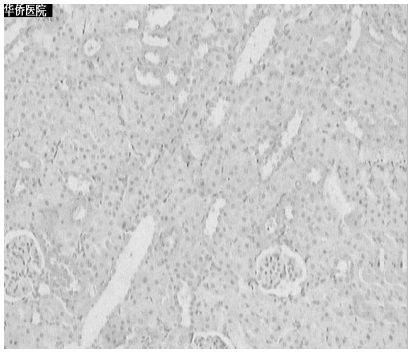
Objectives of Study To investigate the mechanism of renal damage induced by endotoxin and hypoxia .
Methods 10 anesthetized and articially ventilated rats were treated with 2 mg/kg lipopolysaccharide (LPS),FiO2 was reduced after 90 min from 21% to 5% and ventilation continued until 180 min .At the end of the experiment ,the kidney were obtained immediately for immunocytochemistry stain and HE stain.
Results 1. the kidney injury of the rat maily in proximal tubular epitheial cells.Proximal renal tubule injury were more severe than distal proximal tubule. 2.caspase –3 expression in distal renal tubule cells, but not in proximal tubular epitheial cells and glomerular cells.
Conclusion 1. endotoxin and hypoxia induce renal damage main in renal tubale cells .2. expression of caspase-3 in distal renal tubule cells, but not in proximal tubular epitheial cells and glomerular cells .May be there are difference mechanism of the injury and repairment in distal renal tubule cells and in proximal tubular epitheial cells .



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P117

CONSTRUCTION OF RECOMBINAN pLXSN CONTAINING ANTISENSE RAT MCP–1 cDNA AND ITS EXPRESSION IN MESANGIAL CELLS

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Objectives of Study: To transfer antisense MCP-1 cDNA into mesangial cell.

Methods: Monocyte chemoattractant protein–1(MCP-1) cDNA ampilied by reverse transcription and polymerase chain reaction from total RNA of rats measngial cell. Confirmed by the DNA sequence analysis, cDNA fragments were then inserted to mammalian expression plasmid retroviral vector pLXSN. The recombinant retrovirus vector sense pLXSN-MCP-1 and antisense pLXSN-MCP-1 was verified by restriction endonuclease maping. After the retrovirus transfected PA317 packaging celline and selected with G418, the high-titer retroviral supernatants were obtained. The presence of MCP-1 cDNA in genome of the cells was detected by PCR. The rat glomerular mesangial cells were infected with retroviral supernatants containing sense or antisense MCP-1.

Results: Both sense and antisense MCP-1 cDNA were successfully detected in rat mesangial cells by Sorthern blot. Suppression of MCP-1 expression induced by antisense MCP-1 caused decrease of FN and IV type collagen RNA expression.

Conclusions: Retroviral vector pLXSN can transfer MCP-1 cDNA into mesangial cell. Antisense MCP-1 cDNA suppress MCP-1 expression in mesangial cell.

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P118

MUTATIONS OF *NPHS2* GENE IN SPORADIC STEROID-RESISTANT NEPHROTIC SYNDROME IN CHINESE CHILDREN

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Objectives Of Study: Podocin is encoded by *NPHS2* gene in chromosome 1q25-31, which is exclusively expressed in glomerular podocytes. Mutations of *NPHS2* gene are responsible for autosomal recessive steroid-resistant nephrotic syndrome (SRNS). Several mutations have been reported, which demonstrates that sporadic SRNS is also due to mutations of *NPHS2* gene and prompts possible inter-ethnic and geographical differences in the occurrence of mutations of *NPHS2* gene. The study aimed to screen the mutations of *NPHS2* gene in sporadic steroid-resistant nephrotic syndrome in Chinese children.
Methods: Peripheral blood samples were collected for genetic analysis from 23 Chinese children aged from 2.5 to 14 years old with SRNS. The age at onset of disease was 8.9±4.5 years. Among them, there were 7 patients with renal insufficiency. Genomic DNA was isolated from peripheral blood leucocytes. Eight exons of *NPHS2* were amplified by polymerase chain reaction. Mutational analysis was performed using denaturing high-performance liquid chromatography (DHPLC). DNA fragments with aberrant elution profiles revealed by DHPLC were re-amplified and sequenced directly on both strands.
Results: A heterozygous missense mutation of *NPHS2* gene, L361P (1082T>C) in exon 8, was detected in 1 (4.3%) of 23 patients, however, 1082T>C wasn't found in 106 chromosomes of the adults with normal urinalysis. Six polymorphisms of *NPHS2* gene (288C>T, IVS3-46C>T, IVS3-21C>T, IVS7-74C>G, 954T>C and 1038A>G) were also identified in some of the patients and the adults with normal urinalysis. There was not significant difference on the frequencies of IVS3-46C>T, IVS3-21C>T and IVS7-74C>G polymorphisms between the patients and the 53 controls.
Conclusion: The results demonstrated that mutations of *NPHS2* gene were also the causative gene in sporadic SRNS in Chinese children. The mutation of 1082T>C and the polymorphisms of IVS3-46C>T and IVS3-21C>T are novel. The identification of mutations in the relevant gene will be very important to avoid unnecessary steroid or immunosuppressive drugs administration.

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A NOVEL MUTATION OF *NPHS2* IDENTIFIED IN A CHINESE FAMILY WITH STEROID-RESISTANT NEPHROTIC SYNDROME

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Objectives Of Study: Autosomal recessive steroid-resistant nephrotic syndrome (SRNS) is the subgroup of familial nephrotic syndrome. A causative gene has been identified, that is *NPHS2*, in chromosome 1q25-31, which encodes podocin. Several mutations have been reported, which prompts possible inter-ethnic and geographical differences in the occurrence of *NPHS2* mutation. The study aimed to detect *NPHS2* mutation in a Chinese family with SRNS.
Methods: The proband and her sibling were performed renal biopsy for routine histologic and immunohistochemical investigation and electron microscopic examination. The expressions of podocin, nephrin, α-actinin and WT1 in glomeruli of the proband were detected by indirect immunofluorescence. Peripheral blood samples were collected for genetic analysis from the proband and her parents, and 53 adults with normal urianalysis. Genomic DNA was isolated from peripheral blood leucocytes. Eight exons of *NPHS2* were amplified by polymerase chain reaction. Mutational analysis was performed using denaturing high-performance liquid chromatography (DHPLC) and DNA fragments with aberrant elution profiles revealed by DHPLC were re-amplified and sequenced directly of both strands.
Results: The histologic findings on kidney biopsies were focal segmental glomerulosclerosis (FSGS). In controls, the distribution of staining with P35, rabbit against a human podocin recombinant protein (amino acids 135-383=all the C-terminal part of the protein downstream the transmembrane domain), and P21, rabbit against a human podocin recombinant protein (amino acids 15-89=all the N-terminal part of the protein upstream the transmembrane domain) showed a linear pattern along glomerular capillary walls on glomeruli, and the fluorescent intensity of the staining with P35 was intensely positive. The fluorescent intensity of the staining with P21 was positive. In the proband, the distribution of the staining with P35 showed uneven and nonlinear, and the fluorescent intensity of the staining with P35 was weakly positive. The staining with P21 was negative. The area, location, distribution and fluorescent intensity of the staining with nephrin, α-actinin and WT1 on glomeruli of the proband were the same as those in the controls. The DHPLC elution profiles of exon4 of *NPHS2* from the proband and her parents were aberrant. The chromatograms by sequencing detected in the exon4 of *NPHS2* showed a composite heterozygous mutation of both 467_468insT and 503G>A in the proband. Further analysis approved that the mutation of 467_468insT was the maternal source and the mutation of 503G>A was the paternal source.
Conclusion: The results demonstrate that *NPHS2* mutations were also the causative gene in Chinese FSGS patient, but the mutation of 503G>A is novel. Although now we hardly explain the reason and mechanism of the P35 weakly positive and P21 negative staining in glomeruli in the proband, the abnormal expression and distribution of podocin with the normal expression of nephrin, α-actinin and WT1, may be applied as a marker to screen the familiar FSGS.

