RESEARCH PAPER

Hepatitis B Seroprotection in Pediatric Nephrotic Syndrome

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Objectives: To study the prevalence of Hepatitis B seroprotection in children (>1 y) with nephrotic syndrome vaccinated against Hepatitis B vaccine as per the Universal Immunization Program schedule (0,6,10,14 wk); to compare the Hepatitis B seroprotection rates and anti-HBs titers among different phenotypes of nephrotic syndrome; to evaluate the association between Hepatitis B seroprotection status and the immunosuppressive agents; and to study the correlation between anti-HBs titres and proteinuria. **Methods:** Hepatitis B serology and anti-HBs titers were analyzed in 100 children (age-1-18 y) with different clinical phenotypes of nephrotic syndrome (cases) and 100 healthy controls. **Results**: The proportion of seroprotected children among the cases and controls was 37% (*n*=37) and 61% (*n*=61), respectively (*P*<0.04). The median (IQR) anti- HBs antibodies titers among the cases was 75 (62.5, 81) mIU/mL and 112 (56, 367) mIU/mL among the controls (*P*=0.001). The proportion of seroprotected children among the steroid sensitive nephrotic syndrome, steroid-resistant nephrotic syndrome and controls was 40% (*n*=28), 30% (*n*=9) and 61% (*n*=61), respectively (*P*<0.01). No differences in the anti-HBs titers between children receiving steroids *versus* steroids along with other immunosuppressants were found. Weak negative correlation was noted between proteinuria and protective titers (r = -0.155; *P*=0.039). **Conclusion:** Children with nephrotic syndrome, in general, and steroid-resistant nephrotic syndrome in particular, show poor seroprotection with Hepatitis B vaccination.

Keywords: *Corticosteroids, Hepatitis B vaccine, Seroconversion*.

Nephrotic syndrome (NS) in children is classified into steroid-sensitive nephrotic syndrome (SSNS) or steroid-resistant nephrotic syndrome (SRNS); the former being subdivided into infrequently-relapsing (IFRNS), frequently relapsing (FRNS) and steroid-dependent nephrotic syndrome (SDNS). The proteinuria in NS causes loss of albumin as well as various protective immunoglobulins in the body that have been acquired actively or passively [1,2]. In India, 2-7% of individuals are carriers of hepatitis B virus (HBV) that places this region in an intermediate endemicity zone [3]. HBV infection acquired in childhood has higher chance of progression to chronic hepatitis, cirrhosis and hepatocellular carcinoma [4]. The World Health Organization (WHO) recommends hepatitis B vaccination for all children. It has been speculated that children with NS might have decreased hepatitis B seroprotection rates due to prolonged immunosuppressive therapy and proteinuria [5]. However, there is paucity of information regarding the seroprotection status in pediatric NS. The primary objective of this study was to evaluate the prevalence of hepatitis B seroprotection (defined as anti-HBs titers ≥ 10 mIU/mL) in children with NS who had previously been vaccinated against hepatitis B as per the Universal Immunization Program (UIP). The secondary objectives were to compare hepatitis B seroprotection rates among different clinical phenotypes of NS; to evaluate the association between hepatitis B seroprotection and immunosuppressive agents received; and to study the correlation between anti-HBs titers and proteinuria.

METHODS

This cross-sectional study was conducted from March 2016 through July 2018 after obtaining approval from the Institute ethics committee and obtaining written consent from the parents. Consecutively presenting patients were recruited from the pediatric nephrology clinic (for cases) and the general pediatric outpatient department (OPD) (for controls).

Children (≥1 year) with primary nephrotic syndrome who had received full course of Hepatitis B vaccination (as verified by the vaccination card) as per the UIP (at 0, 6 weeks, 10 weeks, 14 weeks) [6,7] were included. Cases

of secondary nephrotic syndrome (*e.g*. IgA nephropathy, lupus nephritis), chronic kidney disease with creatinine clearance <60 mL/min/1.73 m^2, hepatitis B positivity and those who had received any blood product/ intravenous immunoglobulin within 3 months were excluded. Age-matched, healthy, non-proteinuric controls (age 1-18 y) who had received hepatitis B vaccine as per the same schedule as cases were recruited from patients visiting general OPD for minor ailments (*e.g*., upper respiratory tract infections, elective surgery).

Nephrotic syndrome was managed as per Indian Society of Pediatric Nephrology guidelines (8,9). Data were recorded in a structured proforma. The anti-HBs titres were quantitatively determined by immuno-enzymometric assay using a commercially available kit (Elecsys anti-HBs II, Cobas) as per the manufacturer's instructions. The anti-HBs titers were estimated quantitatively. Seroprotection was defined as anti-Hbs titers ≥10 mIU/mL [10].

The sample size was calculated to be 87 for estimating the proportion of hepatitis B seroprotected individuals as 65% [13]; with 95% confidence level and precision of 10%, Accounting for attrition of 10%, we planned to recruit a minimum of 96 participants.

