RESEARCH BRIEF

Non-Autoimmune Subclinical and Overt Hypothyroidism in Idiopathic Steroid-resistant Nephrotic Syndrome in Children

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ABSTRACT

Objective: To evaluate the frequency of non-autoimmune subclinical and overt hypothyroidism in children with idiopathic steroid-resistant nephrotic syndrome (SRNS).

Methods: This cross-sectional study recruited 30 children (age 1-18 y) with idiopathic SRNS; and 30 healthy controls. Serum T3, T4 and TSH were performed in cases as well as controls. Anti-thyroid peroxidase and anti-thyroglobulin antibody tests were performed in all cases.

Results: Non-autoimmune subclinical or overt hypothyroidism was detected in 10 out of 30 children with idiopathic SRNS; 2 had overt hypothyroidism, while 8 patients had subclinical hypothyroidism. Children with SRNS had a mean (SD) TSH value 4.55 (4.64) mIU/L that was higher as compared to controls (1.88 (1.04) mIU/L) (P<0.01). The histopathological profile of these cases included minimal change disease (MCD) in 9 (30%), focal segmental glomerulosclerosis (FSGS) in 13 (43.3%), mesangioproliferative glomerulonephritis in 8 (26.7%). Overall, 36.7 % of SRNS cases were in complete remission, 26.6% in partial remission while 36.7% did not attain remission at the time of enrolment into the study. Children with overt hypothyroidism (2 cases) and grade III subclinical hypothyroidism (1 case) were subsequently started on levothyroxine therapy.

Conclusions: The prevalence of subclinical and overt hypothyroidism seems to be high in idiopathic SRNS, with almost one-third of children having overt or subclinical non-autoimmune hypothyroidism.

Key words: *Glomerulonephritis, Minimal change disease, Thyroid function tests.*

Approximately 10% of children with nephrotic syndrome (NS) are classified as steroid-resistant (SRNS) [1]. Children with SRNS often have protracted proteinuria, which might lead to loss of thyroxine binding globulin (TBG), transthyretin and albumin eventually, resulting in low levels of thyroid hormone [1,2]. Long standing proteinuria in patients with SRNS might damage the renal tubules progressively, resulting in reduced absorption of low molecular weight (LMW) proteins. This might further exhaust the thyroid reserve causing overt hypothyroidism [3]. There is a paucity of data regarding the prevalence of hypothyroidism in SRNS [3-5]. Derangements in thyroid metabolism are known to have effects on renal blood flow, bone mineral density, lipid profile, fluid and electrolyte homeostasis, proteinuria and cardiovascular function, including development of premature atherosclerosis [2]. These parameters are already known to be adversely affected in SRNS; and hypothyroidism can further compromise them. In the present study, we evaluated the prevalence of non-autoimmune subclinical and overt hypothyroidism in SRNS in comparison with healthy controls. The secondary objectives were to determine any

association of non-autoimmune subclinical and overt hypothyroidism with the duration of the disease, serum albumin levels and serum creatinine levels; to study the relationship between histopathological profile and thyroid function tests in children with SRNS; and to compare the characteristics of children with SRNS having overt/ subclinical hypothyroidism versus those without overt/subclinical hypothyroidism.

METHODS

This cross-sectional study was conducted at the Pediatric nephrology outpatient department of JIPMER, Puducherry from March 2015 through July 2016 after obtaining approval from the Institute ethics committee. Written informed consent was obtained from the parents prior to enrolment of the children.

Children (age 1-18 y) with SRNS presenting to the pediatric nephrology clinic were included. Those with secondary nephrotic syndrome (*e.g.*, IgA nephropathy, lupus nephritis, Henoch Schonlein purpura nephritis), hypothyroidism of autoimmune origin, critical sickness requiring intensive care unit treatment and congenital hypothyroidism were excluded. Age- and sex-matched healthy controls were recruited after obtaining informed consent from the parents. They were selected from children attending the general pediatric outpatient department. They were required to have clinically undetectable thyroid swelling; no feature suggestive of hypothyroidism, hyperthyroidism or autoimmune disorders; and not on thyroid hormone or carbimazole; with absence of proteinuria.