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PATHOGENIC GENE "HOT SPOT" MUTATION ANALYSIS OF CHINESE CONGENITAL/FAMILIAL NEPHROTIC SYNDROME

J Ding, Y Shi, JP Huang, JJ Zhang, F Wang, N Guan, JW Ye, JY Yang

Objectives of Study: Congenital/familial nephrotic syndromes mostly manifest as early onset and heavy proteinuria, nephrotic syndrome, resistance to steroid therapy and, rapidly or slowly, progress to end-stage renal disease (ESRD), and often have a poor prognosis. Recent years, several genes associated with them have been cloned, such as NPHS1, NPHS2, ACTN4 and WT1. Their encoding proteins are nephrin, podocin α-actinin-4 and WT1, respectively, which have been identified as proteins of the podocyte slit diaphragm or mainly expressed by podocyte, and play a crucial role to maintain or regulate the structural integrity of the podocyte. Mutations of above genes cause human glomerular disease with massive proteinuria. There have been many reports from other countries that revealed some "hot-spot" mutations among these genes, for example, Fin major (nt121delCT) and Fin minor (R1109X) of two NPHS1 mutations in exon 2, 26 respectively; R138Q, R138X of two NPHS2 mutations in exon 3, R229Q of NPHS2 mutations in exon 5; A682G and C695T mutations in exon 8 of ACTN4 and R394W mutaion in exon 9 of WT1 gene. The present study is designed to screen the "hot-spot" mutations of above genes in nine families of congenital/familial nephrotic syndrome in China, to make clear the mutation frequency of Chinese congenital/familial nephrotic syndromes.
Methods: Clinical and histopathological features of above nine families were analysed. By using PCR amplification, denaturing high-performance liquid chromatography (DHPLC) and direct sequencing, the sequences of "hot-spot" mutations of NPHS2, ACTN4 and WT1 were detected in probands and family members. Above "hot-spot" mutations of NPHS1 were rare in non-Finnish patients, so we didn't screen them.
Results: Three families were diagnosed clinically as congenital nephrotic syndrome according to the disease onset age and clinical presentations. Of them, renal biopsy in II-1 of family A showed diffuse mesangial sclerosis. DNA sequences of reported exon 9 "hot-spot" mutation of WT1 in these families were normal. But renal biopsies of other six families showed FSGS in four families and mesangioproliferative glomerulonephritis in two families. On the basis of genetic pattern and clinical findings, 3/6 families were diagnosed as autosomal recessive FSGS. However, DNA sequences of reported exon 3, 5 "hot-spot" mutations in NPHS2 showed normal. Other three families were diagnosed as autosomal dominant FSGS, and DNA sequences of reported exon 8 "hot-spot" mutation of ACTN4 showed normal.
Conclusion: Chinese congenital/familial nephrotic syndromes were probably not resulted from the "hot-spot" mutations of NPHS2, ACTN4 and WT1 which were reported from other countries. We suspect that there may be some mutations outside the "hot spot" in above genes or, some other novel gene(s) unidentified now might also cause the congenital/familial nephrotic syndrome.

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CHANGES OF SENSITIVE STEREOLOGICAL PARAMETERS OF PODOCYTE FOOT PROCESS IN PAN NEPHROTIC RATS

J Ding, Jh Deng, N Guan, Jj Zhang, Jy Yang

Objectives of Study: The minimal change nephrotic syndrome is the major category of primary nephrotic syndrome occurs in children. The purpose of this study is to investigate the quantitative relationship between proteinuria and the morphological change of glomerular foot process in minimal change nephropathy.
Methods: A well-established rat model induced by puromycin aminonucleoside (PAN) was applied. Using transmission electron microscopy as well as image analysis and taking advantage of morphometric methods, the morphological changes as foot process width, specific surface and volume density of foot processes were studied in different time points after PAN injection. The urinary protein excretion of 24 hours was measured by biuret method. The Pearson correlation analysis was performed to analysis the association of proteinuria and podocyte morphological changes.
Results: At different time points after PAN injection, there was a semblable but not parallel trend between proteinuria and morphological alterations. The twenty-four hours urinary protein excretions increased after 5 days. At 10 days, proteinuria went to the peak level in experimental rats. After 10 days, the proteinuria began to recover. At 20 days, urinary protein excretion returned to the normal level. At the same time, the transmission electron microscopy showed the foot processes became broader from 2 to 5 days after PAN injection. At 10 days, the foot processes became fusion and the slit diaphragms were lost, then the foot processes began to recover at the following days. As to the morphometric parameters, the podocyte foot process widths showed statistical differences between experimental and control groups ($p<0.01$) in different time points. The foot process widths showed no difference between day 2 and day 20. The volume density and the specific surface of the foot process were obviously different between the experimental and control groups ($p<0.01$) in different time points. The surface density was different from day 2 to day 15 between experimental and control groups ($p<0.05$). **)**. In the experimental group, the volume density and specific surface showed no distinct between day 15 and day5 ($p>0.15$). The surface densities showed no differences between day 15 and day 2 ($p>0.1$). The specific surface showed no statistical significance between day 20 and day 2. And a positive correlation was found between protein excretion and foot processes volume density, the Pearson correlation coefficient was $r= 0.9559$ ($p=0.011$). The positive correlation was also showed in protein excretion and foot process width, and the Pearson correlation coefficient was $r=0.9294$ ($p=0.02$). While the relationship between proteinuria and foot process specific surface was negative, the correlation coefficient was $r=-0.9388$ ($p=0.018$).
Conclusion: The volume density and specific surface were
the most sensitive parameters to describe the stereological changes of podocyte foot process in minimal change nephropathy. In addition, a linear correlation existed between the urinary protein excretion and morphological parameters of podocyte in PAN nephropathy rats. Furthermore, the morphological changes presented prior to proteinuria, but the foot process morphology still abnormal when the proteinuria disappeared.

