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SYSTEMATIC REVIEWS

- 1 Vitamin D therapy in children with inflammatory bowel disease: A systematic review
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Vitamin D therapy in children with inflammatory bowel disease: A systematic review

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Abstract

BACKGROUND

Vitamin D deficiency is highly prevalent in children with inflammatory bowel disease (IBD). This may contribute to an increased risk of poor bone health and may also influence the course of disease. An optimal treatment strategy of vitamin D therapy in children with IBD has not yet been established.

AIM

To analyse the published intervention studies of vitamin D therapy in children with IBD.

METHODS

A systematic review was conducted of clinical studies involving children with IBD (including Crohn disease or ulcerative colitis) who had received vitamin D therapy. Studies up to March 31st 2018 were identified through MEDLINE, PubMed, EMBASE and the Cochrane Library. Search terms included synonyms of the following terms: vitamin D, paediatric, supplementation, IBD. References of included articles based on abstract were searched for other relevant articles. All relevant articles were accessed and reviewed in full text. Studies fitting the set criteria were included and the remainder were excluded.

RESULTS

Two hundred and seventy-seven discrete articles were identified. Following assessment of these articles included in the initial search and application of inclusion and exclusion criteria, ten published studies were included in this review. The included studies showed a heterogeneity in study design, inclusion and exclusion criteria, baseline demographics and treatment strategies. Treatment regimens differed in length, supplemented form of vitamin D and factors based upon which dosage was adjusted. Each of the reports included in this review concluded their vitamin D regimens to be safe and well-tolerated. Few of the included studies reported secondary outcomes on the efficacy of vitamin D treatment upon the clinical course of disease or markers of inflammation. The

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majority of included trials were not sufficient in raising serum vitamin D levels to an adequate level (30 ng/mL) in children with IBD with vitamin D deficiency.

CONCLUSION

The included trials featured diverse treatment regimens that were predominantly insufficient in correcting vitamin D deficiency or maintaining adequate levels in children with IBD. Better treatment regimens are required for the management of vitamin D deficiency in children with IBD.

Key words: Child; Inflammatory bowel diseases; Vitamin D; Therapeutics; Systemic review

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Core tip: Vitamin D deficiency is commonly seen in children diagnosed with inflammatory bowel disease (IBD). Correction of deficiency and optimisation of vitamin D levels likely contribute to enhanced bone health and possibly to the underlying disease course. The optimal regimen and target for vitamin D therapy are not yet elucidated. This systematic analysis aimed to review the available reports of vitamin D therapy in children with IBD to assess efficacy and safety of this treatment, address the diversity of the available trials and provide recommendations for future trials in this field on the basis of the evidence reviewed.

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INTRODUCTION

Vitamin D has a pivotal role in both calcium and immune homeostasis^[1,2]. The significance of vitamin D in bone health is widely known: important relationships have been observed between vitamin D deficiency and rickets, osteopenia, osteoporosis, osteomalacia, muscle weakness and an increased risk of fracture^[3,4]. Vitamin D has also been shown to have a key role in immune regulation, primarily with a role in the regulation of T cell development and function^[5]. Recent studies have linked vitamin D deficiency with compromised immunity and an increased susceptibility to cardiovascular diseases, cancer and a variety of autoimmune diseases including rheumatoid arthritis, multiple sclerosis, type 1 diabetes mellitus, and inflammatory bowel disease (IBD)^[6,7].

The IBD, which include Crohn disease (CD) and ulcerative colitis (UC), are characterized by chronic uncontrolled inflammation of the gastro-intestinal tract^[8]. Around a quarter of patients diagnosed with IBD present before the age of 20 years^[9]. Children with IBD typically have more extensive disease, higher rates of extra intestinal manifestations, and greater nutritional consequences than adults with these conditions. Furthermore, the incidence of paediatric IBD is increasing internationally^[9-11].

Vitamin D deficiency is highly prevalent in children with IBD^[12-14]. Some paediatric trials show that this may in part contribute to an increased risk of poor bone health^[15,16], but contrasting conclusions have been shown^[17]. Recent studies have also focused on the relationship between vitamin D deficiency and disease severity in children with IBD; while some limited data suggests an association of vitamin D deficiency with a more severe course of disease, other studies do not report such a relationship^[18-20].

Although there are clearly a number of reasons to consider adequacy of vitamin D an important objective in children with IBD, the optimal regimen to achieve this has not yet been established. In recent years, several publications have assessed the outcomes of vitamin D therapy in children with IBD. While reviews of the reports evaluating vitamin D therapy in adults with IBD have been published, there has not yet been a systematic assessment of the published studies on vitamin D therapy in children with IBD. The aim of the present systematic review was to assess the safety

and efficacy of vitamin D therapy in children with IBD, by reviewing the published studies that have evaluated the administration of vitamin D in this population. Secondary aims were to address the diversity of the available trials, to identify gaps in current knowledge and to identify aspects relevant to future trials of paediatric vitamin D therapy.

MATERIALS AND METHODS

Search strategy

Following establishment of a review protocol, a literature search was conducted of all published studies up to March 31st 2018 through MEDLINE, PubMed, EMBASE and the Cochrane Library. Search terms used included “vitamin D” OR “25-hydroxy vitamin D” OR “vitamin D2” OR “vitamin D3” OR “calcidiol” OR “cholecalciferol” OR “25(OH)D” OR “hydroxycholecalciferol” OR “ergocalciferol” OR “25-hydroxy vitamin D2” OR “dihydrotachysterol” OR “calcifediol” OR “dihydroxycholecalciferol” OR “calcitriol” OR “alfacalcidol” OR “alphacalcidol” OR “hydroxycholecalciferol” OR “colecalfiferol” OR “cholecalciferol” in combination with “child” OR “childhood” OR “children” OR “paediatr*” OR “pediatr*” OR “adolescen*” in combination with “therap*” OR “treatment*” OR “regimen*” OR “supplementati*” OR “maintenance” OR “repleti*” in combination with “Crohn’s” OR “colitis” OR “inflammatory bowel disease” OR “inflammatory bowel diseases” OR “IBD”. References of included articles based on abstract were searched for other relevant articles. Duplicates were subsequently removed.

Eligibility criteria

Trials conducted on patients up to 18 years of age with IBD, CD or UC in which vitamin D therapy was given and where serum (s)-25OHD levels were reported at baseline and follow-up were included in this review. Only published manuscripts were included. Studies were excluded if (1) no vitamin D therapy was given; (2) they were conducted on patients with diseases other than IBD, CD or UC; (3) they were performed on adult patients; or (4) if they were not available in English language. Studies that included both children and adult patients and that did not report separate outcomes for paediatric patients were also excluded.

Data extraction

Key data was extracted independently from each selected article including: authors and date of publication; study design; location of the trial; sample size; condition of the study population (IBD/CD/UC); vitamin D deficiency and/or insufficiency cut-off points; dosage, length and cumulative dose of the vitamin D regimens; mean and standard deviation or interquartile range of s-25OHD levels at baseline and follow-up measurements, primary outcome of the study, and if available, any conclusions of the authors regarding the effect of vitamin D therapy administered in the trial. Vitamin D concentrations reported in nmol were adjusted to and reported in ng/mL, to allow for accurate comparisons across all studies.

RESULTS

Initial and final search findings

A total of 353 articles were identified following the specified searches. Seventy-six duplicates were removed. The remaining 277 article abstracts were analysed and included or rejected according to the protocol criteria. Of the 13 articles remaining, three were subsequently excluded after full-text analysis. A final total of 10 articles were included in this review (Figure 1).

Five studies were designed to investigate the effect of vitamin D therapy on increasing vitamin D levels (Table 1), and four were designed to investigate the effect of therapies on parameters of bone health (Table 2). The final study was designed to investigate vitamin D therapy for maintaining sufficient vitamin D levels [21]. In the studies concerning bone health, vitamin D therapy was not the main intervention given; in one study vitamin D was administered to both the intervention and placebo group.

Findings from studies designed to examine the effect of vitamin D therapy used to treat deficiency

In a randomized trial by Wingate *et al* [22], 83 patients with quiescent CD aged 8-18 years were included. Patients taking corticosteroids (CS) within six weeks before

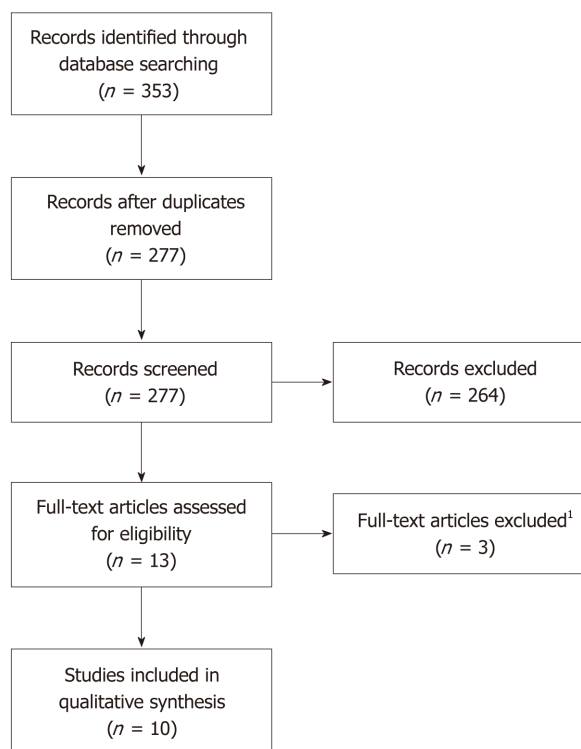


Figure 1 PRISMA flowchart outlining literature selection.¹These full-text articles were excluded because they were either cross-sectional studies with no follow-up s25OHD levels reported or vitamin D therapy was analysed as a dichotomous parameter with no information on dosage or length of regimen.

enrolment and/or vitamin D supplements containing > 1000 IU daily were excluded. In this study, 2000 IU vitamin D₃ was shown to be superior to 400 IU vitamin D₃ in raising mean s-25OHD concentrations during 6 months of supplementation. After 6 months of supplementation, 74% of subjects receiving a 2000 IU daily dose reached s-25OHD levels above 30 ng/mL, compared to 35% of subjects receiving 400 IU daily. During the trial, 2000 IU daily was not always significantly superior to 400 IU daily; however, the higher was never inferior to the lower dose in raising s-25OHD concentrations. There was no difference observed between groups in participants experiencing hypercalcemia, hyperphosphatemia, or exceeded safety cut-off level of urinary calcium to creatinine ratio. There was no observed difference in elevated C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR) concentrations between groups. Pediatric Crohn's Disease Activity Index (PCDAI) scores were only defined at baseline. The authors did not report data concerning the occurrence of adverse events.

The study by Pappa *et al*^[23] included 71 patients aged 5-21 years with a s-25OHD ≤ 20 ng/mL within 8 wk of enrolment. Exclusion criteria were liver or kidney failure, therapy with anticonvulsants metabolized through cytochrome P450, pregnancy, inability to take oral medication, attendance at a tanning salon once weekly or more, or receiving a treatment for vitamin D deficiency. This study showed superiority of both 2000 IU D₃ daily and 50000 IU D₂ weekly, over 2000 IU D₂ daily in changing mean s-25OHD concentrations over a treatment period of 6 wk. Both 2000 IU D₃ daily and 50000 IU D₂ weekly raised s-25OHD concentrations above 20 ng/mL in 95% of the participants, while 75% of participants in the latter group achieved s-25OHD levels above 32 ng/mL (compared to 38% of the subjects who received 2000 IU D₃ daily). The authors did not report whether the change in mean s-25OHD levels was significantly different between the 2000 IU D₃ daily group and the 50000 IU D₂ weekly group. One third of all participants experienced adverse events, but there was no difference in the frequency of adverse events between the three dosing regimens. None of the participants experienced hypercalcemia or hyperphosphatemia.

In a pilot study by Simek *et al*^[24], a weight-based dosing schedule of vitamin D was used for a total of 6 wk. This study included 32 patients aged 8-21 years with a s-25OHD concentration below 30 ng/mL. Patients with a known history of renal or hepatobiliary disease, use of systemic CS within 60 d of enrolment or with an inability to swallow study drug capsules were excluded. A regimen of 10000 IU/10 kg vitamin D₃ per week was compared with 5000 IU/10 kg vitamin D₃ per week. At 8 and 12 wk,

Table 1 Characteristics of included studies: Evaluating vitamin D treatment in children with inflammatory bowel disease

Study	N ¹	Location	Design	Treatment regimen	Vitamin D levels ²		Treatment duration ³	Cumulative dose	Disease activity ⁴
					Before	After			
Wingate, 2014	83 (CD)	Canada	RCT	A: 400 IU/d D ₃ ; B: 2000 IU/d D ₃	24 ± 7.9; A: 26 ± 9.2; B: 22 ± 6.8	A: 28.8 ± 9.2 (3 mo), 28 ± 8.6 (6 mo); B: 34 ± 10 (3 mo), 34.4 ± 10.4 (6 mo)	26 (183)	A: 73200 IU; B: 366000 IU	A: < 10:83 B: < 10:88
Pappa, 2012	71 (IBD)	United States	RCT	A: 2000 IU/d D ₂ ; B: 2000 IU/d D ₃ ; C: 50000 IU/wk D ₂	A: 16.1 ± 1; B: 14.7 ± 0.8; C: 51.3 ± 1	A: 25.7 ± 2.2 (6 wk); B: 31.5 ± 1.9 (6 wk); C: 40.8 ± 2.6 (6 wk)	6 (42)	A: 84000 IU; B: 84000 IU; C: 300000 IU	A: <10:54; B: <10:54; C: <10:78
Simek, 2016	32 (IBD)	United States	RCT	A: 5000 IU/10 kg/wk D ₃ ; B: 1000 IU/10 kg/wk D ₃	A: 24 ± 7; B: 23.7 ± 8.5	A: 41.5 ± 9.6 (8 wk), 30.8 ± 4.2 (12 wk); B: 49.2 ± 13.6 (8 wk), 35.1 ± 8.4 (12 wk)	6 (42)	A: up to 150000 IU; B: up to 150000 IU	Unknown
Shepherd, 2015	76 (IBD)	Australia	Retrospective	< 3 yr: 200000 IU D ₃ ; 3-12 yr: 400000 IU D ₃ ; > 12 yr: 800000 IU D ₃	16.4 ± 3.2	58.4 ± 20.8 (1 mo); 34.8 ± 11.2 (3 mo); 27.6 ± 12.4 (6 mo)	Single dose	< 3 yr: 200000 IU D ₃ ; 3-12 yr: 400000 IU D ₃ ; > 12 yr: 800000 IU D ₃	10 (0-47.5) 0 mo; 2.5 (0-85) 6 mo
Santucci, 2014	61 (IBD)	United States	Retrospective	A: no treatment; B: 400 IU/d; C: 600-1200 IU/d; D: 2000 IU/d or 50000 IU/wk	45% < 30 ng/mL (baseline); 50% < 30 ng/mL; 25% < 30 ng/mL; 88% < 30 ng/mL	Unknown	Unknown	Unknown	Unknown

¹Number of patients recruited (type of disease);²Serum levels expressed as mean ± SD;³Treatment duration expressed as days (weeks);⁴Disease activity scores (Pediatric Crohn disease activity index or Pediatric ulcerative colitis disease activity index) at baseline unless otherwise stated. Expressed as median (range) or percentage of patients with score less than 10 (remission). IBD: Inflammatory bowel disease; CD: Crohn disease; UC: Ulcerative colitis.

mean s-25OHD concentrations in the higher dosage group were maintained at a higher concentration than in the lower dosage group but did not differ significantly. The change in mean s-25OHD concentrations were not affected by participant weight. Calcium and parathyroid hormone (PTH) measurements were not significantly different between baseline and follow-up. In addition, no events of hypercalcemia or serious adverse events were observed. Measures of disease activity were not reported.

