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Original Article

Biochemistry Section

Prevalence of Non-autoimmune Hypothyroidism in Steroid Resistant Nephrotic Syndrome in Paediatric Age Group

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ABSTRACT

Introduction: It was observed that approximately 10% of children with Nephrotic Syndrome (NS) are found as Steroid Resistant NS (SRNS). The data on the prevalence of non-autoimmune hypothyroidism among the SRNS in India is limited.

Aim: To assess the prevalence of non-autoimmune hypothyroidism in the case of SRNS.

Materials and Methods: A case control cross-sectional study was conducted in which 52 cases of SRNS and 52 healthy controls were enrolled. Thyroid profile like serum Thyroid Stimulating Hormone (TSH), Free Triiodothyronine (T3), Free Thyroxine (T4) done in the all cases and controls but anti-Thyroid Peroxidase (TPO), and anti-thyroglobulin antibody test was done in the case and control group with deranged thyroid function test. Low Free T4 (normal: 0.7-2 ng/mL) and elevated serum TSH above the upper limit of the reference range (>4.5 mIU/L) was defined as overt hypothyroidism, whereas elevation in serum TSH with a normal serum FT4 concentration was defined as sub clinical hypothyroidism. MedCalc statistical software Version 19.2.6 was used to do statistical analysis.

Results: Prevalence of Non-autoimmune hypothyroidism was 38.46% (20 out of 52), 16 (30.76%) had subclinical and 4 (7.69%) had overt hypothyroidism in case of SRNS in comparison to 1.96% (1 out of 52) in control group. Out of 16 subclinical hypothyroid patients, two cases with grade 1, 12 cases with grade 2, and two cases with grade 3 found. Patients with SRNS had a mean (SD) TSH value of 4.5±4.7 mIU/L which was significantly higher than control (1.8±1.1 mIU/L). Serum levels of FT4 were within normal range. Anti-TPO and anti-thyroglobulin titre were in normal range in children with hypothyroidism.

Conclusion: The prevalence of non-autoimmune hypothyroidism was high in cases of idiopathic SRNS. So, on the basis of this study estimation of thyroid profile in children with SRNS seems to be the rational approach which will lead to early diagnosis and timely management of hypothyroidism in SRNS.

Keywords: Anti-thyroid peroxidase, Overt, Subclinical, Thyroid stimulating hormone

INTRODUCTION

The Neprotic Syndrome can affect children of any age, from infancy to adolescence, but is most commonly seen among school-aged children and adolescents. It is one of the most common glomerular diseases and is characterised by oedema, massive proteinuria (>3.5 g/24 hours), hypoalbuminemia. Proteinuria results from increased permeability of the glomerular capillary wall and also by impaired reabsorption by the epithelial cells of the proximal tubule. Normally, Low Molecular Weight (LMW) proteins are completely reabsorbed in the proximal tubule. Thyroid function can be affected in case of NS due to excessive loss of urinary protein, which causes loss of albumin and Thyroxine Binding Globulin (TBG) through urine [1]. About 90% of children with NS are steroid responsive; rest of the 10% are SRNS [2]. In SRNS children having prolonged proteinuria causes loss of TBG, transthyretin and albumin which lead to low level of thyroid hormone [2,3]. Prolonged proteinuria in case of SRNS may lead to damage of renal tubules continuously, this lead to decreased absorption of LMW proteins. This may lead further depletion of thyroid reserve causing overt hypothyroidism [4,5]. On the other hand, thyroid hormone metabolism derangement has impact on renal tubular function, the renin-angiotensin system hemodynamic and cardiovascular alterations which subsequently have an effect on renal blood flow, proteinuria and lipid profile [6]. In paediatric age group in patients of NS various forms of dysfunction of thyroid is usually found and it occurs due to loss of thyroid hormones and hormone binding protein. There is paucity of available data regarding the prevalence of hypothyroidism in SRNS in the Paediatric age group and correlation between abnormal thyroid function and various biochemical parameters like spot urinary protein to urinary creatinine ratio [4,7,8]. Therefore, the present study was conducted to assess the prevalence of non-autoimmune hypothyroidism in the case of SRNS.