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THE EFFECT OF THE SPECIFIC KNOCKDOWN OF PODOCIN MRNA ON NEPHRIN AND ALPHA-ACTININ IN MOUSE PODOCYTE

J Ding, QF Fan, JJ Zhang, N Guan, JH Deng

Objectives of Study: Recently, the genes for podocin, nephrin and α-actinin were identified in three types of congenital or family nephrotic syndrome. The proteins have been solely expressed in the podocyte, especially, podocin and nephrin have been localized to the podocyte slit diaphragm. Podocin, nephrin and α-actinin have also been found to be abnormal in some acquired nephrotic syndromes and some types of experimental proteinuria. So, the novel podocyte proteins, podocin, nephrin and α-actinin play a critical role in maintaining the integrity of the slit diaphragm and the normal function of the glomerular filtration barrier, but the relationship between these podocyte proteins remains unclear. In this study, we investigate the molecular interaction between podocin/nephrin and α-actinin by the sequence-specific knockdown of podocin mRNA in the mouse podocyte clone (MPC_5) kindly presented by professor Peter Mundel.
Methods: Firstly, the recombinant RNA interference (RNAi) plasmid-p*Silencer* 2.1U6 specifically targeting podocin mRNA was transfected with siPORT *XP-1* into the cultured podocytes, which were divided into three groups, MPC_5 interference group, MPC_5 negative control group and MPC_5 blank control group. Then, the distribution of podocin, nephrin and α-actinin was revealed by immunofluorescence staining and double-immunolabeling under laser scanning confocal microscope. The mRNAs and proteins expressions of podocin, nephrin, α-actinin and GAPDH/β-actin were detected by semi-quantitative RT-PCR and Western blotting. **Results:** The sequence of the insert fragment of the recombinant plasmid was confirmed by sequencing. The fluorescence intensity of podocin and nephrin in interference group was universally and distinctly lower than that in control groups, whereas that of α-actinin showed no appreciable difference between interference group and control groups. The change of the distributions of podocin and nephrin in interference group was revealed by double-immunolabeling compared with control groups. The staining for podocin and nephrin in control groups was distributed around the nuclei and mainly on the cell membrane surface in a filamentous pattern, whereas their staining in interference group was predominantly localized around the nuclei. Compared with control groups, in interference group the decrease of the mRNAs of podocin and nephrin was remarkably detected by about 65% and 70%, respectively. The protein expression of nephrin in interference group was markedly lower than that in control groups, while that of podocin was not nearly detected in interference group. The mRNA and protein expressions, and the distribution of α-actinin in interference group showed no change compared with control groups, which was predominantly localized to the cytoplasm in a filamentous pattern and also extended to the podocyte processes.
Conclusion: With RNAi the expression of podocin was suppressed successfully and the distribution of podocin also changed evidently. The significant decrease of nephrin expression and the changes of nephrin distribution could be detected with the sequence-specific knockdown of podocin mRNA. These results suggest the molecular interaction between podocin and nephrin, but not α-actinin.

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EFFECT OF TETRANDRINE ON EXTRACELLULAR MATRIX IN NEPHROTIC RATS

DONG Xinggang, AN Zengmei, YANG Haichun, et al

Objective: To investigate the effect of tetrandrine on extra cellular matrix in nephrotic rats.
Methods: Sham operative rats were group 1 as control. Nephrotic rat models, made by unilateral renctomy plus Adriamycin injection twice, were divided into group 2(tetrandrine), group 3(amlodipine) and group 4 (untreated model). After 12 weeks, urinary protein, renal histology, the ratio of glomerular ECM square and gloerular square (ECM/GA) were observed.
Results: Pathological changes of group 2,3 were improved compared with group 4. ECM/GA was lower in-group 2 (0.24±0.02); group 3 (0.29±0.01) than that in-group 4 (0.39±0.02) (P<0.05).
Conclusion: Tetrandrine decrease the glomerular ECM of nephrotic rats and attenuate glomerular sclerosis.

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THE STUDY ON EFFECT OF TRIPTERYGIUM WILFORDII GLYCOSIS BY PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PROTEINURIC PATIENTS

Dong Xinggang, An Zengmei.

Purpose of the study: To evaluate the role of apoptosis on the pharmaccologyic effects of Tripterygium Wilfordii Glycosides (TWG) in proteinuric patients.
Methods: 30 patients in glomernlonephritis with proteinuria were randomized to two groups, control and TWG groups using TWG (1mg/kg/d) for 14days. Apoptosis of peripheral blood mononuclear (PBMC) was detected by gel electrophoresis, HE staining and flow cytometry.
Results: After treatment by TWG, there was typical nuclear condensation and fragments, "DNA ladder" was viewed on agarose gel, "Sub G1" peak was recorded in DNA histogram of flow cytometry at two weeks.
Conclusions: These results suggest that TWG used to treat proteinuric patients can induce apoptotic of PBMC and may be one of the mechanisms for immune inhibition.

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THE EFFECT OF IκBα ON THE MODULATION OF NUCLEAR FACTOR KAPPA B IN THE PERIPHERAL BLOOD MONONUCLEAR CELLS OF CHILDREN WITH STEROID RESPONSIVE SIMPLE NEPHROTIC SYNDROME AND THE ROLE OF IκBα IN IMMUNOSUPPRESSION OF GLOCOCORTOIDS

Guo Yan-Nan, Wang Zheng, Tao Yu-Hong, Li Chan-Sheng

Objective: To study the mechanism of modulation of nuclear factor kappa B (NF-κB) in triggering steroid response simple nephrotic syndrome (SRSNS) via detecting the expression of inhibitory kppa B alapha (IκBα) in the peripheral blood mononuclear cells (PBMCs) of children with SRSNS and investigating its relationship with NF-κB. To attempt to comprehend the role of action IκBα in the suppression of NF-κB with glucocortoids.
Methods: The expression of IκBα was detected by real-time fluorescence reverse transcriptase-PCR (RT-PCR) and western blot analysis. Expression of NF-κB was measured by electrophretic mobility shift assay (EMSA). The relationship between the activation of NF-κB and the levels of IκBα protein was observed.
Results: The expression of IκBα in PBMCs from children with SRSNS in the active stage was lower than not only that of nephritis nephrotic syndrome and secondary nephrotic syndrome in the active stage but that of normal controls and children with SRSNS in the remission stage (P<0.05). Statistically, there is no significant difference between the expression of IκBα in PBMCs from children with SRSNS in the remission stage and that from the normal controls (P>0.05). Compared with the remission of SRSNS, the nephritic nephrotic syndrome, the secondary glomerular disease and the healthy control, the activation of NF-κB in PBMCs of the patients with SRSNS at the active stage was much higher statistically (p<0.05). There was a positive correlation between the IκBα mRNA and the IκBα protein the active stage of SRSNS (r=0.921,p<0.001), but there was a negative correlation between the elevated NF-κB and the decreased IκBα protein at the same time(r=-0.884,p<0.001). Following glucocortoid therapy, the expression of IκBα in PBMCs from children with SRSNS was higher significant than before the using of glucocortoid (P<0.05).
Conclusions: Reduced IκBα expression in PBMCs of children in the active stage has involved in the activation of NF-κB, which may be one of the most important ways of triggering SRSNS by respiratory tract viruses. It is shown that glucocortoid induces the transcription of the IκBα gene, which results in increasing IκBα protein syntheses and reducing the activation of NF-κB.

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INHIBITION OF GENE EXPRESSION OF RESPIRATORY TRACT VIRUSES IN THE PERIPHERAL BLOOD MONONUCLEAR CELLS OF STEROID RESPONSIVE SIMPLE NEPHROTIC SYNDROME WITH ANTISENSE OLIGONUCLEOTIDES

Guo Yan-Nan, Wang Zheng, Zhu Xiao-Shi

Objective: By the inhibition of the gene expression of respiratory tract viruses in the peripheral blood mononuclear cells (PBMCs) of steroid responsive simple nephrotic syndrome (SRSNS) with antisense oligonucleotides (ASONs), we have attempted to provide more proof for the infection of respiratory tract viruses in SRSNS and study the role of viruses in triggering of the disease.
Methods: After transfection by ASONs to PBMCs, the gene expression of respiratory tract viruses was detected by RT-PCR; the expression of NF-κB was detected by electronphoretic mobility shift assay (EMSA).
Results: After transfection by ASONs to PBMCs, the gene expression of respiratory tract viruses was apparently inhibited in the active stage of SRSNS. The gene expression of 14 cases was inhibited (92.3%) in 15 cases of SRSNS with the positive gene expression of respiratory syncytial virus (RSV). The gene of 7 cases was inhibited (87.5%) in 8 cases of SRSNS with influenza virus (Flu). The positive rate of the gene expression of respiratory tract virus was increased significantly in the active stage of SRSNS (including RSV 46.9%, Flu 25.0%) compared with in the remission (0/10), the nephritic nephrotic syndrome (3/12), the purpura nephritis (1/13), the bronchiolitis (15/20) and the normal control (0/10) (p<0.05). After transfection with ASONs of viruses, the expression of NF-κB was inhibited significantly in the active stage of SRSNS compared with the control and the group transfected with SONs (p<0.05).
Conclusion: The study demonstrated that the infection of respiratory tract viruses actually existed in SRSNS in the active stage, and the gene expression of the viruses was inhibited by ASONs that may result in the activation of NF-κB way was interrupted. Therefore we suggested that the infection of respiratory tract viruses be one of the triggers of onset of SRSNS.

