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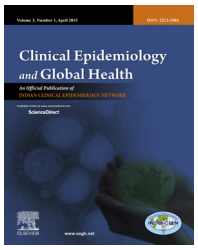

Student's section

Prediction of early response to steroids in nephrotic syndrome patients aged between 2 and 10 years

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ARTICLE INFO

Article history:
Received 4 July 2016
Accepted 2 February 2017
Available online 23 February 2017

Keywords:
Nephrotic syndrome
Prediction model
Remission
Children
CD4+

ABSTRACT

Objective: The primary objective was to predict onset of remission within 10 days of starting corticosteroid treatment in nephrotic syndrome patients aged between 2 and 10 years (either first episode or first relapse) using clinical and laboratory variables. The secondary objective was to compare changes in CD4 count and percentage among newly diagnosed cases.

Method: Prospective cohort study with a nested case–control design was conducted from September 2009 to August 2010 after institutional ethical clearance. Included were cases aged 2–10 years diagnosed as nephrotic syndrome based on standard clinical and laboratory criteria. Controls were age- and sex-matched healthy subjects recruited from the out-patient's department.

Results: Included were 44 cases (26 newly diagnosed and 16 were first relapse) cases of nephrotic syndrome and 38 healthy age- and sex-matched controls. Variables in the linear regression model predicting remission were sex, presence of tuberculosis, Low-Density Lipoprotein (LDL)/High Density Lipoprotein (HDL) ratio and antihypertensive medication, serum LDL, serum triglyceride, and serum creatinine. CD4 count, CD4%, and CD4% rise were significantly high in first episode of nephrotic syndrome as compared to controls. Serum Very Low Density Lipoprotein (VLDL) was raised in late responders of first episode of nephrotic syndrome.

Conclusion: Female gender, concomitant tuberculosis, and raised serum VLDL delayed onset of remission while use of angiotensin converting enzyme inhibitors in hypertensive patients decreased the duration of proteinuria in cases of nephrotic syndrome in children.

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Nephrotic syndrome is an important chronic disease in children. The underlying abnormality in nephrotic syndrome is an increase in permeability of the glomerular capillary wall, which leads to massive proteinuria and hypoalbuminemia. The cause of increased permeability is not well understood, and it is possible that T-cells dysfunction leads to alteration of cytokines, which causes a loss of negatively charged glycoproteins within the glomerular capillary wall. About 80% children with idiopathic nephrotic syndrome show remission of proteinuria following treatment with corticosteroids.[1] Most patients have multiple relapses, placing them at risk for steroid toxicity, systemic infections and other complications.

So a study is needed, which can predict onset of remission after steroid treatment in nephrotic syndrome patients that will guide the physician about the next line of management and hence will prevent overexposure to steroid and its toxicity. It also helps in prognostication and counseling of parents of nephrotic syndrome children because onset of remission after

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http://dx.doi.org/10.1016/j.cegh.2017.02.001
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7–9 days increases risk of relapse in future.[2] This study also gives us a clue about the pathogenesis of nephrotic syndrome in children, which may help pediatrician to guide the treatment in future by targeting CD4 lymphocytes. We hypothesized that it would be possible to predict onset of remission in nephrotic syndrome patients by using clinical and laboratory variables. Therefore, the primary objective was to develop a prediction model of onset of day of remission for response to steroid using pre-steroid peripheral blood CD4 count, serum lipid levels, total leukocyte counts, and other nonrenal factors (such as age, sex, nutritional status and infections like tuberculosis status, and infections like TB) and renal factors (such as hypertension, hematuria, azotemia and Urinary Tract Infection (UTI) secondary objective was to determine peripheral blood CD4 counts, serum lipid levels with serum protein, and serum albumin before initiation of steroid in nephrotic syndrome patients aged 2–10 years and in age- and sex-matched controls.

1. Materials and methods

This study was conducted from September 2009 to August 2010 among nephrotic syndrome patients admitted in Department of Pediatrics, King George Medical University Lucknow after institutional ethical clearance. A total of 42 cases of nephrotic syndrome and 38 age- and sex-matched controls were recruited in this study (Fig. 1). Cases aged between 2 and 10 years were recruited in our study within 72 h of admission and before intake of steroid. Exclusion criteria for cases were intake of steroid for more than 10 days in last 3 months, having suspected measles infection in last 3 months, and children with clinical suspicion of connective tissue disorders, immunodeficiency disease, or HIV-positive children. Children aged between 2 and 10 years not having suspected renal disease, measles infection, or history of steroid intake for more than 10 days in last 3 months and without clinical suspicion of UTI, connective tissue disorders, and immunodeficiency diseases like HIV were recruited as controls in our study.