Shepherd *et al*^[25] showed in their retrospective chart review that a single high age-adjusted dose of vitamin D (stoss therapy) increased s-25OHD concentrations significantly at 1, 3 and 6-mo follow-up in 76 vitamin D deficient children with IBD. Specific exclusion criteria for these participants were not reported. The s-25OHD concentration of all subjects increased to a level above 50 nmol (20 ng/mL) at 1 mo, remaining above 20 ng/mL in 96.6% of the subjects at 3 mo, and in 76.4% of the subjects at 6 months. A s-25OHD concentration above 75 nmol (30 ng/mL) was seen in 98% of the subjects at 1 mo and 63% of the subjects at 3 mo. At 6 mo, 17% of the subjects had not maintained a sufficient s-25OHD concentration of 20 ng/mL or above. The authors stated that there was no evidence of toxicity of vitamin D in any of the participants. Calcium, phosphate and PTH were measured at baseline and during follow-up, and showed no significant changes. Disease activity was reported at baseline.

Santucci *et al*^[26] retrospectively reviewed vitamin and zinc deficiencies in a paediatric IBD cohort of 61 patients. Patients with coexisting medical conditions such as coeliac disease and cystic fibrosis that may affect vitamin D status were excluded. In total, the s-25OHD concentrations of 39 patients were assessed, of whom 21 participants received vitamin D treatment. This study showed that vitamin D supplementation with 2000 IU daily or 50000 IU weekly raised s-25OHD concentration to a level above 30 ng/mL in 88% children, compared to 50% in children receiving 400 IU daily and 25% in patients receiving 600-1200 IU daily. In

Table 2 Characteristics of included studies: Vitamin D treatment and evaluating bone health in children with inflammatory bowel disease

Study	N ¹	Location	Design	Treatment Regimen	Vitamin D levels ²		Main study outcome	Disease activity ³
					Baseline	Follow-up		
Hradsky, 2017	55 (IBD)	Czech Republic	Cohort	2000 IU/d D ₃	23.2 (17.2, 31.6)	34 (24.4, 38.4)	Effect of vitamin D on trabecular BMD and muscle power	5 (0-10)
Benchimol, 2007	72 (IBD)	North America	Prospective	A: no intervention; B: supplemental calcium or supplemental calcium & 50000 IU/m D ₂	A: 27.6; B: 22.8 ± 8 or 24.4 ± 6	B: 26.4 ± 6 or 30 ± 12	Effect of vitamin D on lumbar spine BMD	A: 10.6 ± 10.6; B: 16.5 ± 15.7
Leonard, 2016	121 (CD)	United States	RCT	A: 10 min LMMS/d; B: no LMMS (both 600 IU/d D ₃)	31.5 ± 10.3	34.2 ± 12.4 (12 m); 37.7 ± 15.6 (24 m) ⁴	Effect of LMMS on BMD	< 10:52; 10-30:33; > 30:5
Laakso, 2014	47 (IBD)	Finland	Cohort	Total vitamin D intake: 7.5 (1.6-19.5) µg/d (baseline); 12.6 (1.7-49.2) µg/d (follow-up)	19.6 (8.6, 40.8)	26.8 (10.4, 48)	BMD and BMC	< 10:72; 10-30:23; > 30:4

¹Number of patients recruited (type of disease);²Serum levels expressed as mean ± SD, mean and interquartile range, or mean;³Disease activity scores (Pediatric Crohn disease activity index or Pediatric ulcerative colitis disease activity index) at baseline expressed as mean (range), mean ± SD or percentage of patients with score less than 10, 10-30, or > 30;⁴Calculated in 105 patients who completed the trial. IBD: Inflammatory bowel disease; CD: Crohn disease; UC: Ulcerative colitis; BMD: Bone mineral density; LMMS: Low magnitude mechanical stimulation; BMC: Bone mineral content.

comparison, 45% of participants normalised without any vitamin D supplementation. In total, 52% of the vitamin D deficient children in the cohort had normalisation of vitamin D levels after treatment with different vitamin D dosing regimens over a follow-up period of 6 months to 5 years. This review did not specify whether vitamin D₂ or vitamin D₃ was supplemented and the rates of adverse events in different dosing schedules were not reported.

Findings from the study designed to examine the effect of vitamin D therapy used to maintain sufficient vitamin D levels

Pappa *et al*^[21] compared the efficacy and safety in maintaining s-25OHD above 32 ng/mL in two treatment arms; 400 IU vitamin D₂ daily (arm A), 1000 IU vitamin D₂ daily in summer and 2000 IU vitamin D₂ daily in winter (arm B). Sixty-three patients with IBD aged 5-21 years with a s-25OHD above 20 ng/mL, were followed three-monthly for 12 mo. Exclusion criteria were coexisting liver or kidney disease, pregnancy, use of anticonvulsants metabolized through cytochrome P450 or inability to take oral medications. In both groups, less than 10% of all patients achieved the main outcome of s-25OHD ≥ 32 ng/mL at all follow-up visits; the proportion of patients achieving this cut-off was similar in the two groups. In addition, only 79% of patients in arm A and 73% of patients in arm B with s-25OHD measured at all four follow-up visits maintained a s-25OHD concentration above 20 ng/mL: meaning that respectively 21% and 27% of the subjects presented with a s-25OHD below 20 ng/mL in at least one follow-up visit. The mean s-25OHD of both groups decreased from baseline at every visit with borderline significance. This study found that longer outdoor exposure and higher oral vitamin D intake were independently associated with the achievement of s-25OHD concentration ≥ 32 ng/mL at all follow-up visits. There was no difference observed between groups of participants experiencing adverse events, hypercalcemia, hyperphosphatemia, or exceeding safety cut-off level of urinary calcium to creatinine ratio. Disease activity was measured at baseline, 80% of patients had a PCDAI score below 10 (remission), 16% were between 10 and 30 (mild) and 5% of patients were above 30 (moderate/severe).

Findings from studies designed to examine the effect of bone health related therapies (including vitamin D) in paediatric IBD patients

The cohort study of Hradsky *et al*^[27] was designed to assess the effect of vitamin D₃ therapy on bone health parameters and dynamic muscle function. In total, 55 patients aged 5-19 years with a diagnosis of IBD were included. Patients were excluded if they had another disease with known impact on the muscle-bone unit, if they were noncompliant to treatment, if they were pregnant, if they had undergone extensive small bowel surgery, if they were on total parenteral nutrition, or if they received growth hormone therapy. All patients received 2000 IU of vitamin D₃ daily, for a median of 13.8 mo. Mean s-25OHD concentration at baseline was 58 nmol (23.2 ng/mL). This regimen resulted in 76% of the patients having s-25OHD concentration above 20 ng/mL throughout the study period. In addition, the vitamin D regimen in this cohort was associated with improvements in trabecular bone mineral density (BMD) at the tibia and muscle power. The majority of patients did not show increased PTH levels at follow-up. CRP levels were similar between baseline and follow-up visits. Similarly, PCDAI and the Paediatric Ulcerative Colitis Activity Index scores were not different between baseline and follow-up. The authors concluded that their regimen was safe.

Benchimol *et al*^[28] investigated the effect of supplementation with 1000 mg calcium daily for 12 months to 1000 mg calcium daily for 12 mo along with 50000 IU vitamin D₂ monthly for 6 months on BMD, compared to a control group who received no intervention. Seventy-two patients with IBD were included. Only subjects with an area lumbar spine BMD z score less than -1 were assigned to the intervention group. At baseline, children in the intervention group were shorter, thinner and experienced a greater bone age delay than subjects in the control group. The participants with CD in the intervention group trended towards a higher PCDAI score at baseline compared to controls. The mean s-25OHD of subjects receiving calcium alone and those receiving calcium and vitamin D supplementation together increased, with the latter group trending towards a level of 30 ng/mL, although the change from baseline was not statistically significant in either group. No serious adverse events were reported in any of the participants. No data on disease activity at follow-up or inflammatory markers were reported.

In a trial by Leonard *et al*^[29], the effect of low-magnitude mechanical stimuli on several bone related measures including BMD was analysed in 121 patients. Patients with CD aged 8-21 years who had a tibial trabecular volumetric BMD below the 25th percentile were included. Exclusion criteria were weight > 250 pounds, pregnancy, a medical illness unrelated to CD with potential adverse effects on bone or body composition, plans to travel for more than 4 wk over the study period, a sibling or acquaintance enrolled in the trial, or developmental disorders prohibiting completion of study procedures. Both the control and intervention group were supplemented with 1000 mg calcium and 800 IU vitamin D₃ daily for a period of 12 mo. Additional vitamin D intake in subjects was evaluated; if necessary the vitamin D supplementation was adjusted to prevent the average daily vitamin D intake from exceeding 2000 IU. Mean s-25OHD increased significantly but slightly among all participants. However, the proportion of patients with s-25OHD below 20 ng/mL did not change over the follow up period (7% at 12 mo and 8% at 24 mo). During the 12-month trial, there were 28 hospitalizations in the intervention group (*n* = 69) and 27 hospitalizations in the control group (*n* = 69), most of which were due to disease flares. In addition, 21 and 18 respectively reported symptoms at study visits.

A cohort study by Laakso *et al*^[30] assessed lumbar spine areal BMD and height-adjusted whole body (less head) bone mineral content in 47 children and adolescents with IBD, with a median follow-up of 5 years. Vitamin D intake and s-25OHD were measured at baseline and follow-up. Patients aged 4-20 years with a diagnosis of IBD at least 3 mo before enrolment were included. Patients were excluded if they had other medical conditions unrelated to IBD that could affect growth or bone mass accrual. Total dietary vitamin D intake for all participants differed significantly between 7.5 µg (300 IU) [range 1.6 (64) - 19.5 (780) µg (IU)] at baseline and 12.6 µg (504 IU) [range 1.7 (68) - 49.2 (1968) µg (IU)] at follow-up. The proportion of patients using additional vitamin D supplements was 52% at baseline and 63% at follow-up. As this was a cohort study, vitamin D intake differed substantially between participants. This study found that patients with a low lumbar spine areal BMD had a greater total vitamin D intake than participants with a normal lumbar spine areal BMD, while their s-25OHD concentrations were not significantly higher. The authors suggested that this might possibly reflect impaired absorption of nutrients.

DISCUSSION

Safety

The authors of each of the reports included in this review concluded that their vitamin D regimens were safe and well-tolerated. One study mentioned an adverse event rate of 32%^[23]; however, this rate was similar in all arms in their study and was therefore most likely not related solely to the vitamin D supplementation. Also, only patients with a s-25OHD concentration below 20 ng/mL were included in this study; the severe vitamin D deficiency at baseline may be related to the higher adverse event rate in this study group.

In general, few of the included studies provided quantitative data concerning adverse events. More detailed information on side effects and adverse events of vitamin D therapy is needed to compare safety between regimens and to define an optimal treatment dose. In future trials concerning regular or high dose vitamin D therapy, these markers should be measured strictly and possible side effects should be monitored.

Efficacy

Effects of vitamin D therapy on s-25OHD concentrations: Regarding an optimal cut-off value for vitamin D therapy, the Institute of Medicine has argued that s-25OHD levels above 30 ng/mL do not provide additional benefit^[31]. However, other studies suggest that a level of at least 32 ng/mL is required for optimal intestinal calcium absorption^[32,33]. A level of 30 ng/mL has also been mentioned as to be sufficient to reduce PTH activity^[34]. Based on this evidence, a target s-25OHD level of at least 30 ng/mL for children with IBD should be considered with regards to optimal bone health.

In one of the included studies, 2000 IU D₃ daily raised s-25OHD above 30 ng/mL in 74% of the participants after 6 months of treatment^[22]. Another study showed a similar response after weekly dosing of 50,000 IU D₃ for 6 wk^[23]. In a third study, 2000 IU D₃ daily was able to achieve a mean s-25OHD above 30 ng/mL in the study population throughout the course of treatment^[27]. Both 5000 IU/10 kg vitamin D₃ per week and 10000 IU/10 kg vitamin D₃ per week for a period of 6 wk were able to raise s-25OHD above 30 ng/mL at week 8 in one of the trials; however, this effect was lost by week 12^[24].

The stoss therapy seemed highly effective in raising s-25OHD levels above 30 ng/mL for at least 1 month and was more successful in maintaining concentrations above 30 ng/mL at 3 and 6 mo compared to other trials^[25]. No other trials normalized mean s-25OHD from a deficient level to a concentration above 30 ng/mL. The trial of maintenance of vitamin D levels was not successful in raising s-25OHD levels greater than 20 ng/mL at baseline to a level above 32 ng/mL during a period of 12 months, as less than 10% of the study population achieved this level at each trimonthly visit^[21].

Effects of vitamin D therapy on paediatric IBD: The influence of vitamin D therapy on disease severity is a further potential outcome of supplementation. This is relevant as the primary effects of vitamin D in this population (on factors such as BMD, bone resorption and calcium homeostasis) are inconsistent and the significance of hypovitaminosis D in children with IBD is still unclear^[17]. In adults, more evidence of the role of vitamin D in treatment of IBD is available; small trials show promising results but remain conflicting^[19,35]. Therefore, with the evidence for possible beneficial effects of vitamin D therapy on the clinical course of IBD in childhood being extremely limited, it would have been very useful if more of the included studies had reported disease severity related measures of the participants over time.

Only two of the included studies reported mean PCDAI scores at both baseline and follow-up^[22,25]. However, neither included statistical data to support these findings. One study showed a nonsignificant difference in PCDAI scores between treatment groups^[22]. They reported that the proportion of patients having a PCDAI ≤ 10 (quiescent disease) was smaller at follow-up than at baseline in both treatment arms. No statistical analyses comparing follow-up to baseline levels were reported. The other trial reported PCDAI scores varying from 0 to 47.5 (median 10) at baseline and from 0 to 85 (median 2.5) at 6 mo follow up^[25].

Two included studies reported changes in anti-inflammatory markers subsequent to vitamin D treatment^[21,22]. One reported lower levels of interleukin-6, CRP and ESR in participants taking higher doses of vitamin D^[21]. The authors stated that the study was not designed to investigate the relationships between vitamin D intake and inflammatory markers and that these results should be interpreted with caution. In contrast, the second study reported no differences in CRP and ESR between treatment groups^[22].

Both the limited data on this topic and their contrasting exploratory results call for prospective research that focuses on the effect of vitamin D therapy on disease severity of IBD, concerning clinical factors and anti-inflammatory effects.

Trial differences

While all included studies provide important data on vitamin D therapy in children with IBD, important study differences prevent direct comparisons of treatment regimens or conclusions as to an optimal dose of vitamin D. However, given the variability of disease and patient-related factors (see below), it may be more appropriate to consider personalized treatments rather than one regimen to suit all patients.

Study population: Regarding the studies designed to investigate efficacy of vitamin D therapy, two studies included only patients with a baseline s-25OHD concentration below 20 ng/mL^[23,25]. One study included patients with s-25OHD concentration below 30 ng/mL^[24]. The study focusing on maintenance therapy included only patients with a baseline s-25OHD level above 20 ng/mL^[21]. Another study included only patients with quiescent CD^[22].

Concerning exclusion criteria, patients taking corticosteroids shortly before enrolment were excluded in two studies^[22,24]. Two trials excluded children taking additional vitamin D supplements^[22,23]. Patients with a history of liver or kidney disease were excluded in three trials^[21,23,24]. Two studies also excluded patients taking anticonvulsants metabolized through cytochrome P450^[21,23].