Statistical analysis: Student's *t*-test was used to compare normally distributed continuous variables and proportions were compared using Chi-square test or Fisher exact test as applicable. Median and distribution were compared using Mann Whitney U test. The outcome variables between more than two subgroups of NS were analyzed using ANOVA or Kruskal-Wallis test. Pearson's Correlation coefficient was used to measure linear correlation between two continuous variables. Data were analysed using SPSS version 19.

RESULTS

One hundred and thirty two children with NS were assessed for eligibility. Thirty two cases were excluded from the study. Reasons for exclusion included: 6 cases of secondary nephrotic syndrome (Lupus nephritis 2, IgA nephropathy 2, Membranoproliferative glomerulo-nephritis 1, Henoch Schonlein purpura nephritis); 2 children with creatinine clearance <60 mL/min/1.73 m^2; 16 patients having had no immunization card; and 8 patients who were partially vaccinated against Hepatitis B. Finally, 100 children with NS were enrolled.

The characteristics of children with NS are depicted in ***Table* I.** All the recruited children were on prednisolone and/or alternate immunosuppressive therapy at recruitment. Among 70 SSNS cases, 15 (22%) received steroids alone and 55 (78%) received atleast one additional immunosuppressant at enrolment. All SRNS cases were on immunosuppressants at enrolment. Among SRNS cases, 14 (50%) had received more than one immunosuppressant. Majority (25 of 30) of SRNS patients were in complete or partial remission. The histopathological profile among SRNS included minimal change disease (36.6%), focal segmental glomerulosclerosis (46.7%) and mesangioproliferative glomerulonephritis (16.7%).

TABLE I CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF CHILDREN (*N*=100) WITH NEPHROTIC SYNDROME ENROLLED IN THE STUDY

Characteristic	*Value*
Age at enrolment (y)*	7.5 (4.3,9.3)
Male sex	60 (60%)
Age at onset of NS (y)*	4.6 (2,6.5)
Duration of NS (y)	2.4 (1.7,3.4)
Weight-for-age Z score	-0.66 (0.26)
H eight-for-age Z score	-1.79 (0.6)
Body surface area (m^2)#	0.89 (0.33)
Clinical type of NS	
Infrequently relapsing	20 (20%)
Frequently relapsing	32 (32%)
Steroid dependent	16 (16%)
Steroid resistant	30 (30%)
First episode	2 (2%)
Immunosuppressants received	
Prednisolone	22 (20%)
Levamisole	24 (24%)
Mycophenolate mofetil	20 (20%)
Oral cyclophosphamide	6 (6%)
IV cyclophosphamide	7 (7%)
Cyclosporin	15 (15%)
Rituximab + Mycophenolate mofetil	4 (4%)
Tacrolimus	2 (2%)

*Values in n (%) or *median (IQR) or #mean (SD); NS: nephrotic syndrome.*

The proportion of seroprotected children among the cases and controls was 37% (*n*=37) and 61% (*n*=61), respectively. Anti-HBs titers were higher among cases than controls (***Table* II**). Comparison of the SSNS and SRNS cases revealed significant difference between serum albumin and urine protein:creatinine ratio (Up:Uc) (***Web Table* I**). The proportion of seroprotected children among the SSNS, SRNS and controls was 40% (*n*=28), 30% (*n*=9) and 61% (*n*=61), respectively (*P*=0.002). The median (IQR) anti-HBsAg titers were 76.5 (64.2, 85.5),

WHAT THIS STUDY ADDS?

- Lesser proportion of children with nephrotic syndrome have seroprotection against hepatitis B following 4 doses of vaccine (0,6,10,14 wk) during infancy.
- Children with steroid-resistant nephrotic syndrome have lower anti-HBs titers than steroid-sensitive nephrotic syndrome.

63.5 (55.2, 66.7) and 112 (56, 367) mIU/mL, respectively among these 3 groups (*P*=0.002). The proportion of seroprotected children was similar between IFRNS, FRNS, SDNS and SRNS; as well as among children receiving various immunosuppressants.

On comparison of children with seroprotected (*n*=63) and non-seroprotected titres (*n*=37), significant differences were found between the proportion of children with Up:Uc >2 g/g (22.2% *vs.* 0%, *P*=0.01) and serum cholesterol levels (*P*=0.014). Weak negative correlation was noted between proteinuria and protective titers (r = -0.155; *P*=0.039).

DISCUSSION

In this study, a large proportion of children with NS, who had received hepatitis vaccine as per UIP, had low anti-HBs antibody titers in comparison to healthy controls. Children with SSNS were found to be seroprotected to a greater extent than SRNS. The degree of proteinuria was higher in the non-seroprotected children, implying the central role of proteinuria in the etiology of poor seroprotection.