Nephrotic syndrome was defined as edema, nephrotic range proteinuria (>40 mg/m^2/h on timed sample, spot albumin to creatinine ratio >2 mg/mg), hypoalbuminaemia (<2.5 g/dl), and hyperlipidemia (serum cholesterol > 250 mg/dl).[3] Blood pressure was measured with the help of sphygnomanometer by auscultatory method. The patients were defined as hypertensive if systolic/diastolic blood pressure was above the 95th percentile for age and sex using normograms. The patients were defined as having pulmonary tuberculosis on the basis of diagnosis made by treating physician-based clinical symptoms, signs, and on X-ray of chest postero-anterior view. Urine protein was estimated in the morning urine sample by dipstic method and graded as nil, 1+, 2+, 3+, and 4+. Informed consent was taken from the parent before recruiting the cases and control in our study. 2 ml venous blood was taken on day 1 in a vacutainer containing EDTA for CD4 count estimation during the morning hours (8 am–12 pm) from both cases and controls. The kit used for estimation of CD4+T lymphocytes was PARTEC CD4 easy count kit. Serum cholesterol was measured by CHOD–POD liquid method. Enzymatic colorimetric (LIQUID) method was used to estimate serum Low-Density Lipoprotein (LDL). Serum High-Density Lipoprotein was measured by direct enzymatic colorimetric method. Triglyceride was measured by GPO–POD (liquid) method. Urinary leucocytes count/hpf. – 10 ml. Urine was taken from both cases and control on day 1 and this sample is first centrifuged, followed by addition of methylene blue on precipitate. With the help of light microscope, urine total leucocytes count was done. It was expressed as total number of cells/hpf. Total leukocyte and %lymphocytes count in blood was calculated through manual method.

For the purpose of analysis, nephrotic syndrome cases who were early responders to steroids were defined as those whose proteinuria became nil within 10 days for at least 1 day.[2] The rest were categorized as late responders. Adequate treatment of nephrotic syndrome patients included 6 weeks daily steroid with dose 2 mg/kg followed by alternate day steroid with dose (1.5 mg/kg for 6 weeks).

Data was collected on a preformed pilot tested structured questionnaire. Data was collected on age, sex, religion, history of consanguinity, family characteristics, anthropometry, history of present complaints, past history, and history of treatment taken. Each patient was examined and findings were noted. Investigations were done on day 1 in both cases and controls. CD4% was calculated by CD4 count divided by Absolute Lymphocyte Count (ALC). ALC was calculated by total leukocyte count (TLC) multiplied by lymphocyte% in differential count. CD4% rise was calculated by 45 minus observed CD4% (presuming 45 as a upper limit of CD4% in normal children).

1.1. *Statistical analysis*

Since this was a pilot study, formal sample size calculation was not done a priori. We committed to take all cases of nephrotic syndrome, which fulfilled the inclusion criteria during our study period because of short duration of study and low incidence of disease in study population. Distribution of all outcome and potential explanatory variables was assessed. The data was analyzed by using SPSS16 version. Univariates comparison between cases and controls was done using chi-

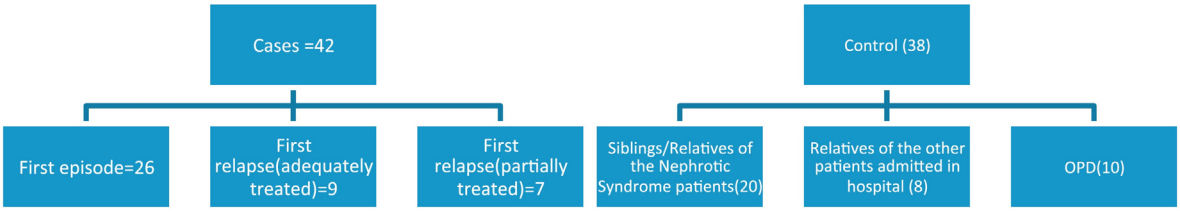

Fig. 1 – Flow diagram of recruited cases and control.

square test for categorical variables and student *t*-test for continuous variables. We used linear regression model to predict duration of proteinuria in cases of nephrotic syndrome because there was no right or left censoring of our outcome data. Potential predictor variables were those which had univariate association with outcome, that is, nephrotic syndrome with a p-value < 0.25. We reported adjusted R^2 of the model.