Regarding the studies on bone health, one study excluded patients with coexisting diseases that might affect vitamin D status^[26]. Another study excluded patients with plans to travel more than four weeks over the study interval^[29]. Three studies excluded patients if they had a history of coexisting medical conditions unrelated to IBD that affected bone mass^[27,29,30]. Patients who had undergone extensive small bowel surgery, patients on total parenteral nutrition and patients on growth hormone therapy were excluded in one study^[27].

Due to these differences in inclusion and exclusion criteria, baseline differences in s-25OHD, co-medications, comorbidities, and disease severity exist between the various reports. Furthermore, *via* the principle of regression to the mean, patients with a lower baseline level of s-25OHD are more likely to achieve a greater mean change in s-25OHD than participants with a higher baseline level. This principle should be considered when comparing treatment regimens and when developing future dosing schedules.

Length of treatment regimen: Two studies both provided vitamin D therapy for 6 wk, whereas two other trials studied the effect of vitamin D supplementation over 6 mo^[22-24,28]. One trial supplemented participants with vitamin D over 12 mo^[29], two studies observed vitamin D levels after a median of respectively 13.8 mo and 5 years of supplementation^[27,30], and one study reviewed the effect of a single dose of vitamin D with follow up for 6 months^[25]. As suggested by the latter study, the duration of the effect of vitamin D could be dependent on the dosage, with higher dosages possibly having longer lasting effects^[25].

Form of vitamin D used: While vitamin D is often still prescribed by the number of international units or IU, which is based on the presumption of an equal bioavailability between vitamin D₂ and vitamin D₃, a growing body of literature recognizes that vitamin D₃ has a 2-3 times greater potency resulting in a higher s-25OHD with a longer duration of effect compared to vitamin D₂^[36,37]. Some trials treated patients using vitamin D₂, while the majority supplemented with vitamin D₃. One study showed superiority of vitamin D₃ compared to the same dosage of vitamin D₂ in raising s-25OHD levels in children with IBD^[23]. These aspects of bioavailability should be considered in future trials, and a distinction in recommended dose for vitamin D₂ and D₃ separately is needed.

Dose adjustment: One study utilised an age-adjusted dosage regimen^[25], while another used weight-adjusted dosing^[24]. The remaining trials did not adjust the dosage for weight or age. One trial suggested that a weight-adjusted dosing regimen might be more suitable, as an increase in s-25OHD was inversely related to weight^[23]. Furthermore, patients in this study who failed to achieve sufficient levels were heavier than average. This finding is in line with observations in healthy adults^[38,39]. In contrast, the study using a weight-adjusted dosing schedule observed no effect of differing weight upon the change in s-25OHD^[24]. One included study postulated that vitamin D is known to sequester in fat tissue and this could explain the variation in achieving sufficiency related to weight^[23]. In contrast, recent literature did not observe a sequestration of vitamin D₂ and vitamin D₃ in fat^[36].

Recent studies that describe an inverse relationship between an increase in mean s-25OHD level during therapy, and baseline s-25OHD concentration^[38,39], would support a dosage regimen based on severity of vitamin D deficiency. Vitamin D may play a role in improving bone health in children with IBD^[23,40]. In addition, one of the

included studies mentioned studies showing a negative effect of glucocorticoids on BMD^[27,41-44]. It seems logical to conclude that children with IBD receiving corticosteroids are likely to benefit from a higher dose of vitamin D. Currently, children with IBD receiving corticosteroids are often excluded from vitamin D trials or comprise a small proportion of study groups.

Taken together, these findings support the need for future studies that assess adjustment of vitamin D treatment based on weight and/or age, severity of vitamin D deficiency, treatment with corticosteroids and gender.

A different way to look at a personalized adjustment of vitamin D treatment is to identify the characteristics of patients failing to achieve sufficiency, such as heavier participants in one included study^[23]. In addition, some patients are prescribed immunosuppressants that are associated with increased sun sensitivity and are therefore counselled to carefully use sunscreen, which negatively influences UV-B synthesis of vitamin D^[45]; for these patients, a higher vitamin D dose might also be appropriate. Moreover, given that genetic variants in vitamin D hydroxylation are related to deficiency and a poor response to treatment^[46], future studies might be needed to identify patients with these genetic variants in order to personalise their treatment. In general, future trials concerning vitamin D therapy in children with IBD should try to identify the characteristics of patients failing to achieve the target level of 30 ng/mL while bearing some of these possible explanations in mind.

Knowledge gaps

While the studies reviewed in this paper give important evidence concerning the efficacy and safety of vitamin D therapy in children with IBD, remaining questions need to be addressed in order to define optimal vitamin D treatment regimens for this patient group.

Safety threshold: Three included studies refer to a safety threshold of s-25OHD at 88 ng/mL, as vitamin D intoxication has not been reported below this level^[21,23,27]. However, other authors mention this concentration was based on trials in healthy adults^[47]; data concerning children and especially those with chronic disease is lacking. As some of the participants in the stoss study had increased levels that exceeded 88 ng/mL^[25], without adverse events seen, continuing these single high-dose regimens might lead to new evidence concerning safety of vitamin D threshold. Participants achieving such high concentrations of s-25OHD should be carefully monitored, as potential risks include hypervitaminosis D with hypercalcaemia, hyperphosphatemia, hypercalciuria, nausea and renal calculi^[48].

One included study referred to a daily upper tolerable intake of vitamin D of 3000 IU in children ages 4-8 years and 4000 IU in children 9 years and older, without reference to the form of vitamin D^[22]. Due to the recent evidence that highlights the differences in bioavailability between vitamin D₂ and vitamin D₃, new studies on this topic should include different recommended intakes for vitamin D₂ or D₃, along with investigating if there is a difference in safety between vitamin D₂ and to vitamin D₃^[37].

Efficacy: With the included trials, vitamin D was introduced as a new therapy for children with IBD. Possible beneficial effects of this treatment are related to bone health, anti-inflammatory markers and disease severity. While the included trials are beginning to establish accurate treatment regimens to achieve sufficient vitamin D levels, the relevance of vitamin D treatment in this population remains controversial^[17]. Where four of the ten included studies in this review focused on bone health related measures as an outcome of therapies including vitamin D^[27-30], only two studies reported disease severity related measures (PCDAI scores)^[22,25], and two studies included anti-inflammatory markers as a secondary outcome^[21,22].

As stated above, the effect of vitamin D on the course of disease in these children is still unclear. Large prospective trials are needed to establish the efficacy of vitamin D treatment in this population, with a focus on the clinical course of disease, bone health and anti-inflammatory related measures.

Absorption: The studies included in this review share different opinions on the absorption of vitamin D in children with IBD compared to healthy children or adults. One study allocated participants to intervention and control groups based on their BMD; the intervention group had a lower BMD than the control group^[30]. The authors discussed that patients with a low lumbar spine areal BMD had a greater total vitamin D intake, while their s-25OHD levels were not significantly greater, reflecting a possible impaired absorption of vitamin D. In contrast, two other studies suggest that the absorption of vitamin D in their study population is likely not different from the healthy population^[22,23]. However, all the participants in one of those trials had baseline s-25OHD levels below 20 ng/mL, and following the principle of regression to the mean these patients are more likely to achieve a greater change in s-25OHD with

relatively lower vitamin D intake than patients or healthy children with a higher average s-25OHD concentration [23]. The participants in the second study all had quiescent CD: absorption in this group may differ to that in children with more active disease [22]. Future studies need to be carried out in order to validate the possibility of impaired absorption of vitamin D in children with IBD.

Adherence: Strict adherence is needed with a daily regimen in order to achieve optimal vitamin D levels. Lowering the treatment burden and improving adherence is likely to enhance s-25OHD status. A noncompliance rate around 30% was reported in both groups in one of the included studies [21]. In addition, another trial reported that 68% and 88% of participants who completed the study had adherence rates greater than 80% and 70%, respectively [22]. Furthermore, one trial stated that adherence in their trial was better with weekly than with either of the two daily regimens (89% *vs* 70% and 78%), therefore the authors recommend a weekly regimen [23]. On the other hand, the authors of the stoss study argued that a single dose treatment of vitamin D may minimise non-adherence to vitamin D treatment [25].

The purpose of the current study was to systematically review the available reports that had evaluated the efficacy and safety of vitamin D therapy in children with IBD. In addition to emphasizing the diversity between the included studies, this review has shown that the majority of included trials were not sufficient in raising s-25OHD concentrations of vitamin D deficient children with IBD to an acceptable level (30 ng/mL). Clearly adequate dosages are needed in order to achieve optimal levels above 30 ng/mL and to maintain these levels long term. The precise dosing regimen should consider various factors such as age, baseline s-25OHD concentration, weight, gender and disease activity. In addition, adherence with therapy is likely enhanced with weekly or single dosed regimens compared to daily regimens. Furthermore, the current data highlight the importance of applying dose adjustments and separate criteria for vitamin D₂ and vitamin D₃, measuring anti-inflammatory markers and further investigating safety in future trials on vitamin D therapy in paediatric patients with IBD.

This review has also emphasized that effects of vitamin D supplementation on disease severity related measures in paediatric IBD are not yet elucidated. As relationships between treatment with vitamin D and factors related to bone health, clinical course of disease and anti-inflammatory markers in paediatric IBD have not yet been clearly established, future trials should assess these topics prospectively.

Several limitations should be acknowledged. Firstly, only three published randomized controlled trials designed to investigate the efficacy of vitamin D therapy on s-25OHD concentrations were identified and included in this review. Because of the lack of comprehensive research on this topic, suggestions concerning treatment regimens in this review need to be confirmed in future trials. Second, given the diversity of included trials, the reviewed vitamin D regimens cannot be compared in a meta-analysis. Therefore, it is not possible to draw a conclusion about an optimal regimen; personalizing vitamin D treatment may be more appropriate. Notwithstanding these limitations, this first comprehensive assessment of vitamin D therapy in children with IBD provides important insights for future research in order to optimize the management of children with IBD.

ARTICLE HIGHLIGHTS

Research background

Vitamin D deficiency is commonly seen in children diagnosed with inflammatory bowel disease (IBD). While vitamin D is important for bone health, inadequate levels may also influence the course of disease.

Research motivation

To date, however, the optimal manner to correct and maintain adequate vitamin D levels in children with IBD have not been established.

Research objectives

The principal objective of this work was to systematically review the published literature focusing on vitamin D therapy in children with IBD. The further objective was to highlight opportunities for future work to augment and enhance the current understanding in this field.

Research methods

The published literature was reviewed systematically for publications that had focused on vitamin D therapy in children with IBD. The available data was then reviewed in detail.

Research conclusions

Ten published articles were identified and reviewed in detail. These reports included very different study designs with varied treatment regimens. Overall, there was no clear optimal regimen or approach that consistently corrected vitamin D deficiency and maintained levels over time.

Research perspectives

Vitamin D sufficiency is an important aspect of managing children with IBD. The available published literature does not provide a clear approach to the management of deficiency of vitamin D in this setting. Various aspects likely influence the response to vitamin D therapy: these are important factors that need to be taken into account in considering future work in this area.

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Applications of lung clearance index in monitoring children with cystic fibrosis

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Abstract

A sensitive, reproducible and feasible measure of lung function for monitoring the respiratory health is a prerequisite for the optimization of management of the patients with cystic fibrosis (CF). Spirometry has been considered the method of choice, although it is applicable only in children older than 6 years of age, as good cooperation is necessary for its proper performance. However, over the last 15 years, scientific interest in gas dilution techniques and particularly in multiple breath wash out (MBW) method has been revived. The most commonly reported index of MBW is lung clearance index (LCI). The aim of this review is to present the most recent developments in the application of LCI as a monitoring index of respiratory status of CF patients. LCI is a sensitive and reproducible marker of ventilation inhomogeneity. It is more sensitive than spirometry and, unlike spirometry; it can be performed across the whole pediatric age range. Since it is dependent on body size, until at least the age of 6 years, the relative and not the absolute changes are more appropriate for providing clinically meaningful conclusion on ventilation inhomogeneity. Until now, MBW has been mainly used as a research tool. Based on the currently available data LCI cannot safely predict high-resolution computed tomography findings in children with CF, especially in infants. It can be used as an end-point measure for the assessment of beneficial effect of interventions. However, its utility as an outcome measure for the efficacy of therapeutic interventions seems to be dependent on the pathophysiologic mechanisms that underlie each intervention. It seems that more studies, especially longitudinal ones, are required in order to fully clarify the clinical usefulness of LCI, not only in the research setting, but also in every day practice

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Core tip: We herein present an overview of the applications of lung clearance index (LCI) in monitoring the respiratory health status of children with cystic fibrosis (CF). LCI is a more sensitive marker than spirometry and unlike spirometry it can be performed across the entire pediatric age range. At present, it is mostly used in research settings. However, as more data become available from longitudinal studies, it may be proved to be a very useful marker of respiratory status monitoring in children with CF, able to identify early those who are at risk for deterioration and allowing the early application of more aggressive interventions.

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INTRODUCTION

Cystic fibrosis (CF) is a chronic inherited disorder that mainly impairs the lung health and the nutritional status of the affected individuals. Advances in the early diagnosis of the disease, optimal monitoring of the lung health and timely implementation of the appropriate available therapies have improved the quality of life of CF patients as well as their longevity^[1]. The use of a sensitive, reproducible and feasible measure of lung function for monitoring the respiratory health and documenting stability or progression of the pulmonary disease is a prerequisite for the optimization of the patients' management. For decades, conventional spirometry, like in many other respiratory disorders, was the technique of choice for evaluating respiratory status in CF patients^[2,3]. However, this technique is not applicable in infants and toddlers as adequate cooperation and coordination are required for performing the test. Additionally, even in cooperative subjects of the appropriate age, peripheral extension of bronchiectasis, as it is seen on the CT scans, may deteriorate faster compared to the spirometric indices^[4,5]. This implies that spirometry is not sufficiently sensitive for evaluating lung health in CF patients. For these reasons, scientific interest in gas dilution techniques, in particular multiple breath wash out (MBW) measurements, has been revived over the last decade.

The aim of this review is to present an overview of the recent developments in the application of lung clearance index (LCI), the main MBW outcome, in monitoring the respiratory status of children with CF.

HISTORICAL-TECHNICAL BACKGROUND OF MBW

MBW test was first described by Fowler *et al*^[6], in 1952. Fowler developed a method, which could measure the extent of uneven ventilation by obtaining pulmonary nitrogen clearance curves of single breath washouts from healthy subjects and from patients with cardiorespiratory diseases^[6]. This method was laborious and initially received little attention. However, technological evolution and modifications in the nitrogen washout curves led to a replenishment of interest in the application of the method in the clinical setting^[7]. The method has been used successfully since 1985 in young children who could not cooperate to perform conventional spirometry, as only spontaneous tidal breathing is required for MBW test^[8].

The MBW procedure consists of two phases, a wash in and a wash out phase^[9]. During the wash in phase the subject breaths an inert exogenous gas of known concentration, more commonly sulfur hexafluoride, until the concentration of the expired gas reaches the concentration of the delivered gas. At this point the wash out phase of the procedure starts. When nitrogen, which is an inert intrinsic gas, is used

there is no wash in period. During the wash out phase, the subject inhales room air (if an exogenous gas was used in the wash in phase), or 100% oxygen (if the gas used in the wash in phase was nitrogen). The wash out period is considered to be complete when the inert gas concentration reaches the $1/40^{\text{th}}$ of the initial level^[10].

A number of parameters have been suggested for the description of the wash out curve (flow and gas concentration plotted against time). The most commonly used is LCI, whereas mixing ratio and moment ratio are less often reported^[11].