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THE EXPRESSION OF NUCLEAR FACTOR-KAPPA B IN PERIPHERAL BLOOD MONONUCLEAR CELLS AND THE LEVEL OF INTERLEUKIN -8 IN PLASMA OF THE CHILDREN WITH STERIOD RESPONSIVE SIMPLE NEPHROTIC SYNDROME

Guo Yan-Nan, Wang Zheng, Li Chan-Sheng,

Objective: This study aimed to study the expression of nuclear factor-Kappa B (NF-κB) in peripheral blood mononuclear cells (PBMCs) and the level of interleukin (IL)-8 in plasma of the patients with steroid responsive simple nephritic syndrome (SRSNS), and the relationship between NF-κB and IL-8. We attempt to insight into the molecular mechanisms on activating of T-lymphocytes by the respiratory tract viruses and triggering of MCNS by the activated T-lymphocytes. We also attempt to reveal the role of the respiratory tract viruses in the triggering of MCNS by the viral transactivation of trsanscription..
Methods: The expression of NF-κB of PBMCs was detected by electrophoretic mobility shift assay (EMSA), and the level of IL-8 in plasma was measured by emyme-linked immunosorbent assay (ELISA).
Results: (1) Compared with all of the healthy control, the expression of NF-κB of PBMCs of the patients with SRSNS in active stage was statistically elevated ($p<0.05$). Compared with SRSNS in convalescence and remission, the expression of NF-κB of SRSNS in the active stage was also significant statistically elevated ($p<0.01$). (2) Compared with the healthy control, the level of IL-8 in plasma of the patients with SRSNS in the active stage was significant statistically elevated ($p<0.001$). There was a positive linear correlation between the expression of NF-κB and the level of IL-8 in the active stage of SRSNS ($r=0.8768$, $p<0.001$). (3) The expression of NF-κB and the level of IL-8 in the patients with the first onset of SRSNS who had not taken glucocorticoids (GCs) were more significant elevated than those of SRSNS in the relapse had taken GCs. (4) There was a positive correlation trend between the expression of NF-κB and the gene expression of the respiratory tract viruses in PBMCs of SRSNS in the active stage. (5) Otherwise, a negative linear correlation existed between the expression of NF-κB and the level of inhibitory Kappa-B (IκB) α and IκBα mRNA in cytoplasma of PBMCs of SRSNS in active stage.
Conclusion: The elevated expression of NF-κB in PBMCs of the patients in the active stage of SRSNS has close relationship with the level of the IL-8 in plasma. The activated and regulation of NF-κB plays the very important role in the triggering of SRSNS by respiratory tract viruses. We speculate that the viral transactivation of transcription through the activating of NF-κB of T-lymphocytes is the key link of the episode and the recurrence of SRSNS.

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THE GENE, ANTIGENS AND ANTIBODIES OF RESPIRATORY TRACT VIRUS OF THE CHILDREN WITH STEROID-SENSITIVE SIMPLE NEPHROTIC SYNDROME

Guo Yan-Nan, Wang Z, Mu Li-Fang

Objective: To investigate the relationship between the infection of respiratory tract viruses and the onset of steroid-responsive simple nephrotic syndrome (SRSNS).
Methods: The gene expression and antigens of respiratory tract viruses in the peripheral blood mononuclear cells (PBMCs) from 26 children with SRSNS were detected by reverse transcription polymerase chain reaction (RT-PCR) and APAAP method, respectively. Meanwhile, the antibodies of respiratory tract viruses of the children with SRSNS were also examined by Elisa test.
Results: In comparison with the remission of SRSNS and the normal controls, the positive rate of the gene expression, antigens and antibodies of the respiratory tract viruses in the relapse were significantly increased ($P<0.05$). The percentage of the genes, antigens and antibodies of the respiratory tract viruses were closely correlated with the urinary protein in SRSNS ($P<0.05$). There was no relationship between the gene expression and antigens of the viruses of PBMCs and the use of prednison in SRSNS, but the positive rates of the antibodies in sera were closely related to the use of prednison.
Conclusion: The study demonstrated that the onset or relapse of SRSNS is related to the infection of the respiratory tract viruses, and the infection may be one of the important triggers of this disease, but its mechanism needs to be confirmed by further studies.

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HLA CLASS II ANTIGENS AS RISK FACTORS FOR THE FREQUENT RELAPSES IN THE CHILDREN WITH STEROID RESPONSIVE NEPHROTIC SYNDROME

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Objectives: The aim of this study is to identify the type of HLA class II antigen as the risk factors for the frequent relapses in the children with the steroid responsive nephrotic syndrome
Methods: HLA class II antigens were examined in 40 frequent relapses nephrotic syndrome patients and 84 children as controls at Hasan Sadikin General Hospital, Jakarta. DNA isolation was performed in Medical Research Unit, Padjadjaran University, Bandung. HLA class II antigens were examined with sequence specific oligonucleotide (SSO) method in Leiden University Medical Center, the Netherlands. Chi square test were used to see the proportion of HLA class II antigens in the frequent relapses nephritic syndrome and control group. The risk for frequent relapses nephritic syndrome was expressed by odds ratio.
Results: The risk for frequent relapses in children with HLA-DQB1*02, HLA-DRB1*03, HLA-DRB1*04, HLA-DQB1*04 are 3.43 ($p=0.002$), 4,43 ($p=0.034$), 4.43 ($p=0.035$), 12.06 ($p=0.013$), consecutively. In children with HLA-DRB1*12 and HLA-DQB1*0301P the OR are 0.34 ($p=0.013$) and 0.35 ($p=0.013$), consecutively.
Conclusion: We conclude that HLA-DQB1*02, HLA-DRB1*03, HLA-DRB1*04, HLA-DQB1*04 are the risk factors for the frequent relapses nephritic syndrome in children, and - HLA-DRB1*12 and HLA-DQB1*0301P have protective effect.