MATERIALS AND METHODS

This case control cross-sectional study was conducted at the Department of Biochemistry and Department of Paediatric, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India, over the period of two years from July 2018 to June 2020 after obtaining approval from the Institutional Ethics Committee vide no 850/IEC/IGIMS. The sample size was calculated to a minimum of 104 (52 cases and 52 control) assuming the proportion of cases with hypothyroidism to be about 30%; the proportion of control with hypothyroidism to be about 2% found in a previous study [7]. Written informed consent was obtained from parents of children prior to enrollment. Consecutive children of age between 1 to 18 years coming to the OPD of the Paediatric Department who fulfilled the definition of SRNS [9] were enrolled in the study. Children with Non-nephrotic proteinuria, Congenital NS, NS secondary due to lupus nephritis, Henoch-Schönlein purpura nephritis, metabolic, infectious, vascular, malignant and cardiac disease, and autoimmune thyroiditis were excluded. Other groups of children were enrolled as control, from the outpatient Department of Paediatrics with undetectable thyroid swelling, no evidence of hypothyroidism or hyperthyroidism, an autoimmune disorder, not on any thyroid hormone or drug like Carbimazole with absence of proteinuria. Age and sex were matched and informed consent from parents was obtained. Characteristic finding in NS are heavy proteinuria, hypoalbuminemia (serum albumin <2.5 g/dL), hyperlipaemia (serum cholesterol >200 mg/dL), and oedema [10]. When the urine protein is 3+/4+ on a dipstick test, spot protein/creatinine ratio >2 mg/mg,

or urine albumin >40 mg/m^2 per hour (on a timed urine sample), proteinuria is considered to be in the nephrotic range. Most of the cases, the finding of 3+/4+ proteinuria (on dipstick or boiling test) is sufficient for defining nephrotic range proteinuria. Precise quantitative assessment of proteinuria was not essential and a 24 hour urine protein measurement was not required for the diagnosis of NS [9].

SRNS is defined as the absence of remission despite therapy with four weeks of daily prednisolone with a dose of 2 mg/kg per day. Initial resistance is defined as a lack of remission within the first episode of NS. Late resistance is defined as the patient remains steroid sensitive initially, but demonstrating steroid resistance during further relapse. Complete remission in SRNS is defined as serum albumin >2.5 gm/dL, urine protein:urine creatinine <0.2 g/g and without oedema. Partial remission in SRNS is defined as serum albumin >2.5 gm/dL, urine protein:urine creatinine between 0.2 to 2.0 g/g or oedema. No remission in SRNS is defined as serum albumin <2.5 gm/dL, urine protein:urine creatinine >2 g/g and or oedema [7,11].

In the condition when low Free T4 (normal: 0.7-2 ng/mL) and elevated serum TSH above the upper limit of the reference range (>4.5 mIU/L) is defined as overt hypothyroidism [12]. When there is an elevation in serum TSH above the upper limit of the reference range with a normal serum FT4 concentration defined as subclinical hypothyroidism. Subclinical hypothyroidism is sub classified as follows-Grade 1: subclinical hypothyroidism is defined as TSH >4.5 mIU/L but <6 mIU/L, Grade 2: TSH between 6-12 mIU/L, Grade 3: TSH >12 mIU/L; with normal FT4 concentration [13]. Serum albumin level and proteinuria in NS patients correlated with subclinical hypothyroidism. The low serum albumin level has a high likelihood of subclinical hypothyroidism [14]. As per Indian Paediatric Nephrology Group guidelines children with SRNS were investigated and treated [9]. The following demographic and clinical parameters were recorded: age of onset of NS, age at the time of presentation, sex, duration of disease, oedema, height, weight, and body mass index, type of immunosuppressants advised, type of steroid resistance, remission state. Fasting intravenous blood sample and first morning urine sample collected and following laboratory parameters were analysed in both groups, T3, T4, Free T3, Free T4, Serum albumin, Serum cholesterol, blood urea, Serum creatinine, urine protein: urine creatinine ratio, anti-thyroglobulin, anti-TPO antibodies and estimated Glomerular Filtration Rate (eGFR).

Tightly stopper was applied at the tube immediately after collecting sample. Samples were stored tightly stoppered at room temperature (15 to 30°C) for up to 18 hours. If the assay was not be completed within 18 hours, the samples were refrigerated at 2 to 8°C. If the assay was not be completed within seven days, freeze at -20°C or colder. Antibodies against TPO and thyroglobulin were measured in those patients having abnormal thyroid profile reported. FT3 and FT4 measurements were performed by competitive immunoassay using direct chemiluminescent technology (Beckman coulter). Intra-assay coefficient of variation was <3.5% for TSH, 2.1% for FT4 and 6.8% for FT3. The inter-assay coefficient of variation was <2.1% for TSH, 2.4% for FT4, and 11.9% for FT3.

STATISTICAL ANALYSIS

All measurements and calculations are mentioned as mean±Standard Deviation (SD). MedCalc statistical software Version 19.2.6 was used to do statistical analysis. Between group differences were assessed by the student's t-test and Wilcoxon test for non-parametric distribution. Pearson correlation coefficient was used for correlation analysis between thyroid hormone profiles and serum albumin or urinary protein/creatinine ratio. The p-value of <0.05 was considered to be statistically significant.