LCI is defined as the number of lung turnovers that are required to reduce the inert gas concentration to the $1/40^{\text{th}}$ of the initial concentration. It is calculated by dividing cumulative expired volume/functional residual capacity. LCI is, therefore, an easy to compute and simple to understand index and it is considered the preferred outcome parameter of ventilation inhomogeneity in CF studies^[12].

CHARACTERISTICS OF MBW TECHNIQUE AND LCI MEASUREMENT

Feasibility

As only relaxed tidal breathing is required for MBW test and the derived LCI index^[10], the test is expected to be feasible even in preschoolers. Quiet breathing is performed through a facemask or a mouthpiece according to the age of the child. The success rate of the test in the clinical setting, in unsedated preschoolers older than 3.5 years of age, ranged from 75%-100%^[13]. Longitudinal monitoring seems to be possible in this age group as preschoolers who completed the first test were also able to complete the test at the follow up visit^[13]. Similar success rate (78%-87%) was observed in the research setting^[14]. However, the test is feasible only under sedation in infants and preschoolers younger than 3.5 years of age. In this age group the success rate ranged from 78.9%-100% in sedated subjects, who were either healthy or suffered from CF and other lung diseases^[15].

Short and long term repeatability of LCI

The mean coefficient of variation of LCI measurements in subjects with CF, within one test occasion, was between 4 to 8% in most studies^[16], with wide, however, range, when reported. These values indicate that LCI is a rather reproducible test.

The knowledge of LCI fluctuation over time is essential for understanding when differences in sequential measurements represent clinically meaningful changes of ventilation inhomogeneity, and not simply measurements variability. The short and long term variability of LCI measurements was acceptable in healthy children and adolescents, ranging from 4.2%-5.1% for one and six months intervals respectively^[17]. These observations indicate that the absolute change of LCI > 1 unit represents a clinical relevant change^[17]. Likewise, Singer *et al*^[18] found that the coefficient of repeatability between tests occasions performed 24 h apart, was 0.96 in children with CF compared to 0.62 in controls. Their results also indicate that a change of LCI of at least 1 unit is clinically meaningful in children with CF. However, later it was shown by Svedberg *et al*^[19] that higher LCI values were associated with higher variability for both intra and inter test measurements. Therefore expressing in absolute numbers the clinical relevant change of LCI may lead to over-representation of clinical meaningful change. According to Svedberg *et al*^[19]'s data a relative increase of > 17% (compared to a previous measurement in a clinically stable CF patient) may be an indicator of lung disease deterioration. Green *et al*^[20] suggested that a change of LCI > 25%, between sessions, indicate a clinically relevant change. As the upper limit of normal Svedberg *et al*^[19] used 1.64 SD whereas Green *et al*^[20] 1.96 SD. In agreement with these findings, Oude Engeberink *et al*^[21] corroborated that in preschool children a relative change of LCI > $\pm 15\%$ is considered clinically relevant and confirmed the observation that LCI variability was proportional to its mean. Therefore, they also supported the view that the expression of LCI change in absolute numbers is biased.

Reference values

Although it was initially considered that LCI was an age independent index in healthy subjects, Lum *et al*^[22] showed that LCI was dependent on body size. Absolute reduction of LCI, which was large enough to be considered of clinical significance, was observed till 6 years of age, whereas thereafter LCI was almost stabilized till early adulthood. Therefore, appropriate reference equations are needed throughout childhood in order to reliably interpret the LCI results. However, it should be noted, that reference data should not be generalized to all different MBW systems or inert gases^[23].

CORRELATION OF LCI WITH FINDINGS FROM LUNG IMAGING

The main structural abnormalities that characterize the CF lung disease are bronchiectasis and small airways disease^[24]. These structural components are clearly depicted by high-resolution computed tomography (HRCT), an accurate modality at delineating not only the extent and severity of advanced lung disease but also early lung disease^[25]. However, its routine use for monitoring CF lung disease has been questioned because of the risk posed by the radiation exposure^[26]. Nevertheless, spirometric indices cannot predict the HRCT findings as structural abnormalities were not uncommon in children with CF and normal spirometric parameters^[4]. By contrast, LCI is a more sensitive indicator than spirometric indices for predicting HRCT abnormalities in CF lung disease^[27]. In a retrospective study of school age children and adolescents with CF^[27] the sensitivity of LCI for detecting HRCT defined abnormalities ranged from 85% to 94% depending on the type of lung damage (bronchiectasis, air trapping, or HRCT score). Therefore, LCI values within the normal range almost precluded the possibility of CF lung disease detectable by HRCT scan. These findings were corroborated by another prospective cross-sectional study in young patients with CF (aged 6-26 years) who had normal FEV1 (> 80% predicted)^[28]. It should be noted that due to technical reasons, in this sample population there was an interval up to 243 d between CT performance and LCI measurement. In this population LCI had a sensitivity of 88% to detect structural lung abnormalities of HRCT, a positive predictive value of 88% but a negative predictive value as low as 63%. Therefore, these findings also supported the notion that an abnormal chest CT is unlikely, but not impossible, in the presence of a normal LCI. Later, another study^[29] was conducted in a population of children with CF, aged 6-10 years, who underwent lung function tests, including LCI measurement, and volumetric HRCT on the same day. This study showed that LCI and HRCT scans have similar sensitivity to detect CF lung diseases with an overall concordance of 81% for total CT score. However, they found a positive predictive value of 88% and a low negative predictive value of 44% for LCI in relation to the detection of HRCT structural abnormalities indicating that a normal LCI did not preclude abnormal HRCT findings. The results of this study are not comparable to the respective results of the two preceding ones as there were methodological differences that are beyond the scope of this review to be presented in detail.

It seems that the age of patients is an important factor for the relation of LCI values with structural lung damage detected by HRCT. In a study (AREST CF program)^[30] that assessed infants with CF diagnosed with newborn screening, it was shown - after controlling for age and infection status - that LCI values were not correlated with the presence of bronchiectasis but only with air trapping.

In accordance to the AREST study^[30], a more recent study^[31] that included pediatric patients with CF across the entire pediatric age spectrum (0-16 years) showed that LCI was not sensitive for the detection of structural abnormalities in infancy. By contrast, in preschool and school age children an elevated LCI had an 85% positive predictive value for the detection of bronchiectasis, but a low negative predictive value of 55% indicating that a normal LCI could not rule out bronchiectasis in almost half of the cases.

Furthermore, a longitudinal three year study^[32], which consisted of patients 6-53 years old, showed that 86% of LCI values in the first year of the study were indicative of the presence or absence of structural lung changes depicted by HRCT scan three years later.

Magnetic resonance imaging has also been used, in research setting, for the detection of early lung disease in children across the entire age range^[33]. It was shown that there was a strong age independent correlation of LCI with airway thickening/bronchiectasis detected by MRI. A moderate correlation with mucus plugging/perfusion abnormalities was also observed. The majority of false negative LCI results were detected in the younger children of the study.

Therefore, at present, LCI could not replace HRCT scans in CF children, particularly the younger ones. More longitudinal studies are needed to explore whether LCI could be used as a potential predictor of the presence and the extent of structural lung damage detected by HRCT, in older children and adolescents.

LCI AS A MEASURE OF EFFICACY OF THERAPEUTIC INTERVENTIONS

As LCI is a sensitive marker of ventilation inhomogeneity and it is easily performed

even in infants, clinical trials were performed to evaluate whether LCI is a reliable outcome measure for the detection of treatment effects of variable interventions.

LCI was improved after the administration of dornase alpha for 28 d, in children with CF, older than 6 years of age, who had mild lung disease ($FEV_1 > 80\%$)^[34]. However, the results of this study should not be generalized in younger children as well as in children with more severe lung disease. Similar findings were observed after the administration of hypertonic saline in pediatric CF patients, aged older than 6 years, with a baseline $FEV_1 > 70\%$ ^[35,36]. However, these results were found only in studies with a duration of at least four weeks^[35], and children with mild lung disease^[36], whereas they were not corroborated in a trial^[37] that assessed the short term LCI change 24 h after hypertonic saline inhalation in children with a baseline $FEV_1 > 40\%$. This discordance may simply indicate that there is an additive effect of multiple doses of hypertonic saline inhalation and/or a different response in treatment in pediatric CF patients' severe lung disease. It also seems that with the use of LCI z-score (z-LCI) change as an outcome measure, a significant treatment effect was observed in infants and preschool children after the twice-daily administration of hypertonic saline for 48 wk^[38]. It needs to be emphasized here that, especially for infancy and early childhood, z-LCI changes should be evaluated in order to adjust for the body size dependence of LCI^[22].

Most recently in a randomized controlled trial^[39] which evaluated the clinical response to ivacaftor in CF children older than 6 years, with mild lung disease ($FEV_1 > 90\%$) and at least one G551D-CFTR allele, it was shown that LCI was more sensitive than spirometry in detecting response in this therapeutic intervention.

In contrast to the above-mentioned findings, the LCI response to antibiotic administration for pulmonary exacerbation was variable. In a recent systematic review^[40], it was shown that a significant but not necessarily clinically relevant treatment effect was observed in patients who received antibiotics for pulmonary exacerbations. Although there was a weak correlation with FEV_1 changes, discordant results between LCI and FEV_1 changes were rather common. There are several hypotheses that could explain the overall small LCI change, the considerable heterogeneity of the observed results, and the discordance with FEV_1 changes which, however, are beyond the scope of this review.

It should be noted, however, that even paradoxical increase of LCI was observed after antibiotic administration for pulmonary exacerbation^[41]. It is speculated that there are non-ventilating lung units during exacerbation that do not contribute to the LCI values. Following antibiotic treatment, and due to the removal of mucus plugging, these areas become ventilated - though not fully but only partially; their partial aeration increases the LCI index^[42].

Overall, it should be recognized that the various therapeutic interventions induce LCI changes to the time interval between the intervention and the assessment of LCI; the severity of the lung disease as it is reflected by baseline spirometric indices; the different mechanisms that underlie the treatment effect of each intervention.

LCI AS A PREDICTOR FOR THE EVOLUTION OF LUNG DISEASE

A question that was raised in the literature was whether LCI values in infancy or early childhood could predict subsequent lung disease status. The London Cystic Fibrosis collaboration (LCFC) followed up a newborn-screened CF cohort up to 2 years of age^[43]. They found that z-LCI at 2 years of age were not associated with the respective results at 3 and 12 mo of age. Therefore, it was recognized in this study that up to 2 years of age LCI could not predict the evolution of the disease. It was, however, also acknowledged that a long-term follow up in preschool and school years is essential for determining whether early measured LCI could be served as a predictor of the lung disease status in childhood.

LCFC also measured LCI and spirometric indices in preschool children (3-5 years) with CF and in healthy controls^[44] and repeated the measurement during early school age (6-10 years). It was found that LCI at preschool age had a positive predictive value of 94% for predicting spirometry or LCI abnormal results, whereas the negative predictive value was as low as 62%. The future repeat of LCI and spirometry in this cohort during late childhood and adolescence will reveal whether preschool age LCI values are predictors for late childhood and adolescence lung health status in CF subjects.

There is also limited data^[45], in children aged 6-19 years, that LCI was a predictor of the occurrence of pulmonary exacerbation during the subsequent 12 mo following the measurement, even in the subgroup of patients with a normal FEV_1 .

It seems therefore that tracking longitudinal changes of LCI may confer to the prediction of lung health status in patients with CF. However the existing data are rather limited and more longitudinal studies are needed for the clarification of this issue.

CONCLUSION

LCI is a sensitive marker for the assessment of respiratory status of children with CF. However, more data from longitudinal studies is needed, in order to clarify which relative change is of clinical importance for identifying the patients who are at risk for respiratory deterioration. Should this information be available, the LCI would be a potential useful marker for monitoring pediatric patients with CF, in every day clinical practice. Until then several obstacles should also be overcome, taking into consideration that the results from different equipment and different inert gasses are not comparable.

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Prevention of necrotizing enterocolitis in premature infants – an updated review

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Abstract

Necrotizing enterocolitis (NEC) is among the most common and devastating diseases encountered in premature infants, yet the true etiology continues to be poorly understood despite decades of research. Recently, gut bacterial dysbiosis has been proposed as a risk factor for the development of NEC. Based on this theory, several best clinical practices designed to reduce the risk of NEC have been proposed and/or implemented. This review summarizes the results of recent clinical trials and meta-analyses that support some of the existing clinical practices for reducing the risk of NEC in premature infants. It is evident that human milk feeding can reduce the incidence of NEC. While most of the studies demonstrated that probiotic supplementation can significantly reduce the incidence of NEC in premature infants, there are still some concerns regarding the quality, safety, optimal dosage, and treatment duration of probiotic preparations. Antibiotic prophylaxis does not reduce the incidence of NEC, and prolonged initial empirical use of antibiotics might in fact increase the risk of NEC for high-risk premature infants. Lastly, standardized feeding protocols are strongly recommended, both for prevention of postnatal growth restriction and NEC.

Key words: Necrotizing enterocolitis; Prevention; Human milk feeding; Probiotics; Empiric antibiotics; Standardized feeding protocols

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Core tip: In this review, we summarize some of the clinical practices recommended to reduce the risk of necrotizing enterocolitis (NEC) in premature infants. Firstly, it is

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evident that human milk feeding can reduce the incidence of NEC. Secondly, while most of the studies demonstrated that probiotic supplementation can significantly reduce the incidence of NEC in premature infants, there are still some concerns regarding the quality of probiotic preparations, safety, optimal dosage, and treatment duration. Thirdly, initial empiric antibiotic use should be restricted in daily practice to reduce the incidence of NEC. Lastly, standardized feeding protocols are recommended both for prevention of postnatal growth restriction and NEC.

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INTRODUCTION

Necrotizing enterocolitis (NEC) is among the most common and devastating diseases encountered in premature infants, yet the true etiology continues to be poorly understood, despite decades of research. Prematurity remains the most consistent risk factor, although term babies can develop NEC with a much lower incidence. Based on a recent large study from the Canadian Neonatal Network, approximately 5.1% (1.3%-12.9%) of infants with a gestational age < 33 wk develop NEC, and the incidence increases with decreasing gestational age^[1]. Despite advances in care in the neonatal intensive care unit (NICU), the estimated mortality rate associated with NEC ranges between 20% and 30%, with the highest rate among infants requiring surgery. Following recovery from the acute phase of NEC, long term complications include intestinal stricture and short bowel syndrome^[2-4].

The classical presentation of NEC includes feeding intolerance, abdominal distension, and bloody stools after 8-10 d of age when feeding enterally. The signs and symptoms are quite variable, ranging from feeding intolerance to evidence of a fulminant intra-abdominal catastrophe with peritonitis, sepsis, shock, and death. Many theories have attempted to elucidate the true pathogenesis since Santulli *et al*^[5] first described a series of NEC cases in premature infants with respiratory distress syndrome. Most theories about the pathogenesis of NEC have focused on the most important risk factors, such as immaturity, formula feeding, and the presence of bacteria^[6]. More recently, gut bacterial dysbiosis has been proposed as the main risk factor for the development of NEC^[7]. Based on this theory, several best clinical strategies are being recommended to reduce the risk of NEC. These include breast milk feeding, restrictive use of antibiotics, supplementation with probiotics, and standardized feeding protocols (SFPs). The purpose of this review is to summarize the results of the recent clinical trials that provide evidence supporting these practices in premature infants as methods to reduce the risk of NEC.