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FUNCTION OF PAX2 RE-EXPRESSION IN CHILDREN WITH PRIMARY NEPHROTIC SYNDROME

Hui-Qiong Zhang, Zhu-Wen Yi, Xiao-Jie He

Objectives of study: To evaluate the expression and function of Pax2 in children with primary nephrotic syndrome and provide the research foundation for the mechanism of steroid-resistance.
Methods: According to the results after glucocorticoid therapy, we divided 40 cases into two groups.20 cases were steroid-responsive and 20 were steroid-resistant. To investigate the expression of Pax2, PCNA, P53 and cell apoptosis in children with primary nephrotic syndrome by immumohistochemistry was made a analysis for the function and mechanism of Pax2 re-expression in steroid- resistance.
Results: Pax2 re-expression showed in all renal tissues of primary nephrotic syndrome. The correlation analysis indicated that Pax2 proper expression in the group of steroid-responsive showed positive correlation with PCNA expression, negative correlation with cell apoptosis. There was no expression of P53 mutation. In the group of steroid-resistant, Pax2 over-expression had no correlation with PCNA expression. Parts of patients showed the expression of P53 mutation and the P53 mutation had a positive correlation with Pax2 expression. Cell apoptosis showed negative correlation with P53 mutation.
Conclusions: The proper expression of Pax2 may repair the pathological changes of renal tubulointerstitial through accelerating cell proliferation and restraining cell apoptosis. The over-expression of Pax2 maybe lead to P53 mutation. That P53 mutation lost its normal activity and reduced cell apoptosis made the repair defeat of damage renal tubule, which might be one of steroid-resistance mechanisms.

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POLYMORPHISM OF INTERLEUKIN-4 ALPHA CHAIN GENE IN JAPANESE CHILDREN WITH MINIMAL CHANGE NEPHROTIC SYNDROME

Y Ikeuchi, Y Kobayashi, H Arakawa, K Tamura, M Suzuki, K Tokuyama and A Morikawa.

Objective of Study: Minimal change nephrotic syndrome (MCNS) in children frequently is associated with allergy and immunoglobulin E production. T helper subtype 2 cytokines, such as interleukin-4 (IL-4) and IL-13, may have an important role in the development of atopy. We have reported previously the associations of polymorphisms of IL-4 related genes with MCNS. We investigated the association of genetic variation of interleukin-4 receptor (IL-4R) gene with MCNS in this study.
Methods: We analyzed the polymorphism in Japanese children with MCNS (n=80: male=50, female=30) and healthy controls with neither allergic nor renal disease (n=61). The polymerase chain reaction (PCR) with two pairs of primers was used for the IL-4R alpha chain gene polymorphism (Ile50Val).
Results: There was a significant difference between the MCNS group and controls in both genotypic and allelic distribution of IL-4R gene polymorphism. The frequency of the Ile allele was significantly lower in the MCNS group than controls. We could not find the differences when we analyzed the MCNS group according to their clinical feature (age of onset, treatment, number of relapse, serum IgE level).
Conclusion: The result suggests that Ile50Val variations in the IL-4R alpha chain genes may be associated with predisposition to MCNS and that in MCNS patients IL-4 may not be involved in IgE synthesis because Ile50 variant was reported upregurates receptor response to IL4 and significantly associated with raised total serum IgE.

Table 1. Allelic and genotypic distribution of IL-4R alpha chain gene in Japanese unrelated controls and patient with minimal change nephrotic syndrome.

	Allele		Genotype		
	I	V	II	IV	VV
Control	70	52	15	40	6
MCNS	61	99	15	31	34
		P<0.01			P<0.001

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RENOPROTECTIVE EFFECTS OF MATRINE ON EXPERIMENTAL GLOMERULOSCLEROSIS IN RATS

Jin Yu, Zhang Hongwen.

Objective: To observe the renoprotective effects of matrine(Mat) on experimental glomerulosclerosis in rats.
Methods: The rats were randomly assigned to following groups: normal control group, model control group, benazepril treatment group, matrine 100mg/kg treatment group and matrine 50mg/kg treatment group. The rats of normal control group were subjected to sham operation and were injected with normal saline after one week through the tail vein. The rats of other groups were uninephrectomized and injected adriamycin (5mg/kg) after one week through the tail vein. The dose of benazepril was 6mg/kg. The level of urinary protein was measured at the 2nd, 4th and 6th week after operation, serum total protein and albumin, serum creatinine (Scr), blood urea nitrogen (BUN) were measured only at the 6th week after operation. Renal pathology changes were evaluated at the 6th week as well. Immunohistochemistry was used to examine the expression of Fibronectin (FN), Laminin (LN), connective tissue growth factor (CTGF) and transforming growth factor-β1 (TGF-β1) in glomeruli.
Results: Matrine and benazepril not only reduced urinary protein, Scr and BUN, but also significantly ameliorated glomerular mesangial proliferation and glomerular sclerosis (P<0.05, respectively). Immunohistochemistry staining indicated that there was an increasing FN, LN, CTGF and TGF-β1 expression in model control group as compared to the three treatment groups (P<0.05). Matrine 100mg/kg treatment group and benazepril treatment group show some advantages over matrine 50mg/kg treatment group (P<0.05), but there were no different between the former two groups (P>0.05).
Conclusion: Matrine has a renoprotective effect on experimental glomerulosclerosis in rats.
Key words: Matrine; Benazepril; glomerulosclerosis; Extracellular matrix

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TUMOR NECROSIS FACTOR GENE $G^{-308}A$ POLYMORPHISM IN CHILDREN WITH NEPHROTIC SYNDROME

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Objectives of Study. The pathogenesis of childhood nephrotic syndrome (NS) and whether it responds to steroid therapy remains unpredictable. Recent knowledge indicates the possible involvement of a T lymphocyte dysfunction with altered cytokine network. Tumor necrosis factor (TNF) is a proinflammatory cytokine already implicated in the severity of many immune mediated diseases, including NS. Moreover, recent evidence suggests that altered TNF production and/or receptor expression may be play a role in determining the clinical response to steroids in childhood NS. The guanine to adenine transition at site -308 of the TNF gene ($G^{-308}A$) has been shown to be associated with enhanced cytokine production. Therefore, it is of interest to estimate the association of this polymorphism with steroid responsiveness/resistance of the disease.
Methods. The $G^{-308}A$ genotype of the TNF alpha gene was determined in 102 children with NS as well as in randomly chosen healthy controls using polymerase chain reaction and restriction fragment length polymorphism methodologies from a 3 ml blood sample. Patients were retrospectively categorized into two groups depending on their response to steroids: 78 of them had steroid sensitive NS (SSNS, proteinuria free following 4 weeks of steroid treatment), while in the remaining 24 the NS was resistant to steroid treatment (SRNS, failure to achieve response after 4 weeks of treatment with standard dosage). The SRNS group included 7 patients with steroid resistant minimal change NS and 17 with focal segmental glomerulosclerosis. The distribution of the genotypes was compared between cases and controls. Significance of differences in the distribution of genotypes was calculated using chi-squared test. The odds ratio (OR) and its 95% confidence interval (CI) were estimated using the exact method.
Results. The prevalence of the TNF alpha A^{-308}allele in patients with NS did not differ significantly from that in the controls (26,4% vs. 22,0%, n. s.). There was, however, a significant difference in the carrier frequency of the A^{-308}allele between the SRNS group and the SSNS group (12/24, 50,0% vs. 15/78, 19,2%, OR: 4,2, CI: 1,64-11,17, p< 0,01). We did not find significant differences between these two patient groups as to their gender distribution, age and age at onset of NS.
Conclusion. Steroid sensitivity is the major determinant of prognosis in childhood NS. Elevated TNF alpha gene expression and synthesis has been found in peripheral blood mononuclear cells (PBNC) taken from children with NS. Moreover, it has been suggested that increased in vitro TNF production from PBMC could be used to discriminate between children with SRNS and SSNS (*Bakr et al, Pediat Nephrol 18, 2003, 516*). Our results are in line with these findings by providing additional support to the hypothesis, that TNF-alpha might be involved in the pathological events that occur in non-inherited forms of childhood NS. Although genotyping reveals an approximately 4-fold increased risk of developing SRNS in carriers of the TNF A^{-308}allele, it may not be sufficient to predict the pathological type of NS and the response of these patients to steroid therapy. However, another prospective study of more patients should be performed to validate these results.

