RESEARCH PAPER

Seroprotection for Diphtheria, Pertussis, Tetanus and Measles in Children With Nephrotic Syndrome

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Objective: To determine seroprotective titres for diphtheria, pertussis, tetanus and measles in children with nephrotic syndrome who had received essential immunization. **Methods**: Children (2-18 years) with steroid sensitive nephrotic syndrome (SSNS) or steroid-resistant nephrotic syndrome (SRNS) who were in disease remission and had received essential childhood immunization were included. Anti-diphtheria, anti-pertussis, anti-tetanus and anti-measles antibody titres were measured. **Results**: Seventy-six (40 with SSNS; 36 with SRNS) children with mean (SD) age 7.54 (3.96) years were enrolled. The time elapsed since last vaccination was >5 years in 68.4% patients. The seroprotection rates for diphtheria, tetanus, pertussis, and measles were 86.8%, 93.4%, 31.6% and 77.6% respectively; lower in SRNS subjects compared to SSNS. Robust seroprotection titers (1.0 IU/mL) for diphtheria were seen in 23.8% SSNS and 17.9% SRNS; *P*=0.04, and for tetanus in 69.3% SSNS and 43.8% of SRNS subjects; *P*=0.03, respectively. **Conclusions**: Children with nephrotic syndrome especially those with SRNS have lower seroprotective titers for diphtheria, tetanus, pertussis and measles, necessitating a booster dose of DPT/DT/Td and MR/MMR.

Keywords: *Antibody titres, DPT vaccine, Immunization, MR vaccine.*

Nephrotic syndrome is a chronic renal disorder in children requiring treatment usually with steroids and occasionally other immunosuppressant agents. Children with nephrotic syndrome have lower seroconversion to various vaccines (both live and killed) due to immune dysregulation, prolonged immunosuppressive treatment, and recurrent prolonged proteinuria [1,2]. These children are also at an increased risk of acquiring vaccine preventable diseases (both bacterial and viral) due to repeated hospital admissions, prolonged immuno-suppression and deranged immune system.

Adequate seroprotection against vaccine preventable diseases (VPDs) has been reported in only 25-56% of immunocompromised children especially those on chemotherapy, with chronic kidney disease, and with underlying congenital and acquired immunodeficiency [3,4]. The level of antibody titers for diphtheria, pertussis, tetanus (DPT) and measles were lower in children with steroid sensitive nephrotic syndrome (SSNS) during an episode of relapse compared to disease remission for all antigens [2]. There is paucity of literature on seroprotection against VPDs in children with steroid resistant nephrotic syndrome (SRNS). An earlier study from our center showed lower seroprotection rates for hepatitis B in SRNS subjects compared to SSNS [5].

The present study was aimed at looking for the seroprotective titers against diphtheria, pertussis, tetanus and measles in both SSNS and SRNS children in remission.

METHODS

This cross-sectional study was conducted in the Departments of Pediatrics and Microbiology of a tertiary care teaching hospital during the period (January, 2016-January, 2017). The study protocol was approved by the institutional ethics committee. A written informed consent and assent was taken from the caregivers.

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All children (2-18 years) with nephrotic syndrome who were in remission and had previously received essential immunization were included. Essential immunization was defined as having received three primary doses of diphtheria, pertussis and tetanus (DPT) with at least one booster, and single or 2 doses of measles and/or measles, mumps, rubella (MMR) vaccines by 2 years of age. The details of prior vaccination were obtained from either the vaccination card or immunization history. Children with congenital nephrotic syndrome were excluded.

A pretested proforma consisting of clinical details,

immunization status and history regarding age of onset, type of disease (SSNS or SRNS), relapse or remission, type and duration of immunosuppressive treatment was filled. Standard definitions were used to define nephrotic syndrome, relapse, remission, SSNS and SRNS [6,7]. Five mL of venous blood was collected from each participant; 2 mL sample was used for estimation of renal function tests, serum protein, serum cholesterol, and sera was separated from the remaining 3ml sample and stored at -70ºC until further tested. Anti-tetanus, anti-diphtheria, anti-pertussis and anti-measles IgG antibodies were measured using an enzyme immunoassay (ELISA) using a commercially available kit DEDIP01 (for diphtheria), DETET01 (for tetanus), DEBPT01 (for pertussis) and DEMAS01 (for measles) (Demeditec Diagnostics GmbH). Results of the anti-diphtheria, anti-tetanus, anti-pertussis and anti-measles antibody titers using the mentioned kits were obtained in OD (optical density) units. These OD units were converted to respective antibody titers in mIU/ml using the manufacturer's instruction. The validation criteria provided by the manufacturer was fulfilled for all samples studied. The cut-offs for seroprotective titers for diphtheria and tetanus were taken as ≥0.1 IU/mL [8,9]; titers >1.0 IU/mL are associated with long-term protection according to WHO [10]. The sero-protective titers for pertussis and measles were defined as levels more than 22 IU/mL and 12 IU/mL, respectively [11,12]. The sensitivity and specificity of the mentioned kits was 94% and 94% for diphtheria, 100% and 84% for pertussis and 90% each for tetanus; the values for measles were 97% and 100%, respectively as per the manufacturer's manual.