LITERATURE REVIEW

A search was conducted in PubMed (<http://www.ncbi.nlm.nih.gov/pubmed/>) for studies published before 15 June 2018. The search included all terms related to NEC and preventive interventions, including human milk feeding, probiotics, prophylactic antibiotics, and SFPs, utilizing PubMed MeSH terms and free-text words and their combinations through the appropriate Boolean operators. Similar criteria were used for searching MEDLINE. The review was limited to clinical studies involving human subjects. All relevant articles were accessed in full text following PRISMA guidelines. The manual search included references of retrieved articles. We reported the results in tables and text.

HUMAN MILK FEEDING

The unique properties of human milk promote an improved host defense and gastrointestinal function. Several well controlled clinical trials have demonstrated that human milk feeding can reduce the incidence of NEC. The results of the recent

randomized trials are summarized in Table 1^[8-12]. Cristofalo *et al*^[9] and Schanler *et al*^[12] demonstrated that human milk feeding could reduce the incidence of NEC in premature infants compared to those fed with preterm formula in their randomized trials. Sullivan *et al*^[10] studied a total of 207 infants and found that feeding with an exclusively human milk-based diet is associated with a significantly lower rate of NEC than a diet of human milk fortified with bovine milk-based products. Human milk feeding also reduces the incidence of late onset sepsis in premature infants^[12].

Human donor milk is considered a safe alternative when the mother's own milk is not available. When the mother's breast milk supply was deficient, the short-term outcomes related to safety and efficacy were similar in very low birth weight (VLBW) infants who were fed with pasteurized donor milk or with preterm formula in the first 10 d of life^[8]. For feeding extremely preterm infants, donor milk offered little short-term preponderance over preterm formula^[8,11]. However, by using the Cochrane Neonatal search strategy, Quigley *et al*^[13] performed a systematic review and meta-analysis of formula versus donor breast milk for feeding preterm or low birth weight (LBW) infants. They identified 11 randomized or quasi-randomized trials in which 1809 infants participated, and they concluded that donor milk feeding decreased the risk of NEC based on the meta-analysis. However, formula-fed infants had higher in-hospital rates of weight gain, linear growth and head growth^[13]. Furthermore, infants fed with donor human milk-based fortifier had approximately 64% lower odds of developing NEC compared to those fed with bovine-based fortifiers^[14]. It is clear that human milk feeding can reduce the risk of NEC. Recently, the policy statement from the American Academy of Pediatrics on the use of human breast milk states that preterm infants should only receive their own mother's milk or pasteurized human donor milk when their own mother's milk is not available^[15].

Human milk colostrum is high in protein, fat-soluble vitamins, minerals, and immunoglobulins. The benefit of colostrum for newborn infants has been well established. However, most extremely premature infants are usually not ready to be fed in the first few days of life for a variety of reasons. Several studies support the use of colostrum for oral care to provide immunotherapy in preterm infants. The efficacy of oropharyngeal colostrum therapy (OCT) in the prevention of NEC in VLBW infants has been reviewed, and a meta-analysis on this topic was recently published^[16]. Only randomized controlled trials and quasi-randomized trials performed in VLBW infants or preterm infants with gestational age < 32 wk were included for the meta-analysis. As a result, a total of 148 subjects (77 in OCT arm and 71 in control arm) in four trials were analyzed, and no statistically significant difference in the incidence of NEC was demonstrated. The authors concluded that the current evidence was not sufficient to enable the recommendation of OCT as a routine clinical practice in the prevention of NEC^[16].

ADMINISTRATION OF PROBIOTICS

Establishment of a normal intestinal microbial colonization after birth is vital for proper maturity of the innate immune system and maintenance of intestinal barrier function. It has been proposed that disruption of the normal gut microbiota formation may play a major role in the pathogenesis of NEC in premature infants^[17]. Probiotics are live micro-organisms that, upon ingestion at certain amounts, confer health to the host. It is known that probiotics can produce bacteriostatic and bactericidal substances, thus having immunomodulatory effects; furthermore, they prevent colonization of pathogens by competing for adhesion to the intestinal mucosa^[18]. One strategy to prevent NEC is oral administration of probiotics to alter the balance of the gut microbiome in favor of non-pathogenic bacteria. In the past two decades, multiple randomized clinical trials in preterm infants have been performed to evaluate the effect of probiotic administration on NEC prevention. The results of these studies are summarized in Table 2. A total of 10520 infants have now been enrolled in probiotic-NEC studies, and a cumulative pooled meta-analysis of the effects of probiotics on NEC was recently published^[19]. In these trials, a wide variety of probiotic strains, dosages, and durations were used. Despite the clinical heterogeneity, the conclusion of the cumulative meta-analysis was that probiotic treatment decreased the incidence of NEC (average estimate of treatment effect, relative risk: 0.53; 95% CI: 0.42-0.66)^[19]. Therefore, it is clear that some oral probiotics can prevent NEC and decrease mortality in preterm infants. However, it is unclear whether a single probiotic or a mixture of probiotics is most effective for the prevention of NEC. Furthermore, some questions remain unanswered regarding the quality of probiotic products, safety, optimal dosage, and treatment duration.

Probiotics are not all equally effective in preventing NEC in preterm infants. A

Table 1 Summary of five randomized controlled trials of human milk feeding on the risk of necrotizing enterocolitis

Ref.	Yr	GA	BW	N	Study	Control	NEC $\geq$ stage II, %			Mortality, %			LOS, %		
							Study	Control	$P < 0.05$	Study	Control	$P < 0.05$	Study	Control	$P < 0.05$
Corpeleijn <i>et al</i> ^[8]	2016	-	< 1500	373	DM	PF	9.3	8.9	No	13.7	12.7	No	36.6	34.7	No
Cristofalo <i>et al</i> ^[9]	2013	-	500-1250	53	HM	PF	3	21	Yes	0	8	No	55	79	No
Sullivan <i>et al</i> ^[10]	2010	-	500-1250	207	HM	Bovine milk	5.8	15.9	Yes	-	-	-	19	13	No
Schanler <i>et al</i> ^[11]	2005	< 30	-	243	DM	PF	6	9	No	3	3.6	No	1	4.8	No
Schanler <i>et al</i> ^[12]	1999	26-30	-	108	HM	PF	1.6	13	Yes	-	-	-	31	48	Yes

BW: Birth weight; LOS: Late onset sepsis; NEC: Necrotizing enterocolitis.

variety of probiotic strains have been tested in different trials. A detailed analysis of the published data on the effects of probiotics for preterm infant regarding specific probiotic strains was recently performed by the ESPGHAN Working Group on Probiotics, Prebiotics and Committee on Nutrition^[20]. They concluded that both *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb-12/B94 appeared to be effective in reducing NEC. Both the combination of *Lactobacillus rhamnosus* GG with *Bifidobacterium longum* BB536 and the combination of *Bifidobacterium lactis* Bb-12 with *Bifidobacterium longum* BB536, however, showed no measurable effect. They suggest that we need to more precisely define the optimal treatment strategies before the routine clinical use of probiotics in preterm infants for NEC prevention can be recommended. Another recent meta-analysis concluded that multiple strains of probiotics were associated with a significantly lower incidence of NEC, with a pooled OR of 0.36 (95%CI: 0.24-0.53; $P < 0.00001$)^[21]. As probiotics are neither drugs nor devices, they fall into a peculiar category of medical intervention and are therefore not strictly regulated. Because the cost of probiotics is low and the consequences of NEC can be devastating, given the available evidence and safety profile of probiotics from the large number of infants studied, a strong argument can be made for the routine use of probiotics in all preterm infants during their NICU stay^[22]. By offering donor milk to infants at high risk when no maternal milk was available, along with the routine use of probiotics, no confirmed NEC cases were reported in a NICU in Canada for over a year^[22]. A much lower incidence of NEC was also observed in Japan where the use of probiotics in preterm infants was routine^[23]. Based upon this strong evidence, Dr. Taylor^[22] argues that NEC can be easily prevented by the routine use of human milk and prophylactic probiotics.

In fact, a large number of commercially available probiotic preparations have been used in different clinical settings. However, all of these products have not been approved as prescription medications through routine vigorous rules and regulations. Concerns regarding the quality of probiotics and the risk of probiotic-associated sepsis have been raised. For example, an increased incidence of NEC associated with routine administration of a particular probiotic preparation, InfloranTM, in extremely preterm infants was recently reported^[24]. In this observational study, the routine use of probiotics was implemented in 2008 in one NICU. Infants born at < 28 wk gestational age were prospectively followed and compared with historical controls. Routine use of InfloranTM in infants was associated with an increase in stage II or higher NEC (13.3% *vs* 5.9%, $P = 0.010$). Surgical NEC was 12.1% *vs* 5.9% ($P = 0.029$). Adjusting for confounders (sex, gestational age, antenatal steroids and human milk) did not change those trends ($P = 0.019$). Therefore, some experts propose that if an NICU plans to pursue the use of probiotics as a routine supplementation for preterm infants, a quality improvement approach should be utilized to measure the desired effect of probiotics on the risk of NEC and to assess their safety^[19].

RESTRICT EMPIRIC ANTIBIOTIC USE

Empiric antibiotics are commonly used in preterm infants immediately after birth due to the possibility that infection caused preterm labor and the relatively high risk for sepsis in VLBW infants. Because the presence of bacteria is one of the main risk factors

Table 2 Summary of 23 randomized controlled trials of probiotics on the risk of necrotizing enterocolitis

Ref.	Yr	GA	BW	N	Study	NEC $\geq$ stage II, %			Mortality, %			LOS, %		
						Study	Control	P < 0.05	Study	Control	P < 0.05	Study	Control	P < 0.05
Shashidhar <i>et al</i> ^[25]	2017	-	750-1499	104	<i>L. acidophilus</i> , <i>L. rhamnosus</i> , <i>B. longum</i> and <i>S. boulardii</i>	4.1	12.5	No	1.9	5.7	No	-	-	-
Güney-Varal <i>et al</i> ^[26]	2017	≤ 32	≤ 1500	110	<i>L. rhamnosus</i> , <i>L. casei</i> , <i>L. plantarum</i> and <i>B. animalis</i>	0	10	Yes	1.4	22.5	Yes	17.1	35	No
Xu <i>et al</i> ^[27]	2016	30-37	1500-2500	125	<i>S. boulardii</i>	0	0	No	-	-	-	7.8	12.2	No
Hays <i>et al</i> ^[28]	2016	25-31	700-1600	199	<i>B. lactis</i> , <i>B. longum</i> , <i>B. lactis</i> and <i>B. longum</i> .	5.5	5.8	No	-	-	-	-	-	-
Costeloe <i>et al</i> ^[29]	2016	23-30	-	1310	<i>B. breve</i>	9.4	10.0	No	8.3	8.5	No	11.2	11.7	No
Patole <i>et al</i> ^[30]	2014	< 33	-	159	<i>B. breve</i>	0	1.3	No	0	0	No	22	16	No
Totsu <i>et al</i> ^[31]	2014	-	< 1500	283	<i>B. bifidum</i>	0	0	No	1.3	0	No	8.5	13.1	No
Benor <i>et al</i> ^[32]	2014	-	≤ 1500	58	<i>L. acidophilus</i> and <i>B. lactis</i>	4	18.2	No	1	2	NO	24	18	No
Oncel <i>et al</i> ^[33]	2014	≤ 32	≤ 1500	454	<i>L. reuteri</i>	4.0	5.0	No	7.5	10	No	6.5	12.5	Yes
Jacobs <i>et al</i> ^[34]	2013	< 32	< 1500	1099	<i>B. infantis</i> , <i>S. thermophilus</i> , and <i>B. lactis</i>	2.0	4.4	Yes	4.9	5.1	No	14.2	16.5	No
Serce <i>et al</i> ^[35]	2013	≤ 32	≤ 1500	208	<i>S. boulardii</i>	6.7	6.7	No	3.8	4.8	No	24.3	18.3	No
Fernández-Carroce- <i>et al</i> ^[36]	2013	-	< 1500	150	<i>L. acidophilus</i> , <i>L. rhamnosus</i> , <i>L. casei</i> , <i>L. plantarum</i> , <i>B. infantis</i> and <i>S. thermophilus</i>	8.0	16.0	No	1.3	9.3	No	-	-	-
Demirel <i>et al</i> ^[37]	2013	≤ 32	≤ 1500	271	<i>S. boulardii</i>	4.4	5.1	No	3.7	3.6	No	14.9	15.4	No
Rojas <i>et al</i> ^[38]	2012		≤ 2000	750	<i>L. reuteri</i>	2.4	4.0	No	5.9	7.4	No	9.1	10.6	No
Al-Hosni <i>et al</i> ^[39]	2012	-	501-1000	101	<i>L. rhamnosus</i> and <i>B. infantis</i>	6.0	7.8	No	6	7.8	No	26.0	31.4	No
Braga <i>et al</i> ^[40]	2011	-	750-1499	231	<i>B. breve</i> and <i>L. casei</i>	0	3.6	Yes	21.8	24.1	No	33.6	37.5	No

Sari <i>et al</i> ^[41]	2011	< 33	< 1500	221	<i>L. sporogenes</i>	6.5	9	No	2.7	3.6	No	26.4	23.4	No
Mihatsch <i>et al</i> ^[18]	2010	< 30	< 1500	183	<i>B. lactis</i>	2.2	4.5	No	2.2	1.1	No	-	-	-
Samanta <i>et al</i> ^[42]	2009	< 32	< 1500	274	<i>B. infantis</i> , <i>B. bifidum</i> , <i>B. longum</i> and <i>L. acidophilus</i>	5.5	15.8	Yes	4.4	14.7	Yes	14.3	19.5	Yes
Lin <i>et al</i> ^[43]	2008	< 34	< 1500	434	<i>B. bifidum</i> and <i>L. acidophilus</i>	1.8	6.5	Yes	0.9	4.1	No	19.8	11.5	No
Lin <i>et al</i> ^[44]	2005	-	< 1500	367	<i>L. acidophilus</i> and <i>B. infantis</i>	1.1	5.3	Yes	3.9	10.7	Yes	12.3	19.3	Yes
Bin-Nun <i>et al</i> ^[45]	2005	-	≤ 1500	145	<i>B. infantis</i> , <i>S. thermophilus</i> , and <i>B. bifidus</i>	4	16.4	Yes	0	20.5	Yes	-	-	-
Dani <i>et al</i> ^[46]	2002	< 33	< 1500	585	<i>L. rhamnosus</i>	1.4	2.8	No	-	-	-	4.7	4.1	No

BW: Birth weight; LOS: Late onset sepsis; NEC: Necrotizing enterocolitis.

for NEC, some believe that the use of prophylactic antibiotics may decrease the risk of NEC. Others feel that the opposite is true and that the altered normal postnatal gut colonization due to antibiotic use may contribute to the pathogenesis of NEC^[17]. Several randomized controlled clinical trials have been performed to evaluate the effect of prophylactic antibiotic administration on the risk of NEC. The results of randomized controlled trials are summarized in Table 3^[47-51]. Although Siu *et al*^[50] found that prophylactic oral vancomycin conferred some protection against NEC in VLBW infants, Tagare *et al*^[47], Kenyon *et al*^[48] and Owen *et al*^[51] found no protective effect of routine antibiotic use in low risk preterm neonates. Rather, their data suggest that antibiotic may increase the risk of NEC. The efficacy of prophylactic antibiotic usage in the prevention of NEC in premature infants was reviewed, and a meta-analysis on this topic was recently published^[52]. Only randomized controlled trials or retrospective cohort studies in LBW infants or preterm infants were included in the meta-analysis. As a result, a total of 5207 infants were included in nine studies. Based on their meta-analysis, the authors conclude that the current evidence does not support the use of prophylactic antibiotics to reduce the incidence of NEC for high-risk premature infants^[52].