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CHANGE OF TGFβ IN PBMC OF CHILDREN WITH NEPHROTIC SYNDROME AND ITS SIGNIFICANCE

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Objective of study: Recent studies indicate that transforming growth factor (TGFβ) is the main cytokine involved in glomerular disease. It plays an important role in the development of glomerulosclerosis. To study changes and significance of TGFβ in children with idiopathic nephrotic syndrome (INS)

Methods: Totally 35 cases with INS (13 males, 22 females) were studied. The active stage group had 35 cases and the remission stage groups were 25 cases. The cases in active stage groups had first onset of the disease with obvious clinical symptoms and abnormal laboratory findings without use of corticosteroids. TGFβwas detected by ELISA in peripheral blood monoeuclear cell (PBMC) culture medium. The TGFβmRNA gene expression was measured by in-situ PCR in PBMC.

Results: (1) Concentration of TGFβ and TGFβmRNA expression in active stage of simple type or nephritis type INS were higher than those of remission stage and control (P<0.01). Concentration of TGFβ and TGFβ mRNA expression in remission stage were higher than that of control (P<0.05). (2) Concentration of TGFβ and TGFβ mRNA expression after therapy was clearly lower than that before therapy in steroid responsive group (P<0.05). Whereas no significant change was seen in steroid resistant group. Both indicators were higher in steroid resistant group than in steroid responsive group whether before or after therapy.

Conclusion: TGFβ may play an important role in the mechanism of INS and its level in PBMC can be used as an immunological indicator for the illness state, therefore, determination of TGFβ level and mRNA may be of some clinical significance.

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THE EFFECTS OF PREDNISONE ON THE MRNA EXPRESSIONS AND THE SEMI-QUANTITY OF PODOCIN IN GLOMERULUS OF ADRIAMYCIN-INDUCED NEPHROPATHIC RATS

Lu Ling, Wang Mingli, Deng Fang , Hu Xu.

Objective: To investigate the possible role of podocin on proteinuria and the effects of Prednisone on the mRNA expression and the quantity of podocin in Glomerulus of Adriamycin-induced nephropathic rats.
Methods: SD male rats (1- month- old ,weighing around 150g) with 24 hrs urinary protein surpassing 30mg were chosen as nephropathic rats by injecting adriamycin 5mg/kg for one time.The rats were randomly divided into three groups (normal control group, nephropathy control group and nephropathy Prednisone-treated group, n=8) .After treated with Prednisone 10mg/(kg.d) by orally for 4 weeks, urinary protein and blood were checked and the rats were then killed to acquire the renal cortex for the mRNA expressions of podocin with the semi-quantity RT-PCR ,and the semi-quantity and location of Podocin with western blot and immunohistofluorescence.
Results: The relative value of Podocin protein in normal control group, nephropathy control group and nephropathy Prednisone-treated group was 5.26±1.19,0.00±0.00,2.46 ±1.32, respectively (P<0.001) with western blot .There was not any expression of Podocin protein in the nephropathy control group; Prednisone could increase the expression quantity of Podocin protein. With immunohistofluorescence the fluorescence intensity of Podocin in glomerulus decreased prominently in nephropathic rats compared with normal control group(P<0.001) while increased in predisone-treated nephropathic rats compared with untreated those(P<0.001).other location is negative.The relative values of Podocin mRNA expression in the three groups (normal control, nephropathy control, Prednisone -treated groups) were 1.06±0.19, 1.96±0.98, 1.31±0.24, respectively. The Podocin mRNA expressions in the nephropathy control group increased significantly in comparison with the normal control group (P<0.05). Prednisone -treated group decreased significantly in comparison with the nephropathy control group(P<0.05) .
Conclusion: There were obvious abnormities in the quantity and mRNA expression of podocin in Glomerulus of Adriamycin-induced nephropathic rats. the reduction of podocin is likely to be one of important facts in the production of proteinuria in Adriamycin-induced nephropathic rats. it is possible that prednisone alleviated proteinuria by affecting podocin in the glomerulus .

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NFκB P65 ANTAGONIZES THE IL4 INDUCTION BY C-MAF IN MINIMAL CHANGE NEPHROTIC SYNDROME

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Objectives of Study: Mechanisms underlying the pathophysiology of Minimal Change Nephrotic Syndrome, the most frequent of glomerular diseases in children, remains elusive although recent arguments suggest that T cell dysfunction may be involved in the pathogenesis of this disease. Recently, we reported that activated T cells of these patients display a downregulation of IL12 receptor β2 chain, suggesting an early commitment toward Th2 phenotype. By subtractive and differential screening of genes upregulated in MCNS relapse, we identified the short form of the proto-oncogene c-maf, a known activator of the IL4 gene. During relapse, c-maf translocates to nuclear compartment and binds to DNA responsive element. Unexpectedly, the nuclear localization of c-maf did not promote the IL4 gene transcription in relapse. The aim of this study is to understand the molecular mechanisms underlying the lack of IL4 induction by c-maf in MCNS relapse.
Methods: we analyzed the IL4 promoter activity in T cell Jurkat transfected with c-maf alone or in combination with NFkB RelA/p50 subunits expression vectors. We determined by mobility shift assays using nuclear extracts from MCNS patients and controls, the nature of protein complexes bound to NFkB and c-maf responsive sites on IL4 promoter
Results and conclusion: we found that RelA blunts IL4 induction in T cells during the relapse in these patients. We demonstrate that the *ex vivo* inhibition of proteasome activity in T cells from relapse, which blocks NF□B activity, strongly increases the IL4 mRNA levels. Overexpression of c-maf in T cells induces a high level of IL4 promoter driven luciferase activity. In contrast, coexpression of c-maf with NF□B RelA/p50, or RelA, but not p50, inhibits the c-maf-dependent IL4 promoter activity. Finally, we demonstrated that in T cell overexpressing RelA and c-maf, RelA expelled c-maf from its DNA binding site on IL4 gene promoter, which results in active inhibition of IL4 gene transcription. Altogether, these results suggest that the involvement of c-maf in Th2 commitment in MCNS operates through IL4 independent mechanisms.