RESULTS

Sixty five paediatric patients were evaluated with SRNS for eligibility, 10 were excluded from the study as per exclusion criteria, out of which 9 were having secondary SRNS, 1 was having hypothyroidism of autoimmune origin as anti-TPO antibody titre was high and three were excluded because consent was not given by parents, 52 children's with SRNS were found eligible, which include 26 (50%) male and 26 (50%), female child. Demography and clinical profile are shown in [Table/Fig-1]. The mean age at the onset of NS was 4.9±3.2 years and the mean age at the time of thyroid hormone profile analysis was 8.1±3.7 years. Comparison of clinical, laboratory, and thyroid hormone profile between cases and controls shown in [Table/Fig-2]. Serum albumin level 2.5±1.0 g/dL and 3.7±0.3 g/dL found respectively in case and control group and was significantly lower in case group (p<0.01). BMI, Serum Creatinine, Serum Cholesterol were significantly different between case and control. The prevalence of hypothyroidism (overt and subclinical) among the case and control group found 38.46% (n=20) and 1.92% (n=1) respectively. Overt hypothyroidism found in 4 (7.6%) and subclinical hypothyroidism found in 16 (30.76%) in SRNS patients. Out of

Characteristic	Value
Age (Y)	8.1 (3.7)
Age of onset of NS (Y)	4.9 (3.2)
Duration of NS (Y)	2.1 (1.9)
Age of onset of steroid resistance (Y)	6.1 (3.1)
Weight (kg)	22.7 (9.3)
Height (cm)	115.2 (22.6)
BMI (kg/m^2)	17.1 (2.5)
eGFR (mL/min/1.73 m^2)	75.5 (31.2)
Type of SRNS	
Initial resistance	20 (38.4%)
Late resistance	32 (61.5%)

[Table/Fig-1]: Clinical profile of children with SRNS (N=52).
Y: Year(s), NS: Nephrotic syndrome, eGFR: estimated Glomerular filtration rate, SRNS: Steroid resistance nephrotic syndrome. Values are expressed in mean (SD) or n (%)

Parameter	Cases (SRNS) (n=52)	Controls (n=52)	p-value
Age (Y)	8.1±3.7	8.0±3.6	0.87
Males*	26 (50%)	27 (52%)	1.00
Body mass index (kg/m^2)	16.9 (3.1)	12.7 (1.7)	<0.01
Body surface area (m^2)	0.9 (0.3)	0.8 (0.2)	0.25
Blood urea (mg/dL)	47 (39.1)	17.9 (4.3)	<0.01
Serum creatinine (mg/dL)	1.1 (0.9)	0.7 (0.2)	0.04
eGFR (mL/min/1.73m^2)	71.7 (32.2)	72.1 (20.1)	0.99
Urine protein: Urine creatinine ratio (g/g)	0.48 (0.49)	0.19 (0.05)	0.029
Serum albumin (g/dL)	2.5 (1.0)	3.7 (0.3)	<0.01
Serum cholesterol (mg/dL)	371.9 (187.6)	139.5 (25.9)	<0.01
T3 (ng/dL)	81.29 (22.98)	121.78 (28.37)	0.01
T4 (µg)	5.36 (1.12)	7.98 (1.49)	<0.01
Free T3 (pg/mL)	3.2 (1.3)	2.9 (0.7)	0.30
Free T4 (ng/mL)	1.7 (1.7)	1.1 (0.2)	<0.05
TSH (mIU/L)	4.5 (4.7)	1.8 (1.1)	<0.01
Hypothyroidism	20 (38.46%)	1 (1.92%)	0.005

[Table/Fig-2]: Comparison of clinical, laboratory, and thyroid hormone profile between cases and controls.
Values are expressed in mean (SD) or *n (%).

16 subclinical hypothyroid patients, two cases with grade 1, 12 cases with grade 2, and two cases with grade 3 found. Cases with overt hypothyroidism (four cases) and grade 3 subclinical hypothyroidism (two cases) were treated with levothyroxine therapy. Serum levels of FT4 were within normal range and TSH value in case and control group was 4.5±4.7 mIU/L and 1.8±1.1 mIU/L, respectively which was significantly different (p< 0.01). Anti-TPO and anti-thyroglobulin titre were in normal range in children with hypothyroidism. Oedema were present in 41 (78.84%) and 2 (3.84%) in cases and controls respectively at the time of presentation. Types of chemotherapy and corticosteroids administered are shown in [Table/Fig-3]. No significant difference was found between the occurrence of hypothyroidism and the type of chemotherapy, corticosteroids, or the number of chemotherapy cycles. When correlating urinary protein/Cr ratio with thyroid hormone profiles, urinary protein/Cr ratio negatively correlated with serum T3, T4, and free T4 levels (r=-0.5874, p=0.002; r=-0.5695, p=0.004; r=-0.5425, p=0.006), respectively. The urinary protein/Cr ratio positively correlated with serum TSH levels (r=0.5102, p=0.013) [Table/Fig-4].