On the other hand, restricting the use of initial empiric antibiotics course may be important. There is increasing recognition that prolonged empirical antibiotic use might increase the risk of NEC for high-risk premature infants. Cotton *et al*^[53] investigated initial empirical antibiotic practices for 4039 extremely low birth weight (ELBW) infants, and 2147 infants in the study cohort received initial empirical antibiotic treatment for more than 5 d. The data suggest that the administration of empiric antibiotics for more than 4 d when the blood culture is negative increases odds of NEC or death in ELBW infants. They suggest that prolonged initial empirical antibiotic therapy for infants with sterile cultures may be associated with increased risk of subsequent death or NEC and should be used with caution. In another retrospective 2:1 control-case analysis from Yale, 124 cases of NEC were matched with 248 controls. Infants with NEC were less likely to have had respiratory distress syndrome ($P = 0.018$) and more likely to have achieved full enteral feeding ($P = 0.028$) than were the controls. The risk of NEC significantly increased with duration of antibiotic exposure when infants with culture-confirmed sepsis were removed from the cohort, and exposure to antibiotics for more than 10 d resulted in an approximately three-fold increase in NEC risk^[54].

Table 3 Summary of five randomized controlled trials of prophylactic antibiotics on the risk of necrotizing enterocolitis

Ref.	Yr	GA	BW	N	Study	NEC $\geq$ stage II, %			Mortality, %			LOS, (%)		
						Stud y	Contro l	P < 0.05	Stud y	Contro l	P < 0.05	Stud y	Contro l	P < 0.05
Tagare <i>et al</i> ^[47]	2010	< 37	-	140	Amoxicillin clavulanic acid and amikacin	13.0	4.2	Yes	2.9	2.8	No	1.4	14.1	Yes
Kenyon <i>et al</i> ^[48]	2002	< 37	-	4809	Co-amoxiclav	1.6	0.3	Yes	5.7	6.2	No	6.2	7.9	No
Oei <i>et al</i> ^[49]	2001	$\leq$ 32	-	43	Erythromycin	4.5	4.8	No	4.5	4.8	No	-	-	-
Siu <i>et al</i> ^[50]	1998	-	< 1500	140	Vancomycin	12.7	27.5	Yes	15.5	18.8	No	-	-	-
Owen <i>et al</i> ^[51]	1993	24-33	-	117	Ampicillin	14	3.5	Yes	6.8	12	No	3.4	10	No

BW: Birth weight; LOS: Late onset sepsis; NEC: Necrotizing enterocolitis.

STANDARDIZED FEEDING PROTOCOL

A current challenge in clinical NEC research is the high variation in feeding practices. It is clear that consistency in approach to feeding intolerance, feeding advancement and breast milk promotion all impact NEC. SFPs address a consistent approach to the: (1) preferred feeding substance; (2) advancement and fortification of feeding; (3) criteria to stop and specifying how to re-start feedings once held; (4) identification and handling of feeding intolerance; and (5) initiation and duration of trophic feeding. SFPs are simple, inexpensive, effective, and transmissible methods for prevention of postnatal growth restriction in premature infants.

In 2015, a total of 482 infants were enrolled in a feeding bundle study, which was a prospective quality improvement project to standardize a protocol for initiating and advancing enteral feeds, and to improve the nutritional care of neonates admitted to the NICU^[55]. In this study, the feeding bundle included breast milk feeding, initiating feedings within 24 h of birth, fortification of breast milk with additional calcium, phosphorus and vitamin D, and the use of trophic feeding for 5 d for ELBW infants followed by daily increases of 10 to 20 mL/kg per day if criteria for tolerance are met. The rate of NEC after bundle implementation was decreased compared to the baseline rate of NEC prior to bundle implementation. Therefore, the authors suggest that early initiation and advancement of enteral feedings does not increase NEC risk, but may actually improve the outcomes^[55]. In 2016, Gephart *et al*^[14] reviewed papers published and found that studies consistently showed lower or unchanged NEC rates when SFPs were used. They combined data from nine observational studies of infants with birth weight < 1500 g and showed overall reduced odds of NEC by 67% (OR = 0.33, 95%CI: 0.17, 0.65, $P = 0.001$) when SFPs were used. Therefore, it is possible that SFPs reduce the risk of NEC.

CONCLUSION

In this review, we summarize the results of the recent clinical trials and meta-analyses that support some of the common clinical practices to reduce the risk of NEC in premature infants. Firstly, it is evident that human milk feeding can reduce the incidence of NEC. We suggest enhanced lactation support in all NICUs, as well as the establishment of more human milk banks in NICUs. Secondly, while most of the studies demonstrated that probiotic supplementation can significantly reduce the incidence of NEC in premature infants, there are still some concerns in regards to the quality of probiotic preparations, safety, optimal dosage, and treatment duration. Thirdly, antibiotic prophylaxis does not reduce the incidence of NEC, and prolonged empirical use of antibiotics may in fact increase the risk of NEC for high-risk premature infants. Therefore, restricting initial empiric antibiotic use should be implemented in daily practice. Lastly, SFPs are recommended both for prevention of postnatal growth restriction and NEC.

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Prospective Study

Prevalence of respiratory syncytial virus infection among children hospitalized with acute lower respiratory tract infections in Southern India

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Abstract

BACKGROUND

Respiratory syncytial virus (RSV) is a leading cause of lower respiratory infections among children.

AIM

To investigate the proportion of RSV and non-RSV respiratory viral infections among hospitalized children ≤ 5 years.

METHODS

Hospitalized children aged < 5 years, with a diagnosis of acute lower respiratory infections (ALRI), admitted between August 2011-August 2013, were included. Cases were defined as laboratory-confirmed RSV and non-RSV respiratory viruses by direct fluorescence assay from the nasopharyngeal wash.

RESULTS

Of 383 1-59 mo old children hospitalized with an acute lower respiratory infection, 33.9% (130/383) had evidence of viral infection, and RSV was detected in 24.5% (94/383). Co-infections with RSV and other respiratory viruses

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(influenza A or B, adenovirus, para influenza 1, 2 or 3) were seen in children 5.5% (21/383). Over 90% of the RSV-positive children were under 2 years of age. RSV was detected throughout the year with peaks seen after the monsoon season. Children hospitalized with RSV infection were more likely to have been exposed to a shorter duration of breastfeeding of less than 3 mo. RSV positive children had a shorter hospital stay, although there were significant complications requiring intensive care. Use of antibiotics was high among those with RSV and non-RSV viral infections.

CONCLUSION

Our study provides evidence of a high proportion of RSV and other virus-associated ALRI among hospitalized children in India. RSV infection was associated with fewer days of hospital stay compared to other causes of lower respiratory infections. A high level of antibiotic use was seen among all respiratory virus-associated hospitalizations. These results suggest the need for implementing routine diagnostics for respiratory pathogens in order to minimize the use of unnecessary antibiotics and plan prevention strategies among pediatric populations.

Key words: Respiratory syncytial virus; Acute lower respiratory infections; Children; Epidemiology; India; Respiratory viral infection

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Core tip: The study shows that a significant proportion of young children hospitalized for acute lower respiratory tract infection were associated with respiratory syncytial virus (RSV) and other viral infections. Early diagnosis of viral infections using a simple test such as the RSV and viral direct fluorescence assay test, in settings where PCR is not feasible, would be useful in the timely institution of appropriate care, minimization of antibiotic overuse, and appropriate follow-up care for complications and sequelae, potentially leading to a reduction of costs of medical care.

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INTRODUCTION

Globally, acute lower respiratory infections (ALRI) are an important cause of morbidity and mortality in children < 5 years of age^[1]. Molecular diagnostic methods have identified respiratory syncytial virus (RSV), as the most common viral cause of ALRI-related death; other prominent viruses are human metapneumovirus, parainfluenza viruses, influenza viruses A and B and adenoviruses^[2,3]. In 2015, Shi *et al*^[4] reported an estimated annual burden of RSV of 33.1 million globally (22% of all episodes of ALRI) resulting in 3.2 million hospitalizations and 59600 deaths of children < 5 years, with about 99% of RSV-related childhood mortality occurring in low and middle-income countries (LMICs)^[4].

Recent literature on pediatric ALRI in LMICs highlights increasing incidence of RSV, a major cause of death of infants < 1 year of age and of ALRI during the first few months of an infant's life^[5]. RSV is also gaining prominence in India; recent hospital-based studies indicate that RSV constitutes up to 16% of children hospitalized with acute lower respiratory tract infections (LRTIs), with incidence highest in infants aged below 6 mo^[6]. The common respiratory symptoms are recurrent episodes of wheezing mimicking early childhood asthma and may persist as lung function abnormality till adolescence^[7]. Known risk factors include low birth weight, smoking during pregnancy, attendance at a child care facility, crowded household, low parental education, exposure to second-hand smoke, history of atopy and lack of breastfeeding^[8]. Furthermore, RSV infections tend to be associated with hospitalization and mortality in high-risk cases^[7].

Detection of RSV using rapid, sensitive and specific diagnostic tests aids good clinical management. Viral isolation by tissue culture is regarded as the gold standard but has limited availability. Real-time reverse transcriptase PCR (RT-PCR) is more sensitive but not routinely performed in clinical diagnostic laboratories in LMICs due to high expense, the need for technical expertise and high laboratory standards to prevent contamination^[9]. Direct fluorescent antibody assay (DFA) has been used as a simple detection tool for RSV antigen detection^[10]. DFA has a sensitivity and specificity of 77.8% and 96.8% respectively and can detect RSV antigens even in conditions where the virus cannot be isolated^[10,11].

The objective of the study was to investigate the proportion of RSV and non-RSV respiratory viral infections as a cause of ALRI among 1-59 mo old children admitted to a tertiary care hospital in southern India. The study also sought to assess the seasonality, clinical features, risk factors and outcome of RSV and non-RSV respiratory viral infections among these hospitalized children.

MATERIALS AND METHODS

Patients and settings

This prospective study was conducted at a tertiary care center in south India between August 2011 and August 2013, on children aged between 1 mo and 5 years, hospitalized for ALRI. The study site, St. John's Medical College Hospital, Bengaluru, Karnataka, India center to local as well as to referred cases from neighboring states like Andhra Pradesh and Tamil Nadu^[12]. ALRI was defined as acute respiratory infection with evidence of respiratory distress and age-specific fast breathing (≤ 2 mo age: ≥ 60 breaths/min; 2-12 mo, ≥ 50 breaths/min; 1-5 years, ≥ 40 breaths/min)^[13]. Features of respiratory distress include chest indrawing, stridor, nasal flaring or grunting, inability to breastfeed or drink, with or without danger signs such as central cyanosis, lethargy or unconsciousness. Bronchiolitis was defined as a viral LRTI in children < 2 years of age characterized by clinical features of small airways obstruction causing symptoms of ALRI. Bronchopneumonia was defined as symptoms of ALRI with chest X-ray findings of hyperinflation with bilateral interstitial infiltrates and peribronchial cuffing. Lobar pneumonia was defined as symptoms of ALRI with confluent lobar consolidation. Reactive airway disease was defined as respiratory illness associated with wheeze secondary to allergen exposure. The study excluded children with empyema, hydropneumothorax or tuberculosis and those with non-respiratory causes of respiratory distress. Informed consent was obtained from the caregivers of eligible children. Detailed patient history and clinical observations were recorded. Vital signs and oxygen saturation were monitored and routine laboratory investigations were performed as indicated. Ethical clearance was obtained from the Institutional Ethics Committee (IRB No 134/2008) at St. Johns Medical College Hospital prior to initiating the study.

Sample collection

Nasopharyngeal (NP) wash were collected using a 5 French infant feeding tube cut to 4 cm length, 1.5 mL of sterile saline was quickly introduced into each nostril and immediately aspirated back into a 2 mL disposable sterile syringe. The aspirated sample was transferred into a sterile centrifuge tube, maintained at 4 °C and transported to the microbiology laboratory immediately on an ice pack (4 °C) within 1 h of collection^[14].

Slide preparation and direct immunofluorescence assay

The NP sample was centrifuged and the pellet was smeared on a slide, air dried and placed in cold acetone (-20 °C) for a minimum of 30 min for fixation. Slides were stained using SimulFluor Respiratory Screen kit (Chemicon International, United States), in accordance with the manufacturer's instructions.

Statistical analysis

The data was compiled in an excel spreadsheet. Descriptive statistics were reported using mean and standard deviation for variables with a normal distribution, and median for variables without a normal distribution. The association between the presence of RSV infection and RSV-related risk factors were assessed by χ^2 test or Fisher's exact test (for categorical variables) and by independent *t*-test (for continuous variables). The analysis was done by using the Statistical software STATA/IC version 12.1 and $P < 0.05$ was considered significant.

RESULTS

Study population

Between August 2011 and August 2013, 9600 children were admitted to the inpatient ward; of these 408 were 1-59 mo old at the time of admission, had ALRI, and were considered eligible for the study. There were 20 caregivers of patients who declined consent citing reasons of non-interest or refusal of permission for the NP wash procedure, and 5 were excluded due to the presence of other diagnoses (2 were diagnosed with pulmonary tuberculosis, and 3 were diagnosed with bacterial pneumonia (one blood culture positive for *Staphylococcus aureus* and two broncho-alveolar lavage fluid positive for *Pseudomonas aeruginosa* and *Enterobacter* species). The remaining 383 children were included in the study. The median age of subjects was 8 mo (inter quartile range 5-15 mo), and 89.0% were less than 2 years of age. There were 68.7% males, and the subjects resided in Karnataka (64.2%), Andhra Pradesh (19.1%) and Tamil Nadu (15.9%). The majority lived in urban areas (66.8%).

Etiology of respiratory illness

Viral etiology (RSV, influenza A or B, adenovirus, para influenza 1, 2 or 3) was confirmed in 130 (33.9%) of 383 children hospitalized for ALRI. RSV was positive in 94 (24.5%), non-RSV viruses in 57 (14.8%), while co-infection with RSV and non-RSV viruses was seen in 21 (5.5%) children (Table 1). Among the children with RSV infection, 86 (91.5%) children were in the age group 1 mo to 24 mo, and 32 (34%) were children 1-6 mo of age. A peak of RSV positive cases was seen after the rainy season during the months of August through November (Figure 1). A smaller peak was also noted during January and February. The seasonality of other viruses mirrored the pattern of RSV infection.

Clinical features and correlates

The predominant physician-assigned clinical diagnosis was bronchiolitis (173/383, 45.1%). Others were bronchopneumonia (135/383, 35.2%), lobar pneumonia (24/383, 6.2%) and reactive airway disease (51/383, 13.3%). Those with RSV infection had fever (23.8%), rhinorrhea (24.6%), cough, (23.5%), chest retractions (25.6%), auscultatory wheeze (22.9%), and auscultatory crepitations (25.7%). Chest radiographs were obtained in 189 of 383 children who had severe ALRI at admission or those who did not respond to first-line treatment. Radiographic abnormalities were detected in 162 children; 135 had evidence of hyperinflation with bilateral interstitial infiltrates suggestive of bronchopneumonia, 24 had focal lung consolidative changes and 3 had evidence of cardiomegaly without lung field abnormalities.

In univariate analysis, RSV infection was significantly associated with being exclusively breastfed for less than 3 mo, compared to those exclusively breastfed for 3-12 mo (unadjusted OR 1.98, 95%CI: 1.69-3.22). There was no significant association between RSV infection and independent variables such as low birth weight, prematurity, complicated neonatal course, family history of asthma, household smoking or indoor wood fuel usage (Tables 2 and 3). There was no significant association between other viruses (influenza A or B, adenovirus, para influenza 1, 2 or 3) and risk factors mentioned in Table 2. Laboratory parameters including total white blood cell count, and absolute lymphocyte count was similar between RSV-positive and RSV-negative patients.