Sero-protection for diphtheria and tetanus in immunocompromised children is reported to vary from 45-80% [2,3]; based on 75% prevalence of seroprotective titers in children with nephrotic syndrome and 95% confidence level with 10% precision, the calculated sample size was 72 and a total of 76 patients were enrolled.

Statistical analyses: All data was compiled in Excel spreadsheet and analyzed using descriptive statistics; mean and standard deviation (SD) were calculated for baseline characteristics and antibody titers. Chi-square test was used to compare groups with categorical data and student t-test or one-way ANOVA was used for comparing continuous data. $P<0.05$ was considered significant.

RESULTS

Seventy-six children (60 boys) with nephrotic syndrome with a mean (SD) age of 7.54 (3.96) years were enrolled; the mean age of SSNS and SRNS was 6.9 (3.6) and 8.4 (4.2) years, respectively; $P=0.1$. Forty (53%) children had SSNS and 36 (47%) had SRNS disease. Twenty-two (55%) children with SSNS had received prednisolone alone, while remaining received drugs like levamisole, oral cyclophosphamide and mycophenolate mofetil. All children with SRNS received cyclosporine or tacrolimus along with prednisolone. At enrollment 33.3% children with SSNS and 8.3% with SRNS were off any treatment while the remaining were on minimal doses of prednisolone and other agents.

The time elapsed since last immunization for DPT was ≥5 years in 52 (68.4%) children, all of whom had received two boosters of DPT vaccine. Overall seroprotection against diphtheria, tetanus, pertussis and measles was seen in (n=66) 86.8%, (n=71) 93.4%, (n=24) 31.6% and (n=59) 77.6% children. The proportion of children with adequate seroprotection for all four antigens among SSNS and SRNS is shown in **Table I**. Good long-term antibody response > 1.0 IU/mL for diphtheria and tetanus was seen in (n=14) 18.4% and (n=41) 53.9% patients respectively; the difference being significant between SSNS and SRNS.

The mean (SD) anti-diptheria, anti-tetanus, anti-pertussis and anti-measles titres were 0.39 (0.12), 1.14 (0.42), 32.05 (10.5), 135.40 (32.89) IU/mL, respectively. The comparative antibody titres between SSNS and SRNS are shown in **Table II**. A higher proportion of children on steroids alone achieved seroprotection compared to those who received other immunosuppressants with prednisolone against diphtheria (91% vs 85.2%), tetanus (91% vs 90.7%), pertussis (36.4 % vs 29.6%) and measles (86.4% vs 74%).

Table I Seroprotection for Diphtheria, Tetanus, Pertussis and Measles in Children With Steroid Sensitive and Steroid Resistant Nephrotic Syndrome

Antibody titres	*SSNS (n=40)*	*SRNS (n=36)*	*P value*
Diphtheria			
> 0.1 IU/mL	38 (95.0)	28 (77.8)	0.03
> 1.0 IU/mL	9 (23.8)	5 (17.9)	0.04
Tetanus			
> 0.1 IU/mL	39 (97.5)	32 (88.9)	0.18
> 1.0 IU/mL	27 (69.3)	14 (43.8)	0.03
Pertussis			
> 22 IU/mL	14 (35.0)	10 (27.8)	0.45
Measles			
> 12 IU/mL	33 (82.5)	26 (72.2)	0.28

All values in no. (%). SSNS: steroid sensitive nephrotic syndrome; SRNS: steroid resistant nephrotic syndrome.

Table II Antibody Titers for different Antigens in Children With Steroid Sensitive and Steroid Resistant Nephrotic Syndrome

Antibody type	*SSNS(n=40)*	*SRNS (n= 36)*
Anti-diphtheria titres (IU/mL)	0.43 (0.05)	0.35 (0.05)
Anti-tetanus titres (IU/mL) *	1.48 (0.23)	0.77 (0.16)
Anti-pertussis titres (IU/mL)	32.3 (5.1)	32 (4.8)
Anti-measles titres (IU/mL)	139.52 (17.7)	137.9 (19.6)

*Values in mean (SD). SSNS: steroid sensitive nephrotic syndrome; SRNS: steroid resistant nephrotic syndrome; *P=0.019.*

DISCUSSION

The present study reports protective antibody titres against diphtheria, tetanus, pertussis and measles in children with nephrotic syndrome. There has been a recent increase in incidence of diphtheria and tetanus despite the universal immunization programme in developing countries like India [13,14]. The literature on seroprotection for vaccine preventable diseases in nephrotic syndrome is scant; the seroprotection rates for diphtheria, tetanus and measles in the study population were similar to the normal comparative population [8,9,13,15].