2. Results

Out of 42 cases, 18 (42.86%) patients were in the age group of 37–60 months, 14 (33.3%) in the age group of 85–120 months, 9 (21.43%) in the age group of 61–84 months, and 1 patient was below 37 months. This showed that nephrotic syndrome most commonly appeared between age 37 and 60 months. Various studies supported our findings.[4] Among 42 cases, 26 (61.90%) were males and the rest were females. A number of studies had shown that the incidence of nephrosis was significantly higher in males than in females.[4] Consanguinity was more common in nephrotic syndrome patient in our study as compared to control, which may be because most of the children of our study belonged to the Muslim community in which consanguineous marriage is very common or this may point out at genetic predisposition of nephrotic syndrome patient. Further study having enough sample size will be needed to establish the role of consanguinity in nephrotic syndrome patients. Mean (±SD) and median duration of proteinuria in our study were 12.34 (±5.1) and 12 days, respectively. Constantinescu also supported similar findings.[5] We found that serum electrolytes and serum creatinine were significantly altered in cases as compared to control, suggesting alteration in renal function in nephrotic syndrome. This was expected in nephrotic syndrome patients. CD4 count in early responders was high (mean ± SD −2022 ± 953) but it was not significantly different as compared to late responders (mean ± SD −1422 ± 736; p value-0.09). Mean (±SD) CD4% in early and late responders was 75 (±2) and 29.71 (±21.6), respectively and CD4% rise in early and late responders was 54 (±2) and 9.55 (±24.4), respectively and this was statistically significant (p value-0.04) (Tables 1–3).

3. Discussion

Presuming 10 days, the median duration to achieve remission after starting steroid, we divided the patients into 2 categories: early responders (those patients who achieved remission within 10 days) and late responders. Mean age of early responders was 50 months whereas mean age of late responders was 80 months. This showed that early responders were younger and this difference was statistically significant ($p = 0.02$). Early responders were mainly males (85.71%). Serum cholesterol, LDL, Very Low-Density Lipoprotein (VLDL), and triglyceride were significantly high in cases as compared to control (Table 2). This was shown by other authors in the past also.[6] In our study, serum high density lipoprotein was not significantly different in cases as compared to control. This finding of ours was similar to Mallik et al.'s study results.[7] There was no significant difference in serum lipid profile of early and late responders (p value > 0.05), but when we divided the first episode patients into early and late responders group, VLDL in late responders was 52.04 (±30.8) mg/dl and in early responders it was 32.90 (±7.6) mg/dl, and this was statistically significant ($p = 0.04$). Newman et al. hypothesized that the disruption in VLDL oxylipid content was a consequence of the nephropathy-associated Lipoprotein lipase deficit, highlighting novel trafficking functions for lipases.[8] VLDL is an indicator of oxidative stress. It was possible that because of oxidative damage, these patients showed prolonged duration of proteinuria. So these patients needed special care, and prognosis was guarded. Also, the role of raised VLDL in delayed onset of remission and role of

Table 1 – Baseline characteristics of cases and controls.

Variable	Case ($N = 42$) (n, %)	Control ($N = 38$) (n, %)	Total ($N = 80$) (n, %)
Age (months)			
<37	1 (2.38)	–	1 (1.25)
37–60	18 (42.86)	16 (42.11)	34 (42.5)
61–84	9 (21.43)	7 (18.42)	16 (20)
85–120	14 (33.3)	15 (39.47)	29 (36.25)
Gender			
Male	26 (61.90)	24 (63.16)	50 (62.50)
Female	16 (38.10)	14 (36.84)	30 (37.50)
Consanguinity			
Yes	9 (21.43)	2 (5.26)	11 (13.7)
No	33 (78.57)	36 (94.74)	69 (86.25)
Religion			
Hindu (first)	9 (21.43)	12 (31.5)	21 (26.25)
Muslim (second)	33 (78.57)	26 (68.42)	59 (73.75)
Residence			
Rural	35 (83.33)	29 (76.32)	64 (80.00)
Urban	7 (16.67)	9 (23.68)	16 (20.00)
Weight in kg mean (±SD)	21.56 (±7.9)	19.68 (±9)	
Height in cm mean (±SD)	101.79 (±18.9)	93.08 (±20.2)	

Table 2 – Comparison of laboratory parameters between cases and controls.