Outcome

Mean hospital stay was 4.6 d (SD 5.1); and 8.3 d (SD 6.5) in RSV-positive and RSV-negative children, respectively ($P = 0.031$) (Table 4). The length of PICU stay ($P = 0.547$), oxygen use ($P = 0.176$), and qualitative antibiotic use ($P = 0.110$) were similar in both groups. Antibiotics were used among 56 (59.5%) and 21 (36.8%) of RSV-positive and non-RSV virus-positive children, respectively. Most commonly used first-line antibiotics were amoxicillin/amoxicillin-clavulanic acid/or ceftriaxone. Respiratory complications such as acute respiratory distress syndrome and respiratory failure requiring PICU admission took place among 13.8% (13/94) of RSV-positive, 8.7% (5/57) of non-RSV positive and 16.6% (42/253) of viral negative children. Of the 57 children admitted to PICU, 46 recovered well while 11 had residual respiratory symptoms including persistent tachypnea and persistent oxygen requirement. There was no mortality recorded in this study (Table 5).

DISCUSSION

Viral-associated respiratory illness among hospitalized children 1-59 mo old at our single center tertiary care hospital in southern India was seen among a third of those

Table 1 Respiratory syncytial virus and non-respiratory syncytial viruses associated with lower respiratory tract infection in young children

RSV	94 (24.5%)
All virus (RSV and/or other virus ¹)	130 (34.0%)
Co-infection with multiple viruses	21 (5.5%)
Non-RSV viruses alone ¹	57 (14.8%)
Virus-negative	253 (66%)
Total enrolled	383

¹Other viruses include infection with parainfluenza viruses, influenza viruses A and B and adenoviruses. RSV: Respiratory syncytial virus.

with ALRI. RSV was the predominant viral etiological agent, and over a quarter had evidence of mixed viral pathogens in the respiratory tract that may be etiologically linked to the respiratory illness. RSV and other respiratory viruses are a major cause of pediatric ALRI in India. ALRI constitutes one of the principal causes of death among children under-five of the developing countries^[8,15] and RSV has been documented as an important cause of ALRI in pediatric age group^[8,14]. In India, studies have shown high ALRI incidence rates of 15.0 per 1000 child years in the under-five age group with the incidence being 3.6 times higher among boys as compared to girls^[16]. The highest rates of ALRI generally occur in the first year of life. RSV infection in the very young causes substantial complications such as respiratory failure, prolonged hospitalization, and high mortality similar to seasonal influenza. Previous hospital-based studies from India reported hospital RSV prevalence of 11.4-26.0% (22.1%^[14]; 20.2%^[17]; 21.3%^[18]; 12.0%^[19]; 26.0%^[20]), although the variability in these studies precludes any direct comparison with the present study. RSV-associated ALRI incidence ranging from 2.4% to 21.2% have been reported in different countries^[21]. Hospital-based studies have reported a significant association between being male and having RSV-related ALRI^[22]. Nair *et al*^[5] concluded in a recent meta-analysis that RSV is the most common cause of childhood LRTI and a major cause of admission to hospital as a result of severe LRTI and that 99.0% of the RSV-related deaths take place among resource-limited countries. The study showed that India, China, Nigeria, Pakistan, and Indonesia together account for a total of 16 million cases of RSV infections, accounting for half of the global cases of under-5 childhood deaths in the world^[1].

In India, routine laboratory diagnoses of viral ALRI remains unavailable and unexplored, even in tertiary care centers. Viral etiology of ALRI largely remains unknown and most cases are empirically treated with antibiotics. Gold standard test like viral isolation and RT-PCR are more sensitive but not routinely performed in clinical diagnostic laboratories in LMICs due to high expense LMICs due to high expense. However, in resource-limited settings, DFA still has value for the diagnosis of ALRI^[23]. Compared to culture, DFA has sensitivities of 72% to 94% and specificities of 95% to 100%^[24]. The DFA kit used in this study, SimulFluor Respiratory Screen Kit (Chemicon) was easy to perform and results were available within 4-5 h. It tested for a panel of seven respiratory viruses; RSV, Influenza A, and B, Parainfluenza 1 to 3 and Adenovirus^[10,11]. Limitations of DFA include the degree of subjectivity in the evaluation of the result, as well as the need for a high level of technical skill and sample quality for the assay^[25].

Early diagnosis facilitates early management and helps combat ALRIs. In India, antibiotics use in pediatric ALRI is very common even if a viral cause is suspected. A Cochrane review on the effectiveness of antibiotics in children fewer than two years old diagnosed with bronchiolitis did not find enough evidence to support the use of antibiotics^[26]. Antibiotics can be used if there is clear documented evidence of secondary bacterial infections^[8]. Early diagnosis of a viral respiratory infection has the potential to reduce the rampant use of antibiotics.

As established by the results, this study demonstrates that exclusive breastfeeding for over 3 mo of age, seems to have some protective effect against RSV and other respiratory viral infections. Significance between exclusive breastfeeding and decreased incidence of RSV positive illnesses have been reported^[27]. However, there is no association between exclusive breastfeeding and wheezing illnesses associated with RSV as wheezing can be secondary to a maternal history of allergy and asthma or pet exposure^[28]. This possibly can be explained by the fact that RSV is caused by infection and breastfeeding is often thought to confer some protection against it^[29], whereas wheezing may be immune to any protective effects of breastfeeding since

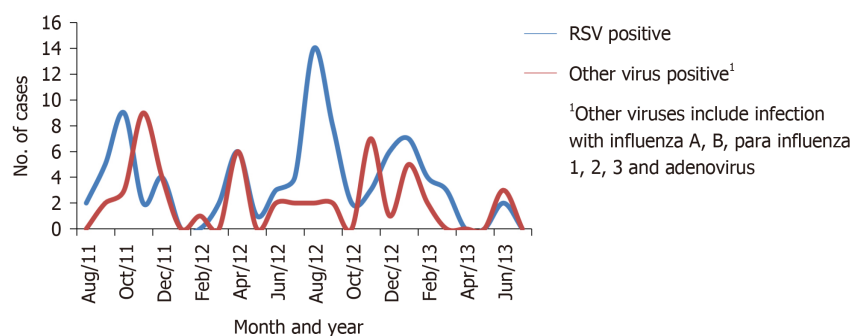


Figure 1 Seasonality patterns of respiratory syncytial virus-positive and non-respiratory syncytial virus viral-associated lower respiratory tract infection in young children.¹Other viruses include infection with influenza A, B, para influenza 1, 2, 3 and adenovirus.

high IgE levels have been found to share an association with the presence of wheezing.

Our finding showed a high rate of respiratory complications in 13.8% (13/94), 8.7% (5/57) and 16.6% (42/253) of children with RSV positive, non-RSV positive and viral negative results. Out of these, 6 children were mechanically ventilated with a median of 4 d (range 3-5 d). Our findings were consistent with previous studies done by Kholy *et al*^[30] in which 10% (24/240) of patients were admitted to PICU with a median duration of 6.5 d and 14 patients required mechanical ventilation.

Limitations of the study

In this study, we focused only on the viral causes of ALRI as they are the most common etiological agents and there are limited studies from south India. However, we did not address key questions about bacterial etiology and the possible role of viral and bacterial co-infections. We had a small sample size which was insufficient to make finer observations regarding differences in age, clinical profile, or factors determining severity between specific viral species.

We find that a significant proportion of young children hospitalized for acute LRTI is associated with RSV and other viral infections. Early diagnosis of viral infections using a simple test such as the RSV and viral DFA test, in settings where PCR is not feasible, would be useful in the timely institution of appropriate care, minimization of antibiotic overuse, and appropriate follow-up care for complications and sequelae, potentially leading to a reduction of costs of medical care.

Table 2 Risk factors for respiratory syncytial virus infection

Variable		RSV negative (n = 289)	RSV positive (n = 94)	P value
Birth weight (≥ 2500 g)	No	55 (75.3)	18 (24.7)	0.980
	Yes	234 (75.4)	76 (24.6)	
Breast fed exclusively for ≥ 3 mo	No	66 (68.0)	31 (32.0)	0.049
	Yes	223 (77.9)	63 (22.1)	
Gestational age (≥ 37 wk)	No	33 (75.0)	11 (25.0)	0.940
	Yes	256 (75.5)	83 (24.5)	
Neonatal complications	No	241 (74.8)	81 (25.2)	0.522
	Yes	48 (78.6)	13 (21.4)	
Family history of asthma	No	256 (75.9)	81 (24.1)	0.532
	Yes	33 (71.7)	13 (28.3)	
History of smoking in the household	No	220 (73.8)	78 (26.2)	0.165
	Yes	69 (81.1)	16 (18.9)	
Kitchen type	Indoor with partition	202 (74.2)	70 (25.8)	0.484
	Indoor without partition	85 (78.7)	23 (21.3)	
	Open air	2 (66.6)	1 (33.4)	
Cooking fuel	Electric	2 (100)	0 (0)	0.695
	Kerosene	6 (66.6)	3 (33.4)	
	Liquefied petroleum gas	241 (74.8)	81 (25.2)	
	Wood/ dung	40 (80.0)	10 (20.0)	
PICU admission	No	245 (75.2)	81 (24.8)	0.741
	Yes	44 (77.2)	13 (22.8)	

RSV: Respiratory syncytial virus; PICU: Pediatric intensive care unit.

Table 3 Risk factors among respiratory viral infection

Variable		No virus detected (n = 253)	Respiratory viral positive (n = 130)	P value
Birth weight (≥ 2500 g)	No	48	22	0.623
	Yes	205	108	
Breast fed exclusively for ≥ 3 mo	No	198	92	0.106
	Yes	55	38	
Gestational age (≥ 37 wk)	No	30	14	0.752
	Yes	223	116	
Neonatal complications	No	214	111	0.836
	Yes	39	19	
Family history of asthma	No	214	114	0.751
	Yes	27	16	
History of smoking in the household	No	195	106	0.315
	Yes	58	24	
Kitchen type	Indoor with partition	174	99	0.313
	Indoor without partition	77	30	
	Open air	2	1	
PICU admission	No	214	112	0.683
	Yes	39	18	

RSV: Respiratory syncytial virus; PICU: Pediatric intensive care unit.

Table 4 Comparison of outcome between respiratory syncytial virus positive and respiratory syncytial virus negative

Days of	RSV		P value
	Positive	Negative	
Hospital stay	4.0 (1.0-17.0)	4.0 (1.0-65.0)	0.031
Oxygen use	1.5 (1.0-9.0)	2.0 (1.0-65.0)	0.176
Antibiotic use	4.0 (1.0-12.0)	4.0 (1.0-29.0)	0.303
Nebulization use	3.0 (1.0-17.0)	4.0 (1.0-65.0)	0.012
PICU stay	2.5 (1.0-10.0)	2.00 (1.0-20.0)	0.547

RSV: Respiratory syncytial virus; PICU: Pediatric intensive care unit.

Table 5 Association of signs and symptoms with respiratory syncytial virus infection

Variable		RSV positive(n = 94)	RSV negative(n = 289)	P value
Fever	Yes	63 (23.8)	201 (76.2)	0.645
	No	31 (26.0)	88 (74.0)	
Rhinorrhea	Yes	81 (24.6)	248 (75.4)	0.931
	No	13 (24.0)	41 (76.0)	
Cough	Yes	85 (23.5)	276 (76.5)	0.066
	No	9 (40.9)	13 (59.1)	
Fast breathing	Yes	62 (24.5)	191 (75.5)	0.981
	No	32 (24.6)	98 (75.4)	
Wheezing	Yes	45 (22.9)	151 (77.1)	0.461
	No	49 (26.2)	138 (73.8)	
Auscultatory crepitations	Yes	73 (25.7)	210 (76.3)	0.338
	No	21 (21.0)	79 (79.0)	
Chest retractions	Yes	52 (25.6)	151 (74.4)	0.604
	No	42 (23.3)	138 (76.6)	

RSV: Respiratory syncytial virus; PICU: Pediatric intensive care unit.

ARTICLE HIGHLIGHTS

Research background

Respiratory syncytial virus (RSV) is the most frequent agent of viral-associated acute lower respiratory diseases (ALRI) and is known to be associated with hospitalization and mortality among high-risk cases.

Research motivation

Early diagnosis of viral infections using a simple test such as the RSV and viral fluorescent antibody assay (DFA) test, in settings where PCR is not feasible, would be useful in the timely institution of appropriate supportive care, minimization of antibiotic overuse, and appropriate follow-up care for complications and sequelae, potentially leading to a reduction of costs of medical care.

Research objectives

The principal objective of the study was to investigate the proportion of RSV and non-RSV respiratory viral infections as a cause of ALRI among 1-59 mo old children admitted to a tertiary care hospital in India. The study also assesses the seasonality, clinical features, risk factors and outcome of RSV and non-RSV respiratory viral infections among these hospitalized children.

Research methods

The prospective study was conducted on hospitalized children aged < 5 years, with a diagnosis of acute lower respiratory infections (ALRI), admitted between August 2011-August 2013, were included. Nasopharyngeal (NP) swabs were obtained from eligible children, and transported to the laboratory in suitable media. Slides were prepared from the media, and DFA staining was performed using SimulFluor Respiratory Screen kit on NP wash samples.

Research results

The median age of subjects was 8 mo (inter quartile range 5-15 mo), and 89.0% were less than 2 years of age. Viral etiology (RSV, influenza A or B, adenovirus, para influenza 1, 2 or 3) was confirmed in 33.9% (130/383) of children hospitalized for ALRI. RSV was positive in 24.5% (94/383) non-RSV viruses in 14.8% (57/383) while co-infection with RSV and non-RSV viruses was seen in 5.5% (21/383) children. A peak of RSV positive cases was seen after the rainy season during the months of August through November. The RSV infection was significantly associated with being exclusively breastfed for less than 3 mo. There was no significant association between RSV infection and independent variables such as low birth weight, prematurity, complicated neonatal course, family history of asthma, household smoking or indoor wood fuel usage. Mean hospital stay was 4.6 ± 5.1 d and 8.3 ± 6.5 d in RSV-positive and RSV-negative children, respectively. The respiratory complications such as acute respiratory distress syndrome and respiratory failure requiring PICU admission, were 13.8% (13/94), 8.7% (5/57) and 16.6% (42/253) of children with RSV positive, non-RSV positive and viral negative results.

Research conclusions

A high proportion of RSV and other virus-associated ALRI were seen among hospitalized children in India. The study demonstrates that exclusive breastfeeding for over 3 mo of age, may have a protective effect against RSV and other respiratory viral infections. The viral DFA test was easy to perform and results were available within 4-5 h.

Research perspectives

Early diagnosis of viral infections using a simple test such as the RSV and would be useful in the timely institution of appropriate supportive care, minimization of antibiotic overuse, and appropriate follow-up care for complications and sequelae, potentially leading to a reduction of costs of medical care.

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CASE REPORT

- 43 Congenital diarrhea in a newborn infant: A case report
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Congenital diarrhea in a newborn infant: A case report

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Abstract

BACKGROUND

Microvillus inclusion disease (MVID) is a rare autosomal recessive cause of severe congenital diarrhea with significant morbidity and mortality. Definitive treatment involves bowel transplant. The diagnosis of this condition can be challenging and a few genetic panels are available for the identification of the most common mutations. We present the case of an infant with MVID due to a mutation not reported in the literature before.