Steroid resistance was defined as failure to achieve remission despite 2 mg/kg/day of daily prednisolone for 4 weeks [6]. Complete remission in SRNS was defined as urine protein: urine creatinine <0.2 g/g, serum albumin >2.5 g/dL and no edema. Partial remission in SRNS was defined as urine protein: urine creatinine between 0.2 and 2 g/g, serum albumin >2.5 g/dL or edema. No remission in SRNS was defined as Up: Uc >2, serum albumin <2.5 g/dL or edema. Overt hypothyroidism was defined as low Free T4 (normal: 0.7-2 ng/mL) and elevated serum Thyroid stimulating hormone (TSH) above the upper limit of the reference range (> 4.5 mIU/L) [7]. Subclinical hypothyroidism was defined as an elevation in serum TSH above the upper limit of the reference range with a normal serum FT4 concentration. Subclinical hypothyroidism was classified as follows— Grade 1: subclinical hypothyroidism was defined as TSH greater than 4.5 mIU/L and <6 mIU/L, Grade 2:TSH between 6 -12 mIU/L, grade 3: TSH >12 mIU/L; with normal FT4 concentration [8]. Initial resistance was defined as lack of remission at the first episode of nephrotic syndrome. Late resistance was defined as being steroid sensitive initially, but demonstrating steroid resistance during a subsequent relapse.

Children with SRNS were investigated and managed as per Indian Pediatric Nephrology Group guidelines [6]. Following clinical parameters were recorded: age, sex, age of onset of nephrotic syndrome, duration of disease, edema, anthropometry (height, weight and body mass index), blood pressure recordings, immunosuppressants being prescribed, type of steroid resistance, remission state, and histopathological profile. Following laboratory parameters were recorded in cases and controls (through

intravenous blood sample and early morning urine sample): blood urea, serum creatinine, urine protein: urine creatinine ratio, serum albumin, serum cholesterol, free T3, free T4 and thyroid stimulating hormone (TSH), and anti-thyroglobulin and anti-thyroid peroxidase (TPO) antibodies. Z scores for height were recorded from the following source*: www.int/growthref/tools/en/*

Fasting blood samples were collected and levels of FT3, FT4 and TSH were analyzed for both cases and controls. In cases with abnormal thyroid profile, antibodies against thyroid peroxidase and thyroglobulin were measured. FT3 and FT4 measurement was performed by competitive immunoassay using direct chemiluminescent technology (ADVIA Centaur CP). Intra-assay coefficient of variation was <2.3% for TSH, 2.3% for FT4 and 7.8% for FT3. The inter-assay coefficient of variation was <2.9% for TSH, 2.5% for FT4 and 12.3% for FT3. Blood samples for anti-thyroid peroxidase and anti-thyroglobulin antibodies were stored at 4^{o}C, and the levels were determined using the standard ELISA (Calbiotech Inc, USA)

Statistical analysis: Data were expressed as mean (SD). Student's *t*-test was used to compare continuous variables and proportions were compared using chi-square or Fisher Exact test. The outcome variables between more than 2 subgroups of SRNS (such as histopathological groups, state of remission, and type of steroid resistance) were analyzed using ANOVA. Correlations between the serum T3, T4 and TSH levels; and duration of disease, serum albumin, serum creatinine, and urinary protein: creatinine ratios were studied using scatter diagrams. Pearson's Correlation coefficient (r) was used to measure linear correlation between two continuous variables. P value <0.05 was considered significant. Data were analysed using SPSS version 19.

The sample size was calculated to be a minimum of 52 subjects (26 cases, 26 controls) assuming proportion of cases with subclinical hypothyroidism to be 30%; proportion of controls with subclinical hypothyroidism to be 2% based on the results of previous study [4,9] with α error 0.05, β error 0.2 and ratio of cases: controls as 1:1.

RESULTS

We assessed 36 children with SRNS for eligibility; 5 were excluded due to secondary SRNS, and one was excluded because of anti-TPO and anti-thyroglobulin positivity. Clinical and biochemical characteristics of included children with SRNS are depicted in ***Table* I**. All children received enalapril for reduction in proteinuria. ***Table* II** compares the characteristics and thyroid profile in cases and controls. The prevalence of hypothyroidism (subclinical or overt) among the cases and controls was 33.3% (*n*=10) and 3.3% (*n*=1), respectively. TSH values between cases and controls were significantly different (***Table* II**). Two SRNS patients had overt hypothyroidism, while 8 SRNS patients had subclinical hypothyroidism (1 case with grade 1, 6 cases with grade 2 and 1 case with grade 3). Only one control child had hypothyroidism (subclinical) with TSH level 5 mIU/L. His T3 level was 3.51 pg/mL and T4 level was

1.21 ng/dL (normal for age). Cases with overt hypothyroidism (2 cases) and grade 3 subclinical hypothyroidism (1 case) subsequently received levothyroxine therapy.