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ALTERED CHOLESTEROL METABOLISM IN RATS WITH SPONTANEOUS FOCAL GLOMERULOSCLEROSIS

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Objectives of study: We investigated key factors involved in cholesterol metabolism. Imai rats exhibit spontaneous FGS (focal glomerulosclerosis), which is marked by heavy proteinuria, severe hyperlipidemia, and progressive renal insufficiency. In an earlier study, we reported severe skeletal muscle and adipose tissue lipoprotein lipase, and VLDL receptor deficiencies, which account for elevated plasma VLDL and triglycerides in Imai rats at 34 weeks of age.
Methods: Male Imai and SD control rats were fed a regular rat chow and observed from age 8 through 34 weeks. HMG-CoA reductase, cholesterol 7 α-hydroxylase, LDL receptor and ACAT were measured by Western blot and plasma LCAT protein was measured by ELISA.
Results: At 34 weeks of age, the Imai rats showed severe proteinuria, hypoalbuminemia, 60% reduction in GFR, elevated plasma total and LDL cholesterol and LDL/HDL ratio. Imai rats showed a twofold elevation of hepatic HMG-CoA reductase, the rate-limiting step in cholesterol biosynthesis, but no significant change in cholesterol 7 α-hydroxylase, the rate-limiting enzyme in cholesterol catabolism to bile acids. This was accompanied by and largely due to a threefold down-regulation of hepatic LDL receptor, which limits hepatic uptake of LDL; and a threefold up-regulation of hepatic ACAT (P< 0.01), which augments esterification of hepatocyte free cholesterol, thus, limiting cholesterol-mediated feedback regulation of cholesterol synthesis and catabolism. Moreover, plasma LCAT concentration was severely depressed (by fourfold) in Imai rats. This abnormality can impair HDL-mediated cholesterol transport from extrahepatic tissues to the liver.
Conclusion: The study revealed marked abnormalities of the key proteins involved in regulation of hepatic cholesterol metabolism. These abnormalities can account for severe dysregulation of cholesterol metabolism in Imai rats with spontaneous FGS, which closely resembles FGS in humans.

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FIBROBLAST GROWTH FACTOR-2 AMONG CHILDREN WITH MINIMAL CHANGE NEPHROTIC SYNDROME

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Objectives of Study: The main pathological hallmark of minimal change nephrotic syndrome (MCNS) is loss of podocyte. Fibroblast growth factor-2 (FGF-2), a key factor, participates in podocyte damage. We examine whether FGF-2 may relate to the recurrence of MCNS.
Methods: The plasma concentrations, urinary excretion and renal distribution of FGF-2 during the course of MCNS were determined, and changes were correlated with clinical and laboratory feature of the disease. Renal biopsy samples were collected from 50 children with MCNS, serial plasma and urine samples were collected from 20 children among them, during the initial (heavy proteinuria in the beginning) and steroid sensitivity (SS) (proteinuria turn to negative after take enough steroid in 8 weeks) and steroid resistent (SR) (proteinuria persist positive after take enough steroid for 8 weeks) and recurrent (appear proteinuriea again in maintain treatment) phases of the disease. 50 children were divided into two groups: SS group and SR group. Among SS group, another two groups were setup with recurrent time: frequent relapse group and non-frequent relapse group. FGF-2 level in plasma and urine were determined with enzyme-linked immunosorbent assays (ELISA). FGF-2 level in renal were determined with immunohistochemistry, and were quantitative analyzed with IMS color image analysis system.
Results: FGF-2 was distributed in podocyte cytoplast along capillary vessel, and in epithelia cytoplast of renal tubule. Positive index (PI) was used expressing sediment degree of FGF-2 in glomerulus. PI level of frequent relapse group (n=18) was higher than that of non-frequent relapse group (n=22) (P<0.001). PI level was correlated to urinary excretion of transferring, albumin and retinol-binding protein (P<0.001, P<0.05, P<0.05, respectively). There was an acute decrease in urinary FGF-2 excretion (P<0.05) during SS phase, which return to higher level (P<0.05) during recurrent phase. Furthermore, there was a progressive increase during SI phase. Plasma FGF-2 concentrations had not obvious change.
Conclusions: Measurements of urinary excretion and renal distribution of FGF-2 among patients with MCNS may be useful indices for calculating recurrent, monitoring course of the disease and guiding scientific treatment.

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GENE EXPRESSION OF HEPARANASE IN PERIPHERAL BLOOD LEUKOCYTES OF NEPHROTIC SYNDROME AND RELEVANCE FOR PROTEINURIA

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Objective: The β-D-endoglycosidase heparanase (Hpa) is HS-specific, which leads to heparan sulfate (HS) degradation. An increased permeability of the GBM for proteinuria is related with decreased HS side chains of the GBM. The purpose of this study was to evaluate the role of Hpa in the development of steroid responsive simple nephrotic syndrome (SRSNS) in children and adriamycin (ADR)-induced nephrotic syndrome (NS) of rats and relevance for proteinuria.
Methods: (1) Forty-three children with SRSNS were selected for the detection of Hpa of peripheral blood leukocytes, including the active stage of SRSNS (n=23), the restoration (n=10), the remission (n=10). The peripheral blood leukocytes (PBLs) from 23 children with nephritic nephrotic syndrome, 15 purpura nephritis and 15 healthy children were served as the control. (2) NS modal was induced in 150~200g Sprague-Dawley rats (n=6 per group) by tail intravenous injection of 6 mg/kg ADR. Rats were housed in metabolic cage for 24 hours to collect urine prior to experiment and at day 7 and 14 following tail intravenous injection of ADR. With the method of reverse transcriptase-PCR (RT-PCR), Hpa gene from PBLs of these groups was assayed respectively.
Results: (1) All of NS showed higher levels of Hpa mRNA than the healthy group ($p<0.05$). The active stage of SRSNS (1.2747±0.3614) was the highest one of all ($p<0.001$). In contrast with the healthy group, the restoration of SRSNS was significant higher ($p<0.05$), but the remission has no difference. There was a significant correlation between the level of Hpa mRNA and the amount of urinary protein in SRSNS and NS model. As the expression level of Hpa mRNA was higher, the amount of urinary protein was more ($r_s=0.751$, $p<0.001$). But there was no difference of Hpa mRNA and urinary protein between the first onset and the recurrent children of SRSNS. (2) The sequence of cDNA of NS modal from RT-PCR amplification was consistent with that of heparanase mRNA of rat in GenBank (accession number AF184963); □The rats at day 7 and 14 showed marked up-regulated of Hpa mRNA and had significant difference in contrast to the normal rats. The levels of Hpa mRNA from the leukocytes of rats at day 14 were the highest (1.1339±0.2211), then at day 7 was 0.6601±0.1724, and the normal was 0.3578±0.1235. The level of Hpa mRNA in these groups showed a linear tendency ($F=57.771$, $p<0.001$); □There was a significant correlation between the level of Hpa mRNA and the amount of urinary protein ($r_s=0.925$, $p<0.001$).
Conclusion: The up-regulated expression of Hpa mRNA may be an important contributor to loss of glomerular negative charge and leads to proteinuria in nephrotic syndrome. The higher is the Hpa mRNA expression, the more the amount of urinary protein is. There was the consistent result of Hpa mRNA in SRSNS of children and the modal of ADR-induced nephrotic syndrome.

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STUDIES THE EFFECT OF CATECHIN ON FREE RADICALS AND THE EXPRESSION OF ENDOTHELIN IN RATS WITH NEPHROTIC SYNDROME

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Objectives of Study: To study the effect of catech on free radicals and the expression of endothelin in rats with nephrotic syndrome.

Methods: Thirty-six female SD rats were randomly divided into six groups , control, nephrotic, dexamethasone-treated, catechin-prevented, catechin-treated, dexamethasone plus catechin-treated groups. at the end of experiment, the excretion of 24hrs urinary protein and the concentrations of·OH, MDA were detected by means of biochemistry assay. The concentrations of the plasma and renal cortex endothelin(ET) concentrations were detected by means of Radioimmunoassay(RIA). A semiquantitative score was used to evaluate the injury degree of glomerular and tubulointerstitium.