While the tissue culture neutralization assay is regarded as the most accurate in vitro procedure for measuring anti-diphtheria antibody, ELISA and passive hemagglutination methods are more widely used due to easier availability and lower costs [10]. A higher cut-off level of >1.0 IU/mL, for both diphtheria and tetanus suggests good long-term protection [8-10]. However, a smaller proportion of children had good response against diphtheria unlike tetanus in the present study, when majority of the patients had also received the second booster of DPT vaccine at five years of age. This information clearly highlights that booster doses of diphtheria vaccine were required in this immuno-suppressed group. The SRNS group also had significantly lower titres than SSNS group against tetanus, with a poor overall seroprotection rate against pertussis (32%) in the present study. Another study [3], which looked at the seroprotective titers for diphtheria, tetanus and pertussis in 146 children who had received chemotherapy showed significantly lower protective titers for diphtheria and tetanus in patients when compared to the healthy subjects; with abysmally lower seroprotection rate for pertussis, similar to our data. The need for booster doses pertussis has been highlighted earlier [15]. The Government of India introduced Td instead of tetanus toxoid alone in the national immunization schedule in 2019 to provide for boosting of waning immunity against diphtheria during adolescence [16].

The seroprotection against diphtheria and tetanus was lower in SRNS than SSNS subjects in this study. This was possibly due to the use of more prolonged immunosuppression and recurrent proteinuria in these subjects. Lower antibody titers against diphtheria, tetanus and pertussis were likewise reported in 18 children with SSNS compared with 20 controls, which were further lower during relapse states for all 3 infections and improved during remission (irrespective of steroid therapy) indicating that proteinuria may decrease the levels of antibodies [2]. However, the difference in seroprotection between SSNS and SRNS was documented even during remission (no proteinuria) in the present study, highlighting the role of immuno-suppressant use in these patients. Lower antibody titers for diphtheria and tetanus were also seen in 400 patients with juvenile idiopathic arthritis compared to the 2176 healthy controls. Prolonged immunosuppression was cited as the reason for lower levels of protection especially for diphtheria in the study participants [17]. The present study also showed that children who received steroids alone had higher seroprotective titers for diphtheria and tetanus as compared to those receiving other immunosuppressant agents as well, similar to a previous study done in children with leukemia and hematopoietic transplant recipients [3]. Another study showed lower protection for hepatitis B in children with nephrotic syndrome compared to controls with lower antibody titres in SRNS subjects [18]. Interestingly the anti-measles antibody titers in our study were comparable in both SSNS and SRNS and could be due to boosting effect provided by subclinical infections in the community unlike diphtheria and tetanus.

The limitations of the present study are lack of control arm and the variability in the timing of antibody test from the time of immunization where role of waning immunity was not discernible. Due to the rarity of the condition, especially SRNS, too stringent criteria would substantially reduce the sample size for any meaningful interpretation.

Based on the results of our study we conclude that children with nephrotic syndrome had lower sero-protective titers for diphtheria, tetanus, pertussis and measles, even during periods of remission and the seroprotection rates were lower for those with SRNS disease. We suggest a booster dose of DPT or Tdap (if age >7 years) and MR/MMR to be administered to all children, especially those with SRNS once the child is in remission or receiving minimal doses of immuno-suppressant, preferably after measuring the antibody titers.

Ethical clearance: Institutional Ethics Committee of Maulana Azad Medical College; No.11/IEC/MAMC/2015/317, dated November 27, 2015.

WHAT THIS STUDY ADDS?

- Seroprotection against diphtheria, tetanus, pertussis and measles is lower in children with SRNS than SSNS.
- There is a need to administer dT/TdaP and MMR boosters, especially in children with SRNS disease, beyond 7 years of age.

Contributors: MM, SY, AC: conceptualization; AM, AA: Methodology; AM, MM, AD: software; AA, MM, SY: validation; AM, AD, MM: formal analysis; AA, AC: investigation; MM, AC: resources; AM, MM, AD: data curation, AM, MM, AD: writing - original draft; AA, MM, AD: writing - review & editing; MM: visualization; MM, SY, AC: supervision; MM, AA: project administration. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.

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