There is paucity of information regarding the degree of hepatitis B seroprotection in children with NS, particularly SRNS. Mantan, *et al.* [11] enrolled 75 children with NS out of whom 56% had SRNS, and 61.3 % had received hepatitis B vaccination through pediatric nephrology services (double dose at 0, 1 and 6 months). The proportion of children with seroprotective titers was higher in SSNS than in SRNS, as well as in those who received steroids alone as against in combination other immunosuppressants. In another study, La Manna, *et al*. [12] vaccinated 18 boys with SSNS and a control group of 21 healthy boys against hepatitis B. The percentage of patients who responded to vaccination was significantly lower than the control group. The differences between the results of these studies [3,10] and our study could be related to heterogeneity in study designs, different patient populations, and variable vaccination schedules doses of vaccine. Repeated and protracted proteinuria in SRNS might have contributed to the low titers in our study [12,13]. Some studies on hepatitis B seroprotection in children with NS have used double dose of the vaccine [11]. Some studies in other immunocompromised states have shown titers after double dose vaccination of hepatitis B to be better [13].

TABLE II BASELINE CHARACTERISTICS AND OUTCOME VARIABLES IN THE STUDY PARTICIPANTS

Characteristic	*Cases* (*n=100*)	*Controls* (*n=100*)	*P value*
Age at enrolment (y)*	7.4 (4.3,9.3)	7.6 (4.35,9.6)	0.93
Male sex	60 (60%)	55 (55%)	0.95
Weight (kg)*	23.6 (17,28.2)	22.8 (16, 27.7)	0.35
Height (cm)#	118.99 (29)	120.5 (35)	0.41
Body surface area (m^2)#	0.88 (0.33)	0.86 (0.35)	0.66
Serum creatinine (mg/dL)#	0.65 (0.2)	0.6 (0.1)	0.36
Serum albumin (g/dL)#	3 (0.3)	3.4 (0.5)	0.001
Serum cholesterol (mg/dL)#	187 (20)	149 (25).	0.001
Anti-HBs titer (mIU/mL)*	75 (62.5, 81)	112 (56, 367)	0.004
Seroprotection	37 (37%)	61 (61%)	0.001

*Values in n(%). *median (IQR) or #mean (SD).*

We observed relatively low seroprotection status among controls (61%). An earlier study from Ghana concluded that all healthy children who received hepatitis B vaccination at 6, 10 and 14 weeks seroconverted initially, but the antibody titers waned with increasing age [14]. In our study, the median age of the controls was 7.6 years; hence, there is a possibility of titers having waned in this group.

The limitations of present study is that considering anti-HBs antibody titer alone as a marker of protection may not be adequate. It is the immune memory that is crucial [15]. In addition, this study may not be adequately powered to draw conclusions regarding the inter-group comparisons.

As the hepatitis B seroprotection status in vaccinated children with NS was found to be suboptimal, it may be worthwhile to screen all children with SSNS as well as SRNS for hepatitis B seroprotection status, and augment it accordingly.

Contributors: NN: collected the data, reviewed the literature and drafted the first version of the manuscript; SK: conceptualized

the study, designed the study protocol, reviewed the literature and revised the manuscript; BJ: participated in protocol preparations and critically reviewed the manuscript; RD: participated in protocol preparations, supervised the laboratory tests and critically reviewed the manuscript. All authors approved the final version of the manuscript, and are willing to be accountable for all aspects of the study. SK: shall act as guarantor of the paper.

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WEB TABLE I CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF CHILDREN WITH STEROID SENSITIVE NEPHROTIC SYNDROME *VS* CHILDREN WITH STEROID RESISTANT NEPHROTIC SYNDROME

Characteristic	*Steroid Sensitive Nephrotic Syndrome (n=70)*	*Steroid Resistant Nephrotic Syndrome (n=30)*	*P value*
Age at enrolment (y)*	8 (4.2, 9.4)	7.3 (4.4, 9.3)	0.97
Age at onset of nephrotic syndrome (y)*	4.6 (1.9, 7.5)	4.6 (2, 8.4)	0.91
Duration of nephrotic syndrome (y)*	2.1 (1.2, 2.9)	2.6 (1.7, 3.6)	0.18
Male sex	44 (62)	16 (53)	0.38
Weight (kg)*	23.4 (16.7,28)	26.9 (17.5,47.9)	0.39
Height (cm)#	117.73 (20.3)	118.6 (24.7)	0.56
Body surface area (m^2)#	0.87 (0.3)	0.96 (0.32)	0.44
Serum creatinine (mg/dL)#	0.6 (0.2)	0.7 (0.3)	0.99
Serum albumin (g/dL)#	3 (0.3)	2.9 (0.4)	0.007
Serum cholesterol (mg/dL)	187 (19.3)	188.5 (29)	0.95
Spot urine protein creatinine ratio >2g/g	5 (7)	9 (30)	0.01
Spot urine protein creatinine ratio (g/g)*	0.13 (0.06,0.17)	0.16 (0.12,0.37)	0.02

*Value in n (%), *median (IQR) or #mean (SD).*