On subgroup analysis (***Web Table* I**) between SRNS children with hypothyroidism versus those without hypothyroidism, there was no difference in terms of age of onset of NS, age of onset of steroid resistance and duration of the disease. There was no association between the prevalence of subclinical/overt hypothyroidism (as well as FT4, FT3 and TSH values) with various histopathological subgroups and with different remission states (complete, partial or no remission). There was a weak positive correlation between proteinuria and serum TSH levels (r= 0.329); and negative correlation between serum albumin and TSH levels (r = -0.375). Weak negative correlations were also noted between proteinuria and serum T3 levels (r= -0.301); and between proteinuria and serum T4 levels (r= -0.129).

DISCUSSION

The prevalence of subclinical or overt hypothyroidism in children with idiopathic SRNS in this study was 33.3% which appears to be higher than previously published reports [4,5]. Pathogenetic mechanisms for hypothyroidism in SRNS include higher urinary excretion of T3 and T4 during nephrosis [10-12]. It has been speculated that TSH (being a LMW protein with molecular weight of 28,500 Daltons) may also be lost in the urine of these children [13,14]. It has also been shown in previous studies that when SRNS deteriorated to end stage renal disease (ESRD), the thyroid hormone profile normalized and the patients could be taken off levothyroxine therapy [3]. This observation indicates the central role of proteinuria and urinary thyroxine loss in the pathogenesis of hypothyroidism in SRNS. However, the results of the present study as well as previous studies [4] indicate that hypothyroidism can occur even in complete or partial remission.

Few studies have evaluated the prevalence of hypothyroidism in SRNS [3-5]. Dagan, *et al.* [3] published a series of 5 children with SRNS aged 3-11 years, who on follow-up (5-42 months) developed non-autoimmune hypothyroidism. All these 5 children eventually deteriorated to ESRD and required dialysis and/or transplantation. Kapoor, *et al. studied* [4] 20 children with SRNS, out of whom 30% had non-autoimmune subclinical hypothyroidism. Sharma, *et al.* [5] enrolled 50 children with SRNS, and the prevalence of subclinical hypothyroidism was 20% with a positive correlation between TSH levels and proteinuria. The differences observed in the prevalence of hypothyroidism between the present study and previously published observational studies [4,5] might be due to heterogeneities in study designs and patient populations.

In our study, only one child with SRNS had grade III subclinical hypothyroidism, in contrast with 9 children who had grade I or grade II hypothyroidism. This could be related to the usage of glucocorticoids, which decrease TRH messenger RNA levels in the hypothalamus leading to lower TSH secretion [15,16]. The role of levothyroxine supplementation in subclinical hypothyroidism is

controversial. The potentially deleterious effects of hypothyroidism and the prospect of prevention of progression to full-fledged clinical hypothyroidism are strong arguments for levothyroxine supplementation in these children. Nevertheless, there are no guidelines for thyroxine supplementation in SRNS children with subclinical hypothyroidism, though studies in adults have found beneficial effects in individuals with TSH > 10 mIU/L [17]. We chose to treat only overt and grade III hypothyroidism; and follow-up grade I and grade II hypothyroidism for possible hormone supplementation.

We recruited a population of exclusively idiopathic SRNS in order to ensure homogeneity with regard to histopathological profile; and therefore excluded secondary SRNS. Thyroid dysfunction has been earlier reported with IgA nephropathy, membranous nephropathy and membranoproliferative glomerulonephritis and is often due to autoimmune mechanisms in these disorders (18-20). We studied the prevalence of non-autoimmune acquired hypothyroidism only; and ruled out autoimmune causes by appropriate investigations. The present study is limited by cross-sectional design. Data regarding prospective development of overt or subclinical hypothyroidism, as well as follow-up serum T3, T4 and TSH levels could not be collected. The study did not venture into molecular and biochemical mechanisms for development of subclinical hypothyroidism e.g., estimation of urinary loss of T3, T4 and TSH levels. Additionally, the study is not powered to examine the relationship between histopathological profile, duration of the disease and thyroid status.

On the basis of the findings of this study, estimation of thyroid hormone status in children with SRNS seems to be a rational approach. This may help in optimizing preventive and therapeutic strategies for early recognition of hypothyroidism in SRNS.

WHAT THIS STUDY ADDS?

- Almost one-third of children with idiopathic steroid-resistant nephrotic syndrome have overt or subclinical non-autoimmune hypothyroidism.

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Contributors: VM and SK: collected the data, reviewed the literature and drafted the manuscript. SK conceptualized the study, reviewed the literature and critically reviewed the manuscript. MR supervised the laboratory tests and critically reviewed the manuscript. All authors approved the final version of the manuscript.