Variables	Hypothyroidism		p-value
	Yes (n=20)	No (n=32)	
Mean chemotherapy cycle (SD)	3 (2)	5 (2)	0.263[1]
Type of Chemotherapy, n (%)			0.089[2]
None	8 (15.38%)	4 (7.69%)	
Cyclophosphamide	10 (19.23%)	28 (53.84%)	
Mycophenolate mofetil	2 (3.84%)	0	
Type of Corticosteroid, n (%)			0.635[3]
Methylprednisolone	16 (30.76%)	28 (53.84%)	
Prednisone	4 (7.69%)	4 (7.69%)	

[Table/Fig-3]: Type of immunosuppressant received.
Analysis done by using [1]Unpaired t-test, [2]Chi-square, [3]Fisher'-exact

Thyroid hormone profile	Coefficient r	Urinary Pro/Cr ratio 95% CI	p-value
T3	-0.5874	-0.8205 to -0.2369	0.002
T4	-0.5695	-0.8026 to -0.2072	0.004
TSH	0.5102	0.1103 to 0.7535	0.013
Free T4	-0.5425	-0.7789 to -0.1576	0.006

[Table/Fig-4]: Relationship between urinary protein/creatinine ratio and serum thyroid hormone in SRNS at onset.
Note: Correlation analysis; CI: Confidence interval
Analysis done by Pearson's Correlation Coefficient (r)

DISCUSSION

Interpretation of the interactions between thyroid and renal function may be a challenge for paediatricians doing the treatment of patients with thyroid and renal disease. In cases of NS, thyroid function gets affected through low circulating thyroid hormone concentration, insufficient binding to carrier proteins or altered iodine storage in the thyroid gland [15]. Among different subtypes of NS, SRNS has a chronic and progressive course. In present study 38.46% of all SRNS patients found to have hypothyroidism. A 30.76% had subclinical hypothyroidism and 7.6% had overt hypothyroidism. The prevalence of hypothyroidism in SRNS in this study was more than reported by a single centre study from north India done by Kapoor K et al., and Marimuthu V et al., from south India were 30% and 33.3% respectively [7,16]. Few other studies have evaluated the prevalence of hypothyroidism in SRNS. Dagan A et al., found in case series of five children with SRNS, who on follow-up for the period of (5-42 months) developed non-autoimmune hypothyroidism. All the five children subsequently developed End Stage Renal Disease (ESRD) and required dialysis and/or transplantation [8]. Guo QY et al., studied on 164 patients and found that the incidence of abnormal thyroid function was 44.51% of paediatric NS patients [13]. Sharma S et al., studied on 50 children with SRNS prevalence of subclinical hypothyroidism was 20% with a positive correlation between TSH level and proteinuria [4]. Studies have also shown that serum albumin and serum TSH to be negatively correlated. The incidence of hypothyroidism increases as the GFR decreases [6,17].

Children having exclusively idiopathic SRNS were enrolled in the present study in order to ensure homogeneity and therefore excluded secondary SRNS. In case of IgA nephropathy, membranous nephropathy and membranoproliferative glomerulonephritis thyroid dysfunction has been earlier reported with and is mainly due to autoimmune mechanisms in these disorders [18]. In present study the prevalence of non-autoimmune acquired hypothyroidism only were evaluated; and ruled out autoimmune causes.

The decision to administer thyroxine to overt and grade 3 subclinical hypothyroidism was based on the important effect of thyroid hormone on growth and metabolism, the cardiovascular and central nervous system and the skeleton in health and disease. Accordingly, other authors reported the administration of thyroxine in infants who have hypothyroidism [16].

Limitation(s)

The study design was cross sectional and so follow-up levels of serum T3, T4 and TSH levels were not measured. It was also not observed that whether there was any development of overt or subclinical hypothyroidism. Molecular and biochemical mechanisms for subclinical hypothyroidism e.g., estimation of urinary loss of T3, T4 and TSH levels were also not assessed. The present study did not examined the relationship between histopathological profile, duration of the disease and thyroid status. Once the mechanism of hypothyroidism in NS is established through large-scale prospective studies, it will help to establish a reasonable basis to develop a treatment protocol to start levothyroxine administration.