CASE SUMMARY

We report the case of an infant transferred to our institution with severe diarrhea of unknown etiology, failure to thrive, and significant metabolic derangements. An extensive work-up including stool studies for common gastrointestinal pathogens, abdominal ultrasound, esophagogastroduodenoscopy with duodenal biopsy and flexible sigmoidoscopy failed to reveal a diagnosis. Multiple dietary and formula regimens were introduced but all resulted in voluminous diarrhea. She remained on total parenteral nutrition (TPN) for the duration of her hospital stay. Genetic testing was done and she was subsequently found to have a novel mutation in the *MYO5B* gene [homozygous mutation for MYO5B c.1462del, p. (Ile488Leufs*93)] giving us the diagnosis of MVID. She remains on TPN while awaiting bowel transplant at the time of the compilation of this case report.

CONCLUSION

We report a novel mutation involved in MVID and highlight the importance of considering this disease when faced with a newborn presenting with life threatening diarrhea. At the time of this publication, 232 allelic variations of this gene (MIM#606540) exist in National Center for Biotechnology Information's database. Our patient's mutation has not been reported in literature as a cause of MVID.

Key words: Case report; Congenital diarrhea; Microvillus inclusion disease; Failure to thrive

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Core tip: Microvillus inclusion disease is a rare autosomal recessive cause of severe congenital diarrhea with significant morbidity and mortality. Infants most commonly present with failure to thrive, severe diarrhea, cholestasis, and electrolyte abnormalities. Diagnosis can be challenging and a few genetic panels are available for the identification of the most common mutations. We present the case of an infant transferred to our institution with severe diarrhea, failure to thrive, and significant metabolic derangements who was subsequently found to have a novel mutation in the *MYO5B* gene [homozygous mutation for *MYO5B* c.1462del, p. (Ile488Leufs*93)]. Infants with microvillus inclusion disease generally require life-long intravenous nutritional support or bowel transplant.

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INTRODUCTION

Microvillus inclusion disease (MVID) is a rare autosomal recessive cause of severe congenital diarrhea with significant morbidity and mortality^[1,2]. Infants most commonly present with failure to thrive, severe diarrhea, cholestasis, malnutrition, and electrolyte abnormalities. Diagnosis can be challenging and a few genetic panels are available for the identification of the most common mutations. We present the case of an infant transferred to our institution with severe diarrhea of unknown etiology, failure to thrive, and significant metabolic derangements who was subsequently found to have a novel mutation in the *MYO5B* gene [homozygous mutation for *MYO5B* c.1462del, p. (Ile488Leufs*93)]. Infants with MVID generally require life-long IV nutritional support with close monitoring of any electrolyte imbalances that may require correction.

The *MYO5B* gene is responsible for the coding of a protein called myosin Vb^[2]. *MYO5B* gene mutations that cause MVID result in a decrease or absence of myosin Vb function^[2]. In the enterocytes of the small bowel, a lack of myosin Vb function leads to abnormal formation of microvilli^[2]. Microvilli are small finger-like projections from the surface of enterocytes that absorb nutrients and fluid from food passing through the intestine. In MVID, affected enterocytes have small clumps of abnormal microvilli mixed with digestive proteins that form microvillus inclusions^[3]. These dysfunctional enterocytes with abnormal microvilli are unable to absorb nutrients and fluids. This subsequently leads to recurrent severe diarrhea, malnutrition, and dehydration in individuals with MVID.

CASE PRESENTATION

A 17-day-old, premature, female infant was transferred to our neonatal intensive care unit (NICU) for failure to thrive, severe metabolic derangements, and severe, intractable diarrhea. She was a 34 5/7 wk gestation infant of African American descent born *via* vaginal delivery to a 22-year-old gravida 3, para 1-0-1-1 woman. Prenatal labs were mostly unremarkable except for a Penta screen that was abnormal with a 1:81 risk of Trisomy 18. Amniotic fluid was noted to be meconium-stained with a duration of rupture of membranes of 5 h. There was no diagnosis of polyhydramnios, although the delivery team did notice a large amount of amniotic fluid. Apgar scores were 8 and 9 at 1 and 5 min, respectively. The birthweight was 2.445 kg (62.7% percentile), length was 47 cm (69.2%), and head circumference was 34 cm (93.1%). The infant was admitted to the NICU for prematurity and respiratory distress. Family history was significant only for a maternal uncle with a history of gastroschisis. There was no known consanguinity.

Upon admission to the outside NICU, the patient was intubated for surfactant administration and subsequently extubated to nasal continuous positive airway pressure. She was treated with ampicillin and gentamicin for 5 d from birth for a diagnosis of clinical sepsis. On day of life (DOL) 1, she was started on feeds of

breastmilk *via* orogastric tube, which were fortified the following day. Overnight, she developed extensive watery diarrhea. On DOL 4 she was noted to be down 25% from birthweight and developed significant metabolic acidosis with a serum CO₂ level of 6 mmol/L and acute kidney injury (creatinine 3.02 mg/dL). She had profound electrolyte derangements including hyponatremia of 157 mmol/L, hyperkalemia of 9.2 mmol/L, hyperchloremia of 128 mmol/L, hyperglycemia of 227 mg/dL. She had unconjugated hyperbilirubinemia (total bilirubin 15.2 mg/dL, direct bilirubin 1.2 mg/dL), was started on phototherapy. Patient had leukocytosis to 25,000/ μ L with immature-to-total (I:T) ratio of 0.10. She was then made nil per os (NPO) while electrolyte abnormalities were corrected with normal saline and sodium bicarbonate replacement. Stool studies were sent and the culture was found to be positive for campylobacter antigen. The patient was subsequently treated with 3 d of azithromycin. Mother's breastmilk was sent for microbial studies and was found to be positive for coagulase negative staph and rare *E. coli*. Patient's blood cultures from admission and DOL 4 resulted negative.

Over the following 2 wk, she was again started on various feeding regimens of donor expressed breast milk, conventional cow's milk formula (Similac Sensitive), and casein-based extensively hydrolyzed formulas (Alimentum). Every feeding trial resulted in a relapse of profuse, watery, non-bloody stools and electrolyte derangements, requiring cessation of enteral feeds. On DOL 17, the patient was transferred to our institution for further work-up and management of refractory diarrhea with feeds associated with dehydration and failure to thrive. At the time of transfer, the patient was receiving TPN with SMOFlipid® (soybean oil, medium chain triglycerides, olive oil, and fish oil). Physical exam on admission revealed signs of moderate dehydration including sunken eyes, dry skin, and capillary refill about 4 s. The patient continued to have significant loose stools (approximately 8/d). Infectious disease and gastrointestinal consults were obtained. With the patient's history of positive campylobacter stool antigen test and leukocytosis, the clinical picture was initially thought to be consistent with infectious diarrhea. Stool studies, a full sepsis work-up, and maternal HIV testing were performed and the results were negative. A gastrointestinal (GI) pathogen, PCR panel which tests for 21 of the most common bacterial, and viral pathogens implicated in infectious diarrhea were also negative. Stool was positive for abundant leukocytes and occult blood. Stool and blood cultures were repeated and found to be negative. Stool osmotic gap was 30 mOsm/kg, and the newborn screen results were positive for glucose-6-phosphate dehydrogenase deficiency, which were subsequently confirmed following further testing. Over the hospital course, she developed worsening unconjugated hyperbilirubinemia, thought to be secondary to TPN-induced cholestasis.

On DOL 19, the patient continued to have diarrhea despite strict NPO, raising suspicion of a secretory diarrhea. An extensive work-up was initiated including EGD and flexible sigmoidoscopy, which were visually normal and the results of a duodenal biopsy showed, "well preserved enterocytes with well-preserved brush borders with no evidence of brush border loss or MIVD." The infant also continued to have electrolyte abnormalities, most significantly hyponatremia and hypochloremia. Stool sodium (Na) and chloride (Cl) were significantly increased while urine Na and Cl were decreased (Figure 1).

Once the infant's electrolytes were stabilized and diarrhea improved, she was started once again on trophic feeds with Enfalyte, which were then advanced to amino acid-based formula (Elecare). Although initially tolerated, relapse of severe diarrhea occurred within 48 h. After this time, a congenital secretory diarrhea genetic panel that was sent weeks earlier returned positive for homozygous mutation for MYO5B c.1462del, p. (Ile488Leufs*93), which caused diarrhea with microvillus atrophy.

Based on the above work-up, she was diagnosed with MIVD. Treatment was supportive with IV nutritional support and correction of electrolyte imbalance. She was transferred to a bowel transplant center for evaluation and subsequently transferred back to our institution to be maintained on intravenous TPN until reaching a weight of 6.5 kg, at which point she will be placed on a waiting list for a bowel transplant.

DISCUSSION

The differential for diarrhea in a neonate is broad. The most common causes include food allergy, gastrointestinal infections, and antibiotic-associated diarrhea followed by congenital defects of ion transport^[4]. Once the most common etiologies have been ruled out, the list of differential diagnoses must be shifted to the various

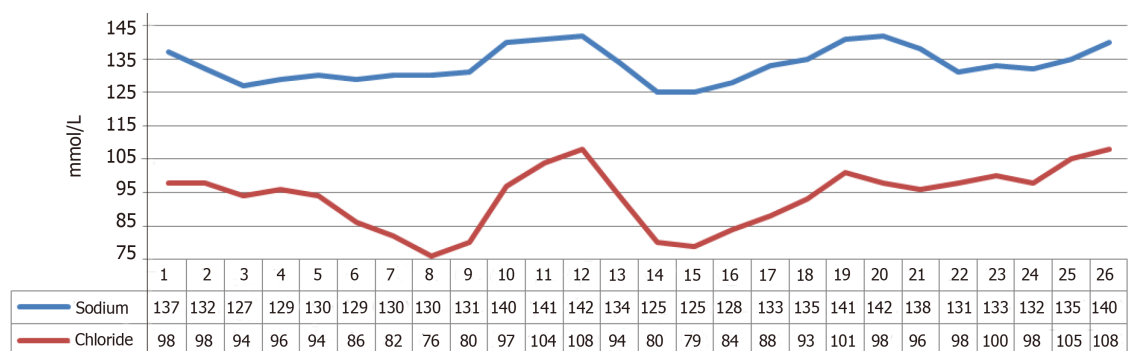


Figure 1 Serum sodium and chloride levels

enteropathies. These include immunodysregulation polyendocrinopathy enteropathy x-linked syndrome, autoimmune enteropathy, intestinal epithelial dysplasia (tufting enteropathy), brush border disaccharidase deficiencies, microvillous inclusion disease, and membrane channel/carrier protein defects such as congenital sodium and chloride channel defects^[5]. Family history of chronic diarrhea, polyhydramnios, or dilated bowel loops on ultrasound should also raise the suspicion for congenital diarrhea^[5].

Initial diagnostic steps include making the patient NPO and measuring the stool osmotic gap to differentiate between osmotic and secretory diarrhea. Stool osmotic gap is defined as $[\text{Osmolality} - 2 \times (\text{stool Na} + \text{stool K})]$ and is normally between 50 and 100 mOsm/kg. Osmotic diarrhea is caused by impaired absorption of osmotically active solutes, which draws water into the intestinal lumen *via* osmosis as seen with lactase deficiency. Osmotic diarrhea improves with fasting and usually involves an osmotic gap > 100 mOsm/kg^[5]. Conversely, secretory diarrhea is the result of either decreased absorption or increased secretion of electrolytes, does not improve with fasting, and usually involves an osmotic gap < 50 mOsm/kg^[5]. This leads to active secretion of water into the intestinal lumen as seen in Congenital channelopathies.

In our patient, the stool osmotic gap was 30 mOsm/kg, consistent with secretory diarrhea. When suspecting secretory diarrheas, an evaluation of thyroid function may be beneficial as hyperthyroidism increases GI motility, decreasing transit time and the absorption of electrolytes. ESR and CRP may be used to screen for very-early onset inflammatory bowel disease, and liver function tests to evaluate for cholestatic liver disease and subsequent bile acid insufficiency. Endoscopy followed by genetic testing for the most commonly involved genes such as SLC26A3 (diarrhea, secretory chloride, congenital), SPINT2 and SLC9A3 (diarrhea, secretory sodium, congenital), and MYO5B (diarrhea with microvillus atrophy) can lead to the diagnosis^[6].

MVID (OMIM 251850) is an autosomal recessive condition causing intractable secretory diarrhea. As in the case of our patient, early-onset MVID is known to cause symptoms within the first few days of life, while late-onset MVID is associated with onset of diarrhea around 2-3 mo of age^[7]. Mutation in Myosin Vb (MYO5B) causes the classical form of MVID^[7,8]. Myosin Vb is an actin motor protein that plays a key role in apical transport and polarization of enterocytes. Defect of this protein leads to failure of microvilli incorporation into apical membrane^[6]. Classical histological features include shortening or loss of microvilli, microvillus inclusions and accumulation of tubulo-/vesicular structures in subapical secretory granules^[7,8]. At the time of this publication, 232 allelic variations of this gene (MIM#606540) exist in National Center for Biotechnology Information's database^[1]. Our patient has a c.1462del that has not been reported in literature as a cause of MVID. Mutations in Syntaxin 3 have been reported as causes for atypical, milder form of MVID^[9,10]. Syntaxin 3 is an apical SNARE receptor protein necessary for microvilli-lined vesicular docking. Histological features in the atypical variant are the same with the exception of location of secretory granules, which are located along the basolateral membrane and microvilli at the lateral surfaces^[9,10]. Decreased expression of sodium hydrogen exchanger, sodium glucose co-transporter leads to impaired water absorption, while expression of cystic fibrosis transmembrane conductance regulator leads to chloride secretion and accounts for high losses of sodium and chloride^[11,12].

Without functional microvilli and appropriate ion channels, enterocytes are unable to absorb ions and nutrients and are largely TPN-dependent with bowel transplant being the gold standard of treatment. MYO5b is found in epithelial cells of other organ systems; however, definitive causation of other disease processes remains

under investigation. Some conditions thought to be associated with MVID include inguinal hernias, aganglionic megacolon, pneumocystic jiroveci pneumonia, autosomal dominant hypochondroplasia, hypophosphatemic rickets, bicuspid aortic valve, coarctation of aorta, liver dysfunction independent of TPN-associated cholestasis, multiple hepatic adenomas, Down's syndrome, renal dysplasia, and nephrocalcinosis^[13-15]. Our patient developed nephrocalcinosis while she was at the transplant hospital for evaluation, which was attributed to dehydration which was managed with fluids being unfused at three-times the maintenance rate. Small bowel transplantation, despite its innate complications and risk of rejection, improves survival (100% in transplanted patients *versus* 40% in patients without transplant at a median follow-up of 3 years) and quality of life as all patients were able to be weaned off TPN^[2].

Congenital diarrheas can pose a diagnostic challenge with high morbidity and mortality from electrolyte derangements and failure to thrive. Clinicians must keep a high index of suspicion for timely diagnosis and management. Prenatal diagnosis may be possible in suspected cases by genetic testing. Infants are at high risk of mortality if disease is not promptly diagnosed. Care for infants with MVID involves a multidisciplinary team approach consisting of gastroenterologists, nutritionists, transplant surgeons, social workers, and case managers. Pediatricians must be vigilant to screen for associated conditions. Parents and caretakers require extensive support, counseling and education in acceptance and management of this rare disorder.

CONCLUSION

MVID is a rare gastrointestinal condition that involves chronic, watery, life-threatening diarrhea caused by mutations in the *MYO5B* gene. Onset of symptoms is as early as the first few hours of life and patients usually require long term intravenous TPN. Consideration of this disease in the differential of any infant with unexplained, severe, intractable diarrhea is essential to ensure timely diagnostic investigations.

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