Results: At the end of experiment, serum·OH , MDA, BUN, TG concentrations(all $P<0.01$) , the renal cortex and plasma ET concentrations($P<0.01$), the excretion of 24hrs urinary protein ($P<0.05$) in the catechin-prevented group were lower and serum Alb concentrations($P<0.01$) were higher than that in the nephrotic group . serum MDA($P<0.01$), TG concentrations($P<0.05$) , the renal cortex and plasma ET concentrations (all $P<0.01$) in the catechin-treated group were lower and serum Alb concentrations($P<0.01$) were higher than that in the nephrotic group . serum·OH , MDA, BUN, TG concentrations(all $P<0.01$) , the renal cortex and plasma ET concentrations($P<0.01$) in the dexamethasone plus catechin-treated group were lower and serum Alb concentrations($P<0.01$) were higher than that in the dexamethasone-treated group. Serum ·OH concentrations were positively correlated well with the renal cortex and plasma ET concentrations ($P<0.01$) . Compared with nephrotic group, the renal pathologic score were significantly different among the catechin-treated group ($p<0.05$), catechin-prevented group ($p<0.01$), dexamethasone-treated group ($p<0.01$), and dexamethasone plus catechin-treated group ($p<0.01$).

Conclusion: Catechin could eliminate ·OH and MDA, decrease the renal cortex and plasma ET concentrations, ameliorate renal function, alleviate progressively renal injury.

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IDENTIFYING NOVEL GENES AND MOLECULAR MECHANISMS IN CHILDHOOD MINIMAL CHANGE NEPHROTIC SYNDROME (MCNS)

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Objectives of Study: to identifying novel genes and molecular mechanisms of MCNS in children by studying renal tissue gene expression profile.
Method: (1) patient group were set up with renal tissues from 7 cases of MCNS diagnosed by renal biopsy. Since the renal tissue available was limited for RNA needed for extraction, the 7 pieces of tissues were further divided into 3 groups (3, 2,and 2 each). The control normal group consisted of 2 sub-groups from the normal renal tissue far off the pathological sites in congenital hydronephrosis and tumor patient. Renal tissues were immediately put into RNAlater Stablization Reagent manufactured by QIAGEN Co. and stored; (2) extraction of RNA, reverse transcription into cDNA, and in vitro transcription and synthesis of single stranded cRNA probe were performed; (3) synthesized cRNA probe were hybridized with 5 pieces of HG-U133A oligonucleotide chips provided by Affymetrix Co, which including equivalent to 18,400 known human genes. They were hybridized for a total of 5 times in patient groups and control group; (4) comparison was carried out between the expression profile of each patient group and control groups respectively, cross-comparative analysis and comprehensive analysis in three kinds of signals, i.e. increased, decreased and without changes, and their intensity were carried out. (5) confirmation of some novel genes were done by RT-PCR and Immunohistochemical studies.
Results: (1) differentially expressed genes were identified by calculating generalized *t* tests ($P < 0.001$). In the 18,400 known human gene, 47 over-expressed genes and 173 low expressed genes were singled out from the 6 comparisons in the results of expression profile; (2) a number of these genes were novel with respect to MCNS in children, while others are known to be involved in cell communication, cell growth, cytokine functions, signal transduction, and so on; (3) human angiopoietin-like 3 (ANGPTL3) gene was further studied by real-time RT-PCR in renal tissues from 27 patients with MCNS, which was significantly higher than that in the renal tissues from 8 isolated hematuria patients. In Wistar rats model with nephrosis induced by adriamycin we found that ANGPTL3 showed significantly increased expression than normal controls both in epithelium of glomerular and renal tubules by Immunohistochemical study; (4) Apolipoprotein H (ApoH) , another new gene, also showed positive expresstion in renal tubules, especially in proximal tubules by Immunohistochemical staining in renal tissues of MCNS patients.
Conclusion: This study represented an important step in identifying new genes and molecular mechanisms in childhood MCNS by renal tissue gene expression profile. ANGPTL3 regulates lipid metabolism and is related to regulation of angiogenesis. ApoH have an effect on endothelium activation. The present data suggest that further study on these novel genes might be helpful in understanding the pathogenic mechanisms of childhood MCNS, especially the mechanisms of glomerular permeability.

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INTERLEUKIN-13 (IL-13) GENE OVEREXPRESSION INDUCES MINIMAL CHANGE-LIKE NEPHROPATHY IN RATS

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Objectives of Study: IL-13 has been implicated in childhood minimal change nephrotic syndrome. The aim of this study was to investigate the effects of IL-13 on development of proteinuria and the expression of genes coding for podocyte-specific proteins associated with nephrotic syndrome, using an *IL-13* gene overexpression rat model.
Methods: Recombinant rat IL-13 was inserted into a mammalian expression vector, pCI, and subsequently transfected into the quadriceps of rats (n=6) by *in-vivo* electroporation every 10 days till day 72, when rats were sacrificed. Control rats (n=6) received the vector without the *IL-13* insert. Body weight, serum albumin, creatinine and IL-13, and urine albumin were measured serially. Following sacrifice, the kidneys were subjected to histological examination by light and electron microscopy (EM). Glomerular expression of the podocyte genes nephrin, podocin, α-actinin-4 (ACTN4), dystroglycan 1 (Dag1) and CD2AP, as well as the IL-13 receptor genes IL-4Rα, IL-13Rα1 and IL-13Rα2, were examined using RT-PCR. Statistical analysis was performed using the Mann-Whitney test.
Results: 4 of the 6 rats were successfully transfected with the *IL-13* gene, as measured by serum IL-13 levels>100 pg/ml. The general characteristics of the rats are shown in the table below as means (SEM):

	Serum IL-13 (pg/ml)	Weight gain (g)	Urine albumin (μg/24 hours)	Serum albumin (g/L)	Serum creatinine (μmol/L)
Control (n=6)	0.0 (0.0)	0.57 (0.02)	252 (35)	52.0 (1.9)	80.3 (4.6)
IL-13 (n=4)	1126.7 (514.5)	0.64 (0.08)	4045 (1840)	31.3 (5.4)	81.5 (5.1)
P value	0.004	0.39	0.011	0.011	0.45

The *IL-13* transfected rats showed significant albuminuria and lower serum albumin, with one rat becoming grossly edematous. No significant glomerular light microscopic alterations were noted, however EM revealed up to 80% fusion of podocyte foot processes in the *IL-13* transfected rats. Glomerular mRNA expression, expressed as an index with cyclophilin as the housekeeping gene, is outlined in the following table as means (SEM):

	IL-4Rα	IL-13Rα1	IL-13Rα2	Nephrin	Podocin	ACTN4	Dag1	CD2AP
Control (n=6)	0.06 (0.02)	0.57 (0.03)	0.028 (0.007)	2.08 (0.19)	1.03 (0.06)	2.67 (0.23)	1.53 (0.15)	3.26 (0.20)
IL-13 (n=4)	0.26 (0.05)	0.64 (0.01)	0.26 (0.12)	0.75 (0.21)	0.58 (0.10)	2.07 (0.05)	0.97 (0.09)	2.30 (0.04)
P value	0.014	0.11	0.078	0.011	0.011	0.088	0.019	0.011

The glomerular expression of the IL-13 receptor gene, IL-4Rα, was significantly increased in the *IL-13* transfected group, and this was associated with significant downregulation of the podocyte genes, especially nephrin, podocin, Dag1 and CD2AP.
Conclusion: Overexpression of *IL-13* gene induced a nephropathy in rats characterized by increased proteinuria, albuminaemia, and minimal glomerular lesion on light microscopy with podocyte foot process fusion on EM. This was associated with downregulation of nephrin and podocin as has been reported previously in minimal change nephrotic syndrome.

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