Laboratory parameters	Case (N = 42) (mean ± SD)	Control (N = 38) (mean ± SD)	P value
Hb% (g/dl)	10.46 (±1.7)	10.62 (±1.6)	0.7
TLC (cells/mm^3)	7960.71 (±2587.7)	7892.11 (±1807.8)	0.7
Polymorphs%	64.05 (±7.5)	60.89 (±9.2)	0.14
Serum urea (mg/dl)	37.93 (±21.7)	29.97 (±8.2)	0.28
Serum creatinine (mg/dl)	1.03 (±0.8)	0.55 (±0.2)	<0.05
Serum sodium (mEq/dl)	130.99 (±20)	136.47 (±2.6)	0.05
Serum potassium (mEq/dl)	5.18 (±5.3)	4.04 (±0.7)	0.01
Serum calcium (mEq/dl)	1.11 (±0.1)	1.06 (±0.1)	0.07
CD4 counts (/μL)	1539.21 (±794.3)	1292.30 (±714.4)	0.13
ALC	2665.87 (±871.6)	2906.37 (±993.6)	0.28
CD4%	57 (±2)	47 (±3)	0.06
CD4% rise	12.9 (±24.9)	2.80 (±23.2)	0.06
Serum cholesterol (mg/dl)	323.70 (±130.4)	107.99 (±19.6)	<0.05
Serum HDL (mg/dl)	43.02 (±25.6)	35.91 (±7)	0.18
Serum LDL (mg/dl)	134.37 (±72.8)	46.46 (±18.2)	<0.05
LDL/HDL	3.62 (±2.2)	1.31 (±0.4)	<0.05
Serum triglyceride (mg/dl)	217.03 (±101.9)	97.41 (±22.6)	<0.05
Serum VLDL (mg/dl)	46.11 (±26.5)	28.03 (±5.4)	<0.05
Serum protein (g/dl)	4.35 (±1.2)	6.82 (±0.4)	<0.05
Serum albumin (g/dl)	2.32 (±0.8)	3.78 (±0.33)	<0.05

Hb-Hemoglobin, TLC-Total Leukocyte Count, ALC-Absolute Leukocyte Count, HDL-High Density Lipoprotein, LDL-Low-Density Lipoprotein, VLDL-Very Low-Density Lipoprotein.

Table 3 – Comparison of CD4 counts between first episode cases vs controls.

Variable	First episode (N = 26)			Control (N = 38)			P value
	Mean (±SD)	Minimum	Maximum	Mean (±SD)	Minimum	Maximum	
CD 4 Count (/μL)	1667.88 (±797.9)	510	3973	1292.30 (±714.4)	117	3511	0.03
CD 4% age	61 (±22)	14	98	47 (±23)	10	96	0.02
CD4% age rise	16.36 (±22.4)	30.3	53.2	2.80 (±23.2)	34.4	51.7	0.02

antioxidants as an adjuvant treatment in patients of nephrotic syndrome should be explored in a larger study.

In our study, hypertension was present in 26% while literature reports that 10–18% of nephrotic syndrome had hypertension at the time of diagnosis and when these patient received angiotensin converting enzyme (ACE) inhibitor beside steroid, duration of proteinuria was less as compared to other groups who had not taken ACE inhibitors. ACE inhibitors modifies glomerular injury, so days of onset of remission is earlier in this group.[9] Hence, all efforts must be made to diagnose hypertension and appropriate treatment with antihypertensive drug in patients with nephrotic syndrome.

CD4 count between cases and controls was not significantly different but CD4% and CD4% rise were more in cases as compared to controls (p value 0.06). In patients with first episode of nephrotic syndrome, we found that CD4 count, CD4%, and percentage rise in CD4 count were significantly higher as compared to healthy controls. So excess number of CD4% cells leads to excess release of cytokines leading to activation of T-lymphocytes leading to glomerular cells dysfunction and proteinuria. Unlike this, Hulton et al. observed that in steroid-sensitive nephrotic syndrome patients (age range 4 months to 16 years) not receiving prednisolone therapy, there was reduction in T-helper (CD4+) percentage in acute untreated relapse.[10] The exact cause of our observation as opposite to Hulton et al. was not clear but may be due to smaller sample size, different study population and age group in this study as compared to our study or beside CD4+T-cells, other factors may have role in relapse of nephrotic syndrome patient. Hence, a larger study will be needed to prove our findings. CD4 count in early responders was high but it was not significantly different as compared to late responders. CD4% and CD4% rise were more in early as compared to late responders and this was statistically significant. The other possibility of high CD4 count and its percentage in early responders might be because these patients were younger and among them high CD4+T lymphocytes percentage was expected or it might be due to smaller no of early responders (No. = 7). Clinically, this observation may show that nonspecific infection might precipitate nephrotic syndrome and that is why CD4 count was high in these patients.