CONCLUSION(S)

On the basis of the findings of present study, subclinical non-autoimmune hypothyroidism was detected in a significant number of children with SRNS. Evaluation of thyroid profile should be done routinely in case of SRNS seems to be rational approach. This can help in planning preventive and therapeutic approach for early recognition of hypothyroidism in SRNS.

REFERENCES

[1] Topham P. Proteinuric renal disease. Clin Med (Lond). 2009;9(3):284-89. doi:10.7861/clinmedicine.9-3-284.
[2] Benvenga S. When thyroid hormone replacement is ineffective? PubMed - NCBI. Curr Opin Endocrinol Diabetes Obes. 2013;20(5):467-77.
[3] Iglesias P, Díez JJ. Thyroid dysfunction and kidney disease. Eur J Endocrinol. 2009;160(4):503-15.
[4] Sharma S, Dabla PK, Kumar M. Evaluation of thyroid hormone status in children with steroid resistant nephrotic syndrome: A north India study. Endocr Metab Immune Disord Drug Targets. 2015;15(4):321-24.
[5] Jung SH, Lee JE, Chung WY. Changes in the thyroid hormone profiles in children with nephrotic syndrome. Korean J Pediatr. 2019;62(3):85-89.
[6] Rhee CM. The interaction between thyroid and kidney disease: An overview of the evidence. Curr Opin Endocrinol Diabetes Obes. 2016;23(5):407-15. doi:10.1097/MED.0000000000000275.
[7] Kapoor K, Saha A, Dubey NK, Goyal P, Suresh CP, Batra V, et al. Subclinical non-autoimmune hypothyroidism in children with steroid resistant nephrotic syndrome. Clin Exp Nephrol. 2014;18(1):113-17.
[8] Dagan A, Cleper R, Krause I, Blumenthal D, Davidovits M. Hypothyroidism in children with steroid-resistant nephrotic syndrome. Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc. 2012;27(6):2171-75.
[9] Bagga A. Revised guidelines for management of steroid-sensitive nephrotic syndrome. Indian J Nephrol. 2008;18(1):31-39.
[10] Jahan KI, Parvez KS, Mia MSH, Khan MMR, Haque MJ. Thyroid function in children with nephrotic syndrome and normal renal function in a tertiary level hospital, Rajshahi. TAJ J Teach Assoc. 2018;31(1):62-69.
[11] Rapa A, Monzani A, Moia S, Vivenza D, Bellone S, Petri A, et al. Subclinical hypothyroidism in children and adolescents: A wide range of clinical, biochemical, and genetic factors involved. J Clin Endocrinol Metab. 2009;94(7):2414-20.

[12] McLean RH, Kennedy TL, Rosoulpour M, Ratzan SK, Siegel NJ, Kauschansky A, et al. Hypothyroidism in the congenital nephrotic syndrome. J Pediatr. 1982;101(1):72-75.
[13] Guo QY, Zhu QJ, Liu YF, Zhang HJ, Ding Y, Zhai WS, et al. Steroids combined with levothyroxine to treat children with idiopathic nephrotic syndrome: A retrospective single-center study. Pediatr Nephrol Berl Ger. 2014;29(6):1033-38.
[14] Nuraeni E, Faisal F, Widiasta A, Novina N. Subclinical hypothyroidism in pediatric nephrotic syndrome: The correlations with albumin, globulin, and proteinuria. Paediatrica Indonesiana. 2020;60(2):90-94.
[15] Gopal B, Anjali M. Interactions between thyroid disorders and kidney disease. Indian Journal of Endocrinology and Metabolism. 2012;16(2):204-13.
[16] Marimuthu V, Krishnamurthy S, Rajappa M. Non-autoimmune subclinical and overt hypothyroidism in idiopathic steroid-resistant nephrotic syndrome in children. Indian Pediatr. 2017;54(11):925-29.
[17] Saha A, Bagri N, Mehera N, Dubey NK, Batra V. Membranoproliferative glomerulonephritis associated with autoimmune thyroiditis. J Pediatr Endocrinol Metab. 2011;24:789-92.
[18] Ito S, Kano K, Ando T, Ichimura T. Thyroid function in children with nephrotic syndrome. Pediatr Nephrol Berl Ger. 1994;8(4):412-15.
[19] Lo JC, Chertow GM, Go AS, Hsu C-Y. Increased prevalence of subclinical and clinical hypothyroidism in persons with chronic kidney disease. Kidney Int. 2005;67(3):1047-52.

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