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TABLE I CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF CHILDREN WITH SRNS ENROLLED INTO THE STUDY (*n*=30)

Characteristic	*Value*
Age at enrolment (y)	7.2 (3.9)
Age at onset of NS (y)	4.5 (3.3)
Duration of NS (y)	2.4 (2.0)
Age of onset of Steroid Resistance (y)	5.8 (3.3)
Weight (kg)	22.2 (9.5)
Height z score	-1.5 (1.1)
Height (cm)	113.3 (22.6)
Type of SRNS	
Initial resistance	12 (40%)
Late resistance	18 (60%)
Immunosuppressants received[*]	
Cyclosporin with prednisolone	23 (73.3%)
IV Cyclophosphamide with prednisolone	14 (46.7%)
Mycophenolate mofetil with prednisolone	10 (33.3%)
Tacrolimus with prednisolone	3 (9.9%)
Rituximab	1 (3.3%)
Remission state	
Complete	11 (36.7%)
Partial	8 (26.6%)
None	11 (36.7%)
Hypertension	
Systolic blood pressure >95th centile	5 (16.7%)
Diastolic blood pressure >95th centile	4 (13.3%)
Histopathological profile	
FSGS	13 (43.3%)
MCD	9 (30%)
MesPGN	8 (26.7%)

NS nephrotic syndrome, SRNS steroid resistant NS, eGFR estimated glomerular filtration rate, FSGS focal segmental glomerular sclerosis, MCD minimal change disease, MesPGN mesangioproliferative glomerulonephritis. $ Indicates the immunosuppressive agents received at various points of time. Hence the total would be more than 100%. All values are expressed in mean (SD) or n (%)

TABLE II COMPARISON OF CHARACTERISTICS (CLINICAL AND LABORATORY) AND THYROID PROFILE IN CASES AND CONTROLS

Parameter	*Cases (SRNS) (n=30)*	*Controls (n=30)*	*P value*
Age (y)	7.2 (3.9)	7.0 (3.8)	0.82
Males*	16 (53.3%)	17 (56.7%)	1.00
Body mass index (kg/m^2)	16.7 (2.9)	13.8 (1.6)	<0.01
Body Surface area (m^2)	0.8 (0.2)	0.7 (0.2)	0.25
Blood urea (mg/dL)	44 (40.1)	18.7 (4.4)	<0.01
Serum creatinine (mg/dL)	1.0 (0.9)	0.7 (0.2)	0.04
eGFR (mL/min/1.73m^2)	72.9 (32.4)	73 (20.3)	0.99
Serum albumin (g/dL)	2.6 (1.0)	3.8 (0.4)	<0.01
Serum cholesterol (mg/dL)	373.9 (187.6)	142.5 (25.9)	<0.01
Free T3 (pg/ml)	3.1(1.3)	2.8 (0.7)	0.30
Free T4 (ng/ml)	1.8 (1.7)	1.2 (0.2)	0.05
TSH (mIU/L)	4.6 (4.6)	1.9 (1.0)	<0.01
Subclinical or overt hypothyroidism*	10 (33.3%)	1 (3.3%)	0.006

*Values are expressed in mean (SD) or *n (%)*

WEB TABLE I. SUBGROUP ANALYSIS COMPARING CLINICAL CHARACTERISTICS OF SRNS CHILDREN WITH AND WITHOUT SUBCLINICAL OR OVERT HYPOTHYROIDISM

Variable	*SRNS with subclinical or overt hypothyroidism (n=10)*	*SRNS without subclinical or overt hypothyroidism (n=20)*	*P value*
Age of onset of SRNS (years)	5.8 (3.3)	8 (4.0)	0.172
Age of onset of NS (years)	3.2 (3.0)	5.8 (3.6)	0.066
Initial resistance*	3 (30%)	9 (45%)	0.694
Duration of onset of SRNS to thyroid status evaluation (months)	10 (14.6)	9.9 (12.0)	0.976
Total duration of illness (years)	2.7 (2.6)	2.1 (1.3)	0.366
Serum albumin (g/dL)	2.2 (0.8)	2.8 (1.0)	0.124
Serum cholesterol (mg/dL)	392.8 (128.1)	364.5 (213.6)	0.700
Histopathology[*] FSGS MCD MesPGN	 2 (20%) 3 (30%) 5 (50%)	 11 (55%) 6 (30%) 3 (15%)	0.373

Values are expressed in mean (SD) or [*]n (%); FSGS-Focal segmental glomerulosclerosis; MCD-Minimal Change Disease; MesPGN-mesangioproliferative glomerulonephritis; SRNS-steroid resistant nephrotic syndrome