Prediction model to predict onset of remission after starting steroid – this model was developed by using backward response linear regression analysis at probability of removal $p > 0.25$. The dependent variable used to create this model was number of days the patients took to achieve remission after starting steroid, that is, days of proteinuria. Independent variables in this model were age, religion, sex, weight, Serum cholesterol, Serum HDL. Serum LDL, Serum VLDL, Serum triglyceride, LDL/HDL ratio, total leukocyte counts in urine, serum urea, serum creatinine, total leucocytes count in blood, CD4 count, CD4%, CD4% rise, presence of tuberculosis, and patients taking oral antihypertensive drugs.

In this model (Table 4), females took longer duration to achieve remission as compared to males while days of proteinuria in Muslim patient were less as compared to Hindu

Table 4 – Multivariable linear regression model to predict onset of remission in cases of acute attack of nephrotic syndrome.

Variables	Coefficient	95% confidence limit	P value	Coding
Constant	2.4	−4.65 to 9.51	0.49	
Religion	−4.57	−7.96 to −1.18	0.01	1 = Hindu 2 = Muslim
Sex	5.36	2.53 to 8.18	<0.05	1 = male 2 = female
Wt. (kg)	0.33	0.164 to 0.51	<0.05	
LDL/HDL ratio	0.35	0.27 to 0.97	0.26	

LDL-Low density Lipoprotein, HDL-High-Density Lipoprotein.

Table 5 – Multivariable linear regression model to predict onset of remission in cases of first episode of nephrotic syndrome (adjusted R^2 = 0.73).

Variable	Coefficient	95% confidence interval	P value	Coding
Age (years)	0.03	−0.009; 0.08	0.10	
Sex	7.99	4.4; 11.52	<0.05	1 = male 2 = female
Tuberculosis	4.30	0.28; 8.32	0.03	1 = yes 0 = no
Hypertension	−3.48	−6.95; 0.01	0.04	1 = yes 0 = no
LDL/HDL ratio	3.79	2.23; 5.35	<0.05	
LDL (mg/dl)	−0.07	−0.11; 0.04	<0.05	
Triglyceride (mg/dl)	−0.03	−0.05; −0.01	<0.05	
Creatinine (mg/dl)	−2.35	−5.37; 0.67	0.11	
Constant	4.56	−2.21; 11.33	0.17	

LDL-Low density Lipoprotein, HDL-High-Density Lipoprotein.

patient. Weight and LDL/HDL ratio were positively correlated with duration of proteinuria. The significance of these findings was unknown, so further study will be needed to prove this.

Predictor of onset of remission in first episode of nephrotic syndrome: In first episode of nephrotic syndrome, various factors such as age, sex, presence or absence of tuberculosis, serum LDL, LDL/HDL ratio, and serum triglyceride might play a role in early or late onset of remission. Those patients who were taking angiotensin converting enzyme inhibitor showed early remission, suggesting the role of angiotensin converting enzyme inhibitor as protein sparing agent. Duration of proteinuria was less in males as compared to females. Presence of tuberculosis delayed remission suggesting that immunological derangement might be a cause of delayed onset of remission. In this prediction model (Table 5), patients having raised serum LDL and triglyceride had early onset of remission. The association of these findings is not known. So further study is needed to establish the role in early clearance of proteinuria. According to this model, the patient who has high serum creatinine has early onset of remission. Significance of this finding was not known. Further study will be needed to know the role of serum creatinine in early onset of remission.

3.1. *Strengths and limitations of the study*

This was the first type of prospective matched nested case control study on nephrotic syndrome patients till date on prediction of onset of remission. Limitations of the study were: small sample size, pilot type study, and multiple factors controlling CD4 counts blood level in children. So we need a study having enough sample size to prove our findings for future use of our prediction model.

4. Conclusion

The onset of remission can be predicted by a model in which variables are sex, religion, weight, presence of tuberculosis, LDL/HDL ratio, use of ACE inhibitors, serum LDL, serum triglyceride, and serum creatinine. CD4% and CD4% rise were higher in nephrotic syndrome patients as compared to control. CD4 count, CD4% and CD4% rise in first episode of nephrotic syndrome were raised as compared to control. Serum cholesterol, serum LDL, serum HDL, serum VLDL, and serum triglyceride were elevated in cases as compared to control. Serum VLDL was elevated in early responders of first episode. The role of VLDL in delayed onset of remission in late responders of first episode of nephrotic syndrome should be investigated in further studies.

Author contribution

Vinay Kumar Rai was involved in designing the study, data collection, laboratory work, data analysis and interpretation, and manuscript writing. Professor Shally Awasthi was involved in designing the study, data analysis and interpretation, and manuscript writing. Professor Vimala Venkatesh was involved in laboratory work and manuscript writing.

Conflicts of interest

The authors have none